AGING

From Fundamental Biology to Societal Impact

Edited by Paulo J. Oliveira and João O. Malva

Aging

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Edited by

PAULO J. OLIVEIRA

CNC - Center for Neuroscience and Cell Biology,
CIBB - Centre for Innovative Biomedicine and Biotechnology,
University of Coimbra, Coimbra, Portugal

JOÃO O. MALVA

Coimbra Institute for Clinical and Biomedical Research (iCBR),
Center for Innovative Biomedicine and Biotechnology (CIBB),
Institute of Pharmacology and Experimental Therapeutics,
Faculty of Medicine, University of Coimbra, Coimbra, Portugal

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Contents

Section 3 Aging-related physiology, disease and prevention of aging-related diseases

List of contributors

Margarida Abrantes
Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Raquel Alves
Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal; Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal

Sharath Anugula
Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

Veronica Aran
Paulo Niemeyer State Brain Institute of the Health Secretariat of the State of Rio de Janeiro, Rio de Janeiro, Brazil

Lina Badimon
Cardiovascular Program ICCC - Research Institute Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, Barcelona, Spain; Centro de Investigación Biomédica en Red Cardiovascular (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain; Autonomous University of Barcelona (UAB), Barcelona, Spain

Jorge N. Barreto
Directorate National of Health, Ministry of Health and Social Security, Praia, Cabo Verde

Grzegorz Bartosz
Department of Bioenergetics, Food Analysis and Microbiology, Institute of Food Technology and Nutrition, College of Natural Sciences, Rzeszow, Poland

Fernanda Borges
CIQUP, Department of Chemistry and Biochemistry, School of Sciences, University of Porto, Porto, Portugal

Consuelo Borrás
Freshage Research Group, Department of Physiology, Faculty of Medicine, University of Valencia, CIBERFES-ISCIII, INCLIVA, Valencia, Spain

Maria Filomena Botelho
Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Jean Bousquet
MACVIA-France, Montpellier, France

António M.D. Brehm
Human Genetics Laboratory, University of Madeira, Funchal, Portugal

Gerly A.C. Brito
Department of Morphology, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil

Daan Bultje
Healthy Ageing Network Northern Netherlands (HANNN), Groningen, The Netherlands

Ana Cristina Cabral
Pharmacology and Pharmaceutical Care Laboratory, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

André Caetano
CNC - Centre for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

Helena Canhão
Lisbon AHA Reference Site, CHRC, NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal

Isabel Marques Carreira
Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Ana Maria Carriazo
Andalusia Reference Site, Regional Ministry of Health and Families of Andalusia, Seville, Spain

Cristina Carvalho
CNC - Centre Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; IIIUC -Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

Eugenia Carvalho
CNC - Centre Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal

Margarida Castel-Branco
Pharmacology and Pharmaceutical Care Laboratory, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; Coimbra Institute for Clinical and Biomedical Research (iCBR), University of Coimbra,Coimbra, Portugal

Yaohua Chen
Global Brain Health Institute, Trinity College Dublin, Dublin, Ireland; University of Lille, Inserm UMR-S1172, Lille Neurosciences & Cognition, Degenerative and Vascular Cognitive Disorders, CHU Lille, LiCEND, Lille, France; Department of Geriatrics, CHU Lille, Lille, France

João M. Coelho-Filho
Department of Clinical Medicine, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil

Vera Constâncio
Cancer Biology and Epigenetics Group, Research Center of IPO Porto (CI-IPOP)/ RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Centre (Porto.CCC), Porto, Portugal; Department of Pathology and Molecular Immunology, School of Medicine and Biomedical Sciences, University of Porto (ICBAS-UP), Porto, Portugal

António Correia e Silva
Faculty of Social Sciences, Humanities and Arts, University of Cabo Verde, Praia, Cabo Verde

Deiziane V.S. Costa
Department of Morphology, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil; Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, VA, United States

Elísio Costa
UCIBIO REQUIMTE, Faculty of Pharmacy and Competence Center on Active and Healthy Ageing (Porto4Ageing Reference Site), University of Porto, Porto, Portugal

Inês Costa
Coimbra Institute for Clinical and Biomedical Research (iCBR), CIBB - Center for Innovative Biomedicine and Biotechnology, Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Teresa Cunha-Oliveira
CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Cantanhede, Portugal

Paulo de Carvalho
CISUC, Centre for Informatics and Systems of University of Coimbra, DEI, Coimbra, Portugal

Vicenzo De Luca
Dipartimento di Sanità Pubblica, Università degli Studi di Napoli Federico II, Naples, Italy

Edoardo R. de Natale
Neurodegeneration Imaging Group, University of Exeter Medical School, London, United Kingdom

Jorge Dias
Institute of Systems and Robotics, University of Coimbra, Coimbra, Portugal; Center for Autonomous Robotic Systems, Khalifa University, Abu Dhabi, UAE

Ronaldo P. Dias
Laboratory of Biology of Tissue Healing, Ontogeny and Nutrition, Department of Morphology, Center of Biomedicine, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil

Ana I. Duarte
CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; CNC - Center for Neuroscience and Cell Biology, Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

John Farrel
Reference Site Collaborative Network (RSCN), Brussels, Belgium

Fernando Fernandez-Llimos
Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal; Center for Health Technology and Services Research (CINTESIS), University of Porto, Porto, Portugal

Lino Ferreira
CNC - Center for Neuroscience and Cell Biology, CIBB — Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Coimbra, Portugal

Luigi Ferrucci
National Institute on Aging, Baltimore, MD, United States

Isabel Vitoria Figueiredo
Pharmacology and Pharmaceutical Care Laboratory, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; Coimbra Institute for Clinical and Biomedical Research (iCBR), University of Coimbra, Coimbra, Portugal

Jamie K. Ford
School of Public Health, Imperial College London, London, United Kingdom

Claudio Franceschi
Institute of Information Technologies, Mathematics and Mechanics and Photonics Center, Department of Fundamental and Applied Research, National Research Lobachevsky State University of Nizhni Novgorod, Nizhny Novgorod, Russia

Alex A. Freitas
School of Computing, University of Kent, Canterbury, United Kingdom

Maja Furlan de Brito
University of Coimbra, Faculty of Medicine, Coimbra, Portugal; King's College London, Cicely Saunders Institute of Palliative Care, Policy & Rehabilitation, London, United Kingdom

Karla C. Giacomin
International Longevity Centre, Brazil. Centre for Studies in Public Health and Aging, Oswaldo Cruz Foundation, Belo Horizonte, MG, Brazil

Henrique Girao
University of Coimbra, Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Centre of Coimbra (CACC), Coimbra, Portugal

Ilias Gkikas
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Greece; Department of Biology, University of Crete, Heraklion, Greece

Bárbara Gomes
University of Coimbra, Faculty of Medicine, Coimbra, Portugal; King's College London, Cicely Saunders Institute of Palliative Care, Policy & Rehabilitation, London, United Kingdom

Rodrigo M. Gomes
Department of Physiological Sciences, Biological Sciences Institute, Federal University of Goias, Goiania, Brazil

Ana Cristina Gonçalves
Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal; Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal

Peter Goulden
Icahn School of Medicine at Mount Sinai, Division of Endocrinology, Diabetes & Bone Disease, Department of Medicine, Mount Sinai Morningside & West, New York, NY, United States

Marcus Grant
Environmental Stewardship for Health of People and Planet, Bristol, England; Cities & Health, Bristol, England

Luís F. Grilo
CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; PDBEB - PhD Programme in Experimental Biology and Biomedicine, IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal

Nick Guldemond
Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, The Netherlands; Faculty of Preventive Medicine, Department of Public Health and Healthcare NA Semashko, IM Sechenov First Moscow State Medical University, Moscow, Russia

Jorge Henriques
CISUC, Centre for Informatics and Systems of University of Coimbra, DEI, Coimbra, Portugal

Manoela Heringer
Paulo Niemeyer State Brain Institute of the Health Secretariat of the State of Rio de Janeiro, Rio de Janeiro, Brazil

Maddalena Illario
Dipartimento di Sanità Pubblica, Università degli Studi di Napoli Federico II, Naples, Italy

Carmen Jerónimo
Cancer Biology and Epigenetics Group, Research Center of IPO Porto (CI-IPOP)/ RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Centre (Porto.CCC), Porto, Portugal; Department of Pathology and Molecular Immunology, School of Medicine and Biomedical Sciences, University of Porto (ICBAS-UP), Porto, Portugal

John G. Jones
CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

Joana Jorge
Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal; Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal

Yaschar Kabiri
Institute of Toxicology and Environmental Hygiene, Technical University of Munich, School of Medicine, Munich, Germany

Agnieszka Karkucinska-Wieckowska
Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland

George Kelly
Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Campus for Ageing and Vitality, Newcastle Upon Tyne, United Kingdom

James L. Kirkland
Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, United States; Division of General Internal Medicine, Department of Medicine, Mayo Clinic, Rochester, MN, United States

Kristina Kitaeva
University of Nottingham, School of Veterinary Medicine and Science, Nottingham, United Kingdom

Andrey Kiyasov
University of Nottingham, School of Veterinary Medicine and Science, Nottingham, United Kingdom

Viktor I. Korolchuk
Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Campus for Ageing and Vitality, Newcastle Upon Tyne, United Kingdom

Mafalda Laranjo
Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Thomas J. LaRocca
Department of Health & Exercise Science, Colorado State University, Fort Collins, CO, United States

Marta Lavrador
Pharmacology and Pharmaceutical Care Laboratory, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; Coimbra Institute for Clinical and Biomedical Research (iCBR), mUniversity of Coimbra, Coimbra, Portugal

Magdalena Lebiedzinska-Arciszewska
Laboratory of Mitochondrial Biology and Metabolism, University of Coimbra, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Zhiquan Li
Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

Yuezhong Liu
Ageing Research Institute for Society and Education (ARISE), Nanyang Technological University, Singapore

Alexandre Lourenço
Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal; NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisbon, Portugal; Comprehensive Health Research Center (CHRC), Lisbon, Portugal

Lina Ma
Xuanwu Hospital Capital Medical University, Beijing, P.R. China

João O. Malva
Coimbra Institute for Clinical and Biomedical Research (iCBR), CIBB - Center for Innovative Biomedicine and Biotechnology, Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Christopher R. Martens
Department of Kinesiology & Applied Physiology, University of Delaware, Newark, DE, United States

Cristina Mas-Bargues
Freshage Research Group, Department of Physiology, Faculty of Medicine, University of Valencia, CIBERFES-ISCIII, INCLIVA, Valencia, Spain

Paulo Matafome
CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Coimbra Institute for Clinical and Biomedical Research (iCBR) and Institute of Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal

Joana Barbosa Melo
Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Laboratory of Cytogenetics and Genomics, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal

Maria L. Lima Mendonça
National Institute of Public Health, Praia, Cabo Verde

Athanasios Metaxakis
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Greece

Lefkos T. Middleton
School of Public Health, Imperial College London, London, United Kingdom; Public Health Directorate, Imperial College NHS Healthcare Trust, London, United Kingdom

Paula I. Moreira
CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Laboratory of Physiology – Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Judite M. Nascimento
Faculty of Science and Technology, University of Cabo Verde, Praia, Cabo Verde

Vivaldo M. Neto
Paulo Niemeyer State Brain Institute of the Health Secretariat of the State of Rio de Janeiro, Rio de Janeiro, Brazil

Paulo J. Oliveira
CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

Alessandro Ori
Leibniz Institute on Aging, Fritz Lipmann Institute (FLI), Jena, Germany

Reinaldo B. Oriá
Laboratory of Biology of Tissue Healing, Ontogeny and Nutrition, Department of Morphology, Center of Biomedicine, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil

Susan E. Ozanne
University of Cambridge Metabolic Research Laboratories and MRC Metabolic Diseases Unit Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, United Kingdom

Miguel Padeiro
Centre of Studies in Geography and Spatial Planning (CEGOT), Coimbra, Portugal; Department of Geography and Tourism, Faculty of Arts and Humanities, University of Coimbra, Portugal

Teresa Padro
Cardiovascular Program ICCC - Research Institute Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, Barcelona, Spain; Centro de Investigación Biomédica en Red Cardiovascular (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain

Carlos M. Palmeira
CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Yiming Pan
Xuanwu Hospital Capital Medical University, Beijing, P.R. China

Simão Paredes
Polytechnic of Coimbra, Coimbra Institute of Engineering, Coimbra, Portugal; CISUC, Centre for Informatics and Systems of University of Coimbra, DEI, Coimbra, Portugal

João F. Passos
Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, United States; Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, United States

Edith Pereira
IHMT, Universidade Nova de Lisboa, Lisbon, Portugal

Francisco B. Pereira
Polytechnic of Coimbra, Coimbra Institute of Engineering, Coimbra, Portugal; CISUC, Centre for Informatics and Systems of University of Coimbra, DEI, Coimbra, Portugal

Susana P. Pereira
CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Laboratory of Metabolism and Exercise (LametEx), Research Centre in Physical Activity, Health and Leisure (CIAFEL), Laboratory for Integrative and Translational Research in Population Health (ITR), Faculty of Sport, University of Porto, Porto, Portugal

Paolo Pinton
Department of Medical Sciences and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy

Joana F. Pires
Multidisciplinary Institute of Ageing, University of Coimbra, Coimbra, Portugal

Salomé Pires
Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

M. Cristina Polidori
Ageing Clinical Research, Department II of Internal Medicine and Center for Molecular Medicine Cologne, University Hospital of Cologne, Cologne, Germany; Cologne Excellence Cluster on Cellular Stress-Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany

Marios Politis
Neurodegeneration Imaging Group, University of Exeter Medical School, London, United Kingdom

Nuno Raimundo
Multidisciplinary Institute of Ageing, University of Coimbra, Coimbra, Portugal; Penn State University College of Medicine, Department of Cellular and Molecular Physiology, Hershey, PA, United States

João Ramalho-Santos
Department of Life Sciences, University of Coimbra, Coimbra, Portugal; CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

Lene Juel Rasmussen
Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

Fernando J. Regateiro
Institute of Medical Genetics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal

Dario D. Reis
Private Medical Practice, Praia, Cabo Verde

Caio Ribeiro
School of Computing, University of Kent, Canterbury, United Kingdom

Ilda Patrícia Ribeiro
Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Laboratory of Cytogenetics and Genomics, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal

Teresa M. Ribeiro-Rodrigues
Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Centre of Coimbra (CACC), Coimbra, Portugal

Albert Rizvanov
University of Nottingham, School of Veterinary Medicine and Science, Nottingham, United Kingdom

Teresa Rocha
Polytechnic of Coimbra, Coimbra Institute of Engineering, Coimbra, Portugal; CISUC, Centre for Informatics and Systems of University of Coimbra, DEI, Coimbra, Portugal

Susanne Röhr
Global Brain Health Institute, Trinity College Dublin, Dublin, Ireland; Institute of Social Medicine, Occupational Health and Public Health (ISAP), Medical Faculty, University of Leipzig, Leipzig, Germany

Anabela P. Rolo
CNC - Center for Neuroscience and Cell Biology, Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Department of Life Sciences, University of Coimbra, Coimbra, Portugal

Aurora Román-Domínguez
Freshage Research Group, Department of Physiology, Faculty of Medicine, University of Valencia, CIBERFES-ISCIII, INCLIVA, Valencia, Spain

Roman Romero-Ortuno
Global Brain Health Institute, Trinity College Dublin, Dublin, Ireland; Discipline of Medical Gerontology, School of Medicine, Trinity College Dublin, Dublin, Ireland; Mercer's Institute for Successful Aging, St James's Hospital, Dublin, Ireland

Manuel Santos Rosa
Institute of Immunology, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal

Catrin Rutland
University of Nottingham, School of Veterinary Medicine and Science, Nottingham, United Kingdom

Izabela Sadowska-Bartosz
Laboratory of Analytical Biochemistry, Institute of Food Technology and Nutrition, College of Natural Sciences, Rzeszow, Poland

Paula Santana
Centre of Studies in Geography and Spatial Planning (CEGOT), Coimbra, Portugal; Department of Geography and Tourism, Faculty of Arts and Humanities, University of Coimbra, Portugal

Deolinda Santinha
CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Coimbra, Portugal; Inter-University Doctoral Program in Aging and Chronic Diseases, Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Coimbra, Portugal

Marcos Santos
Department of Dental Prosthesis and Materials, School of Dentistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil

Ana Bela Sarmento Ribeiro
Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal; Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal; Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal; Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal

Deisa S.R.C. Semedo
Faculty of Social Sciences, Humanities and Arts, University of Cabo Verde, Praia, Cabo Verde

João Sequeira
Institute of Systems and Robotics, IST–University of Lisbon, Lisbon, Portugal

Felipe Sierra
Inspire, CHU Toulouse, Toulouse, France

Maria Natalina L. Silva
Directorate National of Health, Ministry of Health and Social Security, Praia, Cabo Verde

Rafael Solana
Immunology and Allergy Group, Maimonides Institute for Biomedical Research of Cordoba (IMIBIC), Córdoba, Spain; Department of Cell Biology, Physiology and Immunology, University of Córdoba, Córdoba, Spain; Immunology and Allergy Service, Reina Sofia University Hospital, Córdoba, Spain

Valeriya Solovyeva
Kazan Federal University, Kazan, Russia

Hélder Spínola
Human Genetics Laboratory, University of Madeira, Funchal, Portugal

Renata S. Tavares
CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal

Nektarios Tavernarakis
Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Greece; Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece

Tamar Tchkonia
Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, United States

Yin-Leng Theng
Ageing Research Institute for Society and Education (ARISE), Nanyang Technological University, Singapore

Vicente Traver-Salcedo
ITACA, Universitat Politecnica de Valencia, Valencia, Spain

Chi Udeh-Momoh
School of Public Health, Imperial College London, London, United Kingdom

Rakhi Verma
Nanyang Business School, Nanyang Technological University, Singapore

Stella Victorelli
Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, United States; Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, United States

Andreia Vilaça
CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal; CARIM School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands; Department of Molecular Genetics, Faculty of Sciences and Engineering, Maastricht University, Maastricht, Netherlands; PDBEB - PhD Programme in Experimental Biology and Biomedicine, IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal

Gemma Vilahur
Cardiovascular Program ICCC - Research Institute Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, Barcelona, Spain; Centro de Investigación Biomédica en Red Cardiovascular (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain

José Viña
Freshage Research Group, Department of Physiology, Faculty of Medicine, University of Valencia, CIBERFES-ISCIII, INCLIVA, Valencia, Spain

Thomas von Zglinicki
Newcastle University Biosciences Institute, Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne, United Kingdom

Devin Wahl
Department of Health & Exercise Science, Colorado State University, Fort Collins, CO, United States

Berenice Maria Werle
Global Brain Health Institute, Trinity College Dublin, Dublin, Ireland; Moriguchi Institute, Rio Grande do Sul, Brazil

Mariusz R. Wieckowski
Laboratory of Mitochondrial Biology and Metabolism, University of Coimbra, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland

Heather Wilson
Neurodegeneration Imaging Group, University of Exeter Medical School, London, United Kingdom

Erin O. Wissler Gerdes
Robert D. and Patricia E. Kern Center for the Science of Health Care Delivery, Mayo Clinic, Rochester, MN, United States

Lucyna A. Wozniak
Lodz EIP AHA Reference Site, Medical University of Lodz, Lodz, Poland

Barbara Zavan
Department of Translational Medicine, University of Ferrara (c/o CUBO), Ferrara, Italy

Yi Zhu
Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, United States

Hans Zischka
Institute of Toxicology and Environmental Hygiene, Technical University of Munich, School of Medicine, Munich, Germany; Institute of Molecular Toxicology and Pharmacology, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany

Foreword

The opening chapter of this book is titled "Global aging and health determinants in a changing world." The world is of course always changing, but events of the recent past have brought fresh perspective to the traditional Spanish saying "que no haya novedad" (may no new thing arise). The COVID-19 pandemic has changed so much, particularly as it concerns the health of older people, whose increased susceptibility to the SARS-CoV-2 coronavirus has been alarmingly apparent. The implications of this emergency are properly considered in the book, but the majority of its chapters reflect the fact that the societal impacts of global aging have been and will remain a continuing challenge for life scientists, health experts, and those involved in social organization. Of course, there are crises to distract us, such as pandemics, the climate emergency, and geopolitical upheavals, but in the background the demographic changes caused by a combination of improved survival into old age and reduced birth rates mean that there are inevitable shifts still to come, for which we need to be better prepared.

A global scan of flagship initiatives for healthy living and active aging provides a good grouping of four chapters (on Europe, North America, Asia and Oceania, and Central and South America), which collectively set a positive tone to provide context for the rest of the book. The direction of travel is from fundamental biological mechanisms to aspects of age-related health and disease. The coverage is impressively comprehensive. For the reader with focused interests, the selection of chapters should offer the opportunity to dig in detail into the latest research. In addition, the collection as a whole might provide the impetus to think about connections. Aging is a process characterized by exceptional complexity, where not only is there the need for the best of reductionist enquiry but also it is essential to appreciate how the parts fit together. Mechanisms interact, often synergistically and with consequences at various levels of physiology. The subtitle of the book—From Fundamental Biology to Societal Impact—is therefore a call to arms as much as a description. We need to work together in interdisciplinary initiatives that span the traditional silos.

One of the fastest growing areas of science at present is that involving artificial intelligence technology—robotics, machine learning, and the exploitation of "big data." Some hints of what is to come from these areas is described in two contrasting chapters on socially assistive robots and on machine learning to improve healthcare in aging. Further work, ongoing but not yet examined here, concerns the extension of machine learning to aid the exploration of these mechanisms.

Another traditional saying, this time an English one, tells us "a stitch in time saves nine." Everything we are learning about the intrinsic biology of aging reinforces the idea that prevention is better (and probably more easily attainable) than a cure within the context of age-related diseases and frailty. Thus the focus on integrated care and preventive medicine toward the end of the book makes perfect sense, as does a well-informed assessment of myths and realities of potential antiaging interventions.

Returning to the COVID-19 pandemic, there are many important lessons still to be observed about how older people fit within our experience and management of what has hit us. Those involved in aging research have long appreciated how age is the single largest risk factor for so many illnesses, and how the presence of underlying multimorbidity makes the outlook worse. The harsh statistics of COVID-19 mortality have made everyone more clearly aware of this. At the same time that we work to protect older people from the virus, we must not fall into the age-old trap of simply regarding old people as being frail and vulnerable. The emphasis of this book, as a whole, is to display how aging is proving to be a scientifically tractable challenge, the solutions to which will create great benefits for individuals and across society.

Tom Kirkwood is an emeritus professor in aging at Newcastle University and affiliate professor in the Center for Healthy Aging at the University of Copenhagen.

Tom Kirkwood

Preface

In recent decades, research in biomedicine has been delivering massive information about health determinants that are of utmost importance to inspire individuals and decision makers to fine-tune behaviors and public policies supporting healthy living. Science-based practices are the stepping stones to build healthier societies and support increased healthy life span expectations for all.

Population aging brings together the challenges and the opportunities to fight disease and to support healthy lifestyles, decreasing inequalities and delivering health. Population aging is a global growing phenomenon which is a major challenge creating the urgent need to deliver healthy aging opportunities for individuals and families, contributing to decrease the burden of aging and delivering sustainability of social and health-care support systems. Population aging is also an opportunity because it opens new windows for individuals to live longer and healthier and also because it brings opportunities for innovators and entrepreneurs to deliver new services and products for a growing silver market. Aging research and increases in a healthy life span are a new horizon for human kind, creating an entire new perspective for the deterministic view of health/disease dichotomy, broadening horizons for health maintenance as the default biological and societal program for all citizens. In fact, we are born to be alive and should be in good health until the last day of our life.

In this interdisciplinary book, we bring together a panel of highly recognized aging experts in different research and business fields, which tackles challenges and opportunities in an interdisciplinary and multisectoral perspective approaching the quest for healthy aging in a holistic manner.

Different elements of "Aging: From Fundamental Biology to Societal Impact" approach include the socioeconomic angle of aging, the role of collaborative innovation networks and their societal impact in different macroregions, the role of fundamental research and research-based knowledge to better understand molecular, metabolic, and cellular determinants of aging, cell reprograming and increased healthy life span, the burden of chronic diseases and multimorbidity restricting healthy life span, the use of innovation and technology assistance in delivering health and independent living technology-based support systems, the role of science awareness and health literacy to support behavioral changes in society, and inspiring citizens to adopt healthy lifestyles practices during the entire life course. At the end, the reader can find a very nice comic, which will guide them through a summary of the book's contents.

We hope that the readers of this book will find the content accessible, informative, and useful. This book is part of the mission for science to pursue the limits of

knowledge, opening new windows in our understanding of human life and its limits. By providing a collection of excellent chapters written by some of the best-known aging researchers, we expect to inspire students, young researchers, and citizens to join hands and support the aging research community.

Paulo J. Oliveira
João O. Malva

SECTION 1

The societal burden of aging

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CHAPTER 1

Global aging and health determinants in a changing world

Miguel Padeiro[1,2], **Paula Santana**[1,2] **and Marcus Grant**[3,4]
[1]Centre of Studies in Geography and Spatial Planning (CEGOT), Coimbra, Portugal
[2]Department of Geography and Tourism, Faculty of Arts and Humanities, University of Coimbra, Portugal
[3]Environmental Stewardship for Health of People and Planet, Bristol, England
[4]Cities & Health, Bristol, England

1.1 Introduction

Never before have humans lived as long as they do today. Life expectancy has more than doubled since 1900. Aging is rapidly accelerating worldwide and by 2050, the number of people aged 65 and older will have more than doubled to reach 1.5 billion and represent 16% of the global population.[1] While this trend is more intense in developed countries, with 26% of the population in Europe and North America aged 65 and older, it has now become a global phenomenon affecting developing countries as well.

With improving living conditions and an overall, yet patchy, improvement in healthcare, demographic aging has been accompanied by an increase in healthy life years. This has, in many cases, extended working life and provided new modalities of consumption and a flourishing age-related technological and service industry, the so-called "silver economy." However, as the limits of human life are extended, aging is also characterized by an increasing number of older adults with socially and spatially-patterned frailty and chronic diseases and mortality and morbidity burdens, raising many health equity questions.

This chapter offers an overview of how the environment shapes health and well-being in later life. After briefly presenting the global demographic trends associated with aging, we examine how health and well-being in later life are related to the physical, social, and service environments in which older adults are embedded, and discuss how place of residence acts as a health determinant in later life. In the last section, we discuss four key stressors and their influence on health inequities in old age, including the changing role of welfare state, climate change, migrations, and discriminations.

1.2 The geographies of a global trend

1.2.1 Global patterns and projections

While 6% of the world population was aged 65 and older in 1990, that proportion grew to 9% in 2019.[2] Until recently, societal problems associated with new patterns of

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aging were frequently viewed as an issue only in western countries. In a United Nations Department of Economic and Social Affairs (UNDESA) report in 2015, the proportion of people aged 65 and older was 17.5% in UNDESA-defined more developed countries, and only 6.3% in less developed countries.[3] When assessed by sustainable development goals (SDG) region, the countries with the largest proportion of people aged 65 and older were found in Europe, with the notable exception of Japan, the country with the longest life expectancy, 84.4 years in 2019.[4] It is tempting to draw conclusions based on the world map (Fig. 1.1) and on general data (Table 1.1). Estimates for 2020 show that Europe and North America have the largest proportions of people aged 65 and older (18.3%), followed by Australia and New Zealand (16.2%). The lowest proportion is found in Sub-Saharan Africa (3.0%).

These figures, however, should not obscure that, in absolute numbers and alongside the estimated 143 million people aged 65 and older living in Europe and the 62 million in North America, in 2020 there are estimated to be more than 60 million in Africa, 60 million in Latin America and the Caribbean, 124 million in Central and Southern Asia, and 272 million in Eastern and South-Eastern Asia. This brings the global total estimate of people aged 65 and older in 2020 to 727.6 million (Table 1.1).

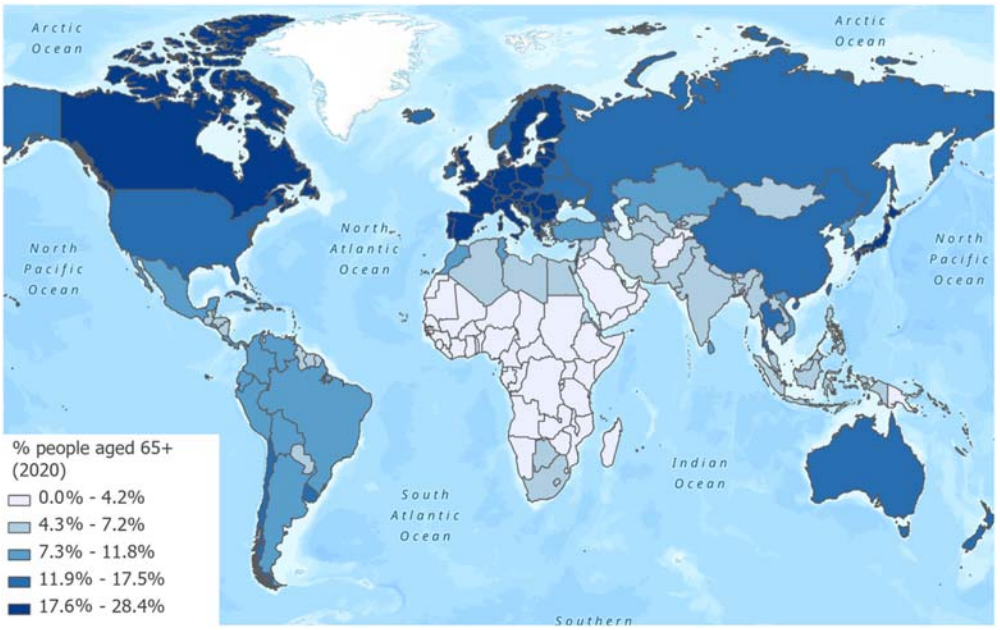

Figure 1.1 Estimates of people aged 65 and older in 2020. *Data from standard population projections (UNDESA.* World population prospects 2019 (dataset). *United Nations; 2019).*

Table 1.1 Population aging estimates in 2019 by Sustainable Development Goals region (2020–50).

SDG region	2020		2050		2020–50	
	People aged 65 and older (millions)	% Total population	People aged 65 and older (millions)	% Total population	Change (millions)	% Change
Sub-Saharan Africa	32.9	3.0	101.4	4.8	68.5	208.3
Northern Africa and Western Asia	30.6	5.8	95.8	12.7	65.2	213.4
Central and Southern Asia	123.6	6.1	328.1	13.1	204.5	165.4
Eastern and South-Eastern Asia	271.6	11.6	572.5	23.7	300.9	110.8
Latin America and Caribbean	58.7	9.0	144.6	19.0	86.0	146.6
Australia and New Zealand	4.9	16.2	8.8	22.9	3.9	79.0
Oceania[a]	0.5	4.2	1.5	7.7	0.9	178.6
Europe[b]	142.9	19.1	199.9	28.1	57.0	39.9
North America[b]	61.9	16.8	96.3	22.6	34.4	55.5
Total	727.6	9.3	1,548.8	15.9	821.2	112.9

[a]Here Oceania does not include Australia and New Zealand.
[b]Here Europe and North America are considered separately, contrary to SDG regions.
Source: Data from UNDESA. *World population prospects 2019 (dataset)*. United Nations; 2019.

Current forecasts indicate that population aging will continue to grow (Table 1.1). Globally, the proportion of people aged 65 and older is expected to reach 16% by 2050. By that time, 1.5 billion people are expected to be aged 65 and older, representing a 113% increase in 30 years. Reviewing absolute numbers is fundamental to assessing needs and determining future policies. In 2050, almost 200 million people aged 65 and older will be living in Sub-Saharan Africa, Northern Africa, or Western Asia; 900 million in Asia (Central and Southern Asia, Eastern and South-Eastern Asia); and almost 150 million in Latin America and the Caribbean. This totals 1.25 billion people aged 65 and older, compared to 200 million in Europe and 96 million in North America.[2]

Demographic aging has not only resulted from increased life expectancy, but is also related to fertility decline, with fertility rates declining in almost every country. In developed countries, total fertility rates (TFRs) are below the replacement level: 1.66 in Europe and North America for the period 2015–20, 1.84 in Australia and New Zealand, and 1.83 in Eastern and South-Eastern Asia (Fig. 1.2).[2] Other regions show higher numbers, but they also are declining. For example, the TFR in Sub-Saharan Africa was estimated at 4.72 for 2015–20 (vs. 5.88 in 1995–2000) and is expected to decrease to 2.67–3.66 in 2045–50 based on low and high variants projections.[2]

At the same time, life expectancy is increasing in all parts of the world (Table 1.2). While Europe (78.3 years), North America (79.1 years), Australia and New Zealand

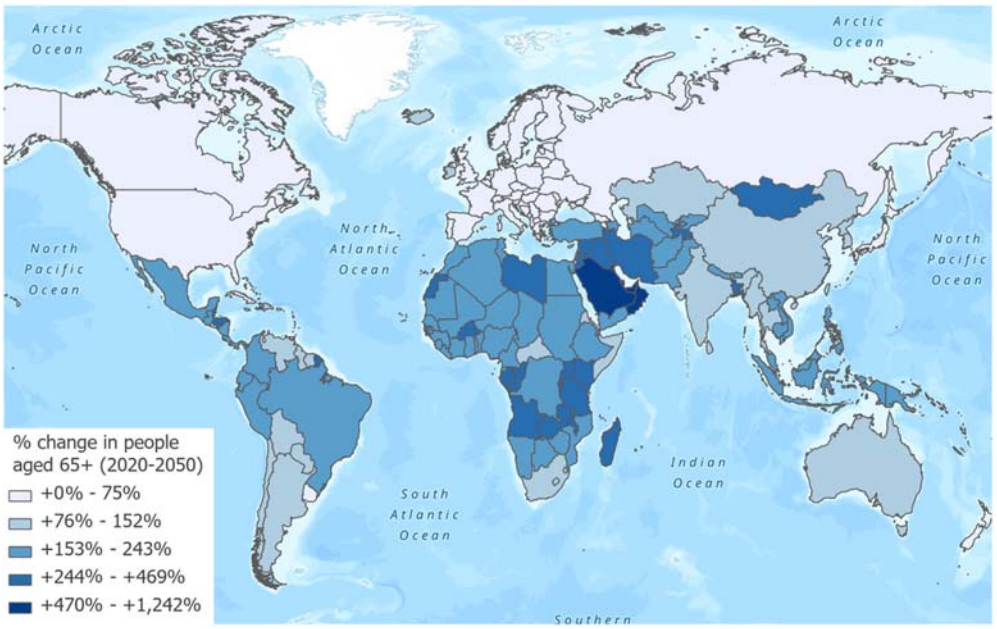

Figure 1.2 Predicted population growth of people aged 65 and older (2020–50). *Data from standard population projections (UNDESA.* World population prospects 2019 (dataset). *United Nations; 2019.*

Table 1.2 UNDESA estimates of total fertility rate and life expectancy, by Sustainable Development Goals region (2015–20 and 2045–50).

SDG region	Total fertility rate		Life expectancy at birth		Life expectancy at age 60	
	2015–20	2045–50	2015–20	2045–50	2015–20	2045–50
Sub-Saharan Africa	4.72	3.17	60.5	67.1	16.1	17.8
Northern Africa and Western Asia	2.93	2.28	73.4	77.6	19.9	22.5
Central and Southern Asia	2.41	1.91	69.5	74.0	18.2	20.4
Eastern and South-Eastern Asia	1.83	1.79	76.3	79.8	21.07	23.9
Latin America and Caribbean	2.04	1.75	75.2	79.7	21.95	24.8
Australia and New Zealand	1.84	1.72	83.0	86.2	25.57	28.4
Oceania[a]	3.46	2.65	66.3	70.4	15.98	17.4
Europe[b]	1.61	1.72	78.3	82.0	22.6	25.6
North America[b]	1.75	1.78	79.1	82.7	23.7	26.9
Total	2.47	2.21	72.3	76.8	20.7	22.9

[a]Here Oceania does not include Australia and New Zealand.
[b]Here Europe and North America are considered separately, contrary to SDG regions.
Source: Data from UNDESA. *World population prospects 2019 (dataset)*. United Nations; 2019.

(83.0 years) are leading in projected life expectancy at birth in 2015–20, other SDG regions are expected to see their life expectancy at birth approach 80 years by 2050. Northern Africa and Western Asia had a life expectancy at birth in 2015–20 of 73.4 years, which is projected to increase to 77.6 years by 2045–50, Eastern and South-Eastern Asia are projected to increase from 76.3 years to 79.8 years, and Latin America and the Caribbean are projected to increase from 75.2 years to 79.7 years.[2] Other regions are also expected to make significant gains in life expectancy at birth: Sub-Saharan Africa is projected to increase from 60.5 to 67.1 years, and Central and Southern Asia are projected to increase from 69.5 to 74.0 years.[2] Increasing longevity also means that the number and proportion of people aged 80 and older is projected to dramatically increase in the next decades (Table 1.3). While 5.3% of people living in Europe and 4.0% in North America are aged 80 and older in 2020, their proportion is projected to reach 10.1% and 8.7%, respectively, in 2050. In Eastern and South-Eastern Asia, this proportion is projected to rise from 2.1% to 7.3% in the same period. On a global scale, the projected proportion of people aged 80 and older is expected to increase from 145.5 million in 2020 to 426.4 million in 2050.

1.2.2 Multiscale intraregional variations of the aging process

Beyond these global figures, it is important to bear in mind two kinds of spatial variations: within regions and within countries. The first one concerns the existing diversity of drivers and situations across countries located in the same SDG region. These intraregional variations are explained by differences in fertility levels, quality of healthcare provision, socioeconomic development, social conditions, and population policies. In Europe, for example, the proportion of people aged 65 and older varies from 12.5% (Moldova) to 23.3% (Italy). In Eastern and South-Eastern Asia, the proportion ranges from 4.3% (Laos) to 28.4% (Japan), and in Latin America and the Caribbean from 5.0% (Honduras) to 21.7% (Martinique, an Overseas department of France).[2]

The second kind of variation exists between urban and rural areas within an individual country. Aging has traditionally been associated with a demographic decline in low-density rural areas, because there is a trend for younger adults to relocate to cities in search of jobs.[5,6] This trend is frequently accentuated by a parallel relocation of retired people, who often leave cities and move to the countryside or coastal areas.[7] While these processes still exist and are likely to increase in absolute numbers,[8–10] and in many countries proportions of people aged 65 and older are higher in rural areas than in urban areas, increasing urbanization rates anticipate an increase in the number of older people in urban areas where they have been settled for several years or decades. This is already apparent in the proportion of urban dwellers in the total. Of people aged 65 and older, globally 58.8% of them lived in urban settings in 2015 versus 48.3% in 1990. The proportion is even higher in UNDESA-defined more developed

Table 1.3 UNDESA projected population of people aged 80 and older, by sustainable development goals region (2020–50).

SDG Region	2020		2050		2020–50	
	People aged 80 and older (millions)	% Total population	People aged 80 and older (millions)	% Total population	Change (millions)	% Change
Sub-Saharan Africa	3.7	0.3	12.4	0.6	+ 8.8	+ 238.9
Northern Africa and Western Asia	5.2	1.0	20.3	2.7	+ 15.0	+ 286.5
Central and Southern Asia	18.7	0.9	62.6	2.5	+ 43.9	+ 234.3
Eastern and South-Eastern Asia	49.7	2.1	177.0	7.3	+ 127.3	+ 255.9
Latin America and Caribbean	12.4	1.9	41.4	5.4	+ 29.0	+ 234.6
Australia and New Zealand	1.2	4.1	3.3	8.5	+ 2.0	+ 161.8
Oceania[a]	0.1	0.5	0.2	1.1	+ 0.2	+ 274.6
Europe[b]	39.6	5.3	71.9	10.1	+ 32.3	+ 81.6
North America[b]	14.8	4.0	37.2	8.7	+ 22.4	+ 150.9
Total	145.5	1.9	426.4	4.4	+ 280.9	+ 193.0

[a]Here Oceania does not include Australia and New Zealand.
[b]Here Europe and North America are considered separately, contrary to SDG regions.
Source: Data from UNDESA. *World population prospects 2019 (dataset)*. United Nations; 2019. Projections based on medium variant.

regions, where 75% of people aged 65 and older live in urban areas, compared to 50% in less developed regions.[3]

1.3 Environmental health in later life

1.3.1 From successful aging to the role of place and inequities

The World Health Organization (WHO) defines health as "a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity."[11] This approach recognizes the existence of multiple determinants of individual health status and the mutual relationship between physical and mental health, which involves physiological, behavioral and social mechanisms.[12,13] The deterioration in the health of older people is often described through the model of the disablement process.[14,15] This corresponds to the gradual deterioration of various bodily functions, including: physiological (respiratory, cardiovascular, endocrine), motor (locomotion, resistance to effort), cognitive (memory, executive functions), and sensory (vision, hearing). This has long been seen as an intrinsic and inevitable evolution of aging, and has been used to justify a geriatric and socio-medical approach to health. However, this view neglects the transactional, social, and environmental determinants of health. Until the 1980s, the dominant social and behavioral theory of aging was that which was proposed by Cumming and Henry.[16] Their disengagement theory described how older people progressively withdrew from activities and social relations, preparing for their own death and leaving the way for younger people.

Meanwhile, Havighurst's activity theory of aging,[17] later developed by Lemon, Bengtson and Peterson, Bengtson,[18] asserted that keeping active was necessary to maintain one's self-identity and meaningful life. While this theory shared with the disengagement perspective a common view of curtailing activities and social interactions,[19] it departed from it by emphasizing the social needs of aging individuals. In this it would pave the way for the concept of successful aging.[20,21] Far from being locked in a slow and unavoidable disappearance, individuals had to proactively undertake changes in their habits such as diet, physical activity, and social engagement. The difference between usual and successful aging resided in their own attitudes, and the individual's choice could lower the probability of disease and disease-related disability by increasing or maintaining a high cognitive and physical functional capacity, and by actively engaging with life. This concept became commonly accepted in public discourse and in research on aging.[22]

This approach, however, was criticized for its overemphasis on the role of individuals and agency. Anchored in a Western researcher-driven perspective, this conception of successful aging and the new gerontology paradigm have led to an individualistic and normative approach. This has led to a stereotyped view of successful aging, and excluded others, such as ill or disabled older adults, from this vision of

successful aging.[23,24] The conceptualization of aging, health, and well-being as embedded in an individuals' socio-cultural context was not fully recognized, and neither were socio-spatial inequities. Nonetheless, other perspectives already offered such an embedded view of aging. Environmental gerontology, traditionally developed by the psychological sciences,[25–28] was the first discipline to develop a conceptual model linking people and their immediate environment, initially at the scale of their homes. The person-environment fit theory describes the interaction between an individual and their environment.[29,30] If a person's needs are met by their environment, this facilitates better health and well-being.

Researchers have gradually expanded the scale of the residential environment to include the context of the neighborhood[31–33] and then link local contextual elements to major societal, economic, and political developments. Part of this evolution is linked to Bronfenbrenner's socio-ecological approach[34] that places the person in a multiscalar context, extending from the micro-system (the local context surrounding the individual) to the macro-system (the larger cultural and social context). This model, further developed and adapted to numerous fields of inquiry, provides a useful and popular framework for analyzing the ways in which a complex set of individual, interpersonal, and environmental factors (institutional, community/society, and policy) affect an individuals' health and behavior. It is at the origin of the famous Dahlgren-Whitehead rainbow,[35] which focuses on social inequalities, and it has been applied to the built environment in Barton and Grant's ecosystem model of health determinants (Fig. 1.3).[36]

The way that aging is viewed and managed has thus evolved to a more holistic approach that incorporates the multiscale environment. This affects individuals' choices, chances, behaviors, and opportunities. The emergence of geographical gerontology[38–40] also allowed the incorporation of place and its multiple, multiscalar interrelationships (from home to global scale) as explanatory elements of aging. This brought into recognition the role of the "where" in the aging process, and with it the social inequities that are spatially-dependent.

1.3.2 How environment affects physical health in old age

Older people are the group that spends the most time at home and in their residential area (around the home).[32,41] Recent work has somewhat questioned our certainties about the narrowness of their living space, in particular because new cohorts of older people are now more multianchored than the previous ones.[42] However, older people still rely more on local resources than other groups, and therefore on their residential environment. In many instances, driving cessation and a decrease in number of trips using public transit lead to a drop in social interactions, which is associated with mental health consequences.[43]

The environment can be characterized by three categories of attributes: physical, social, and service environment. The physical environment includes elements such as

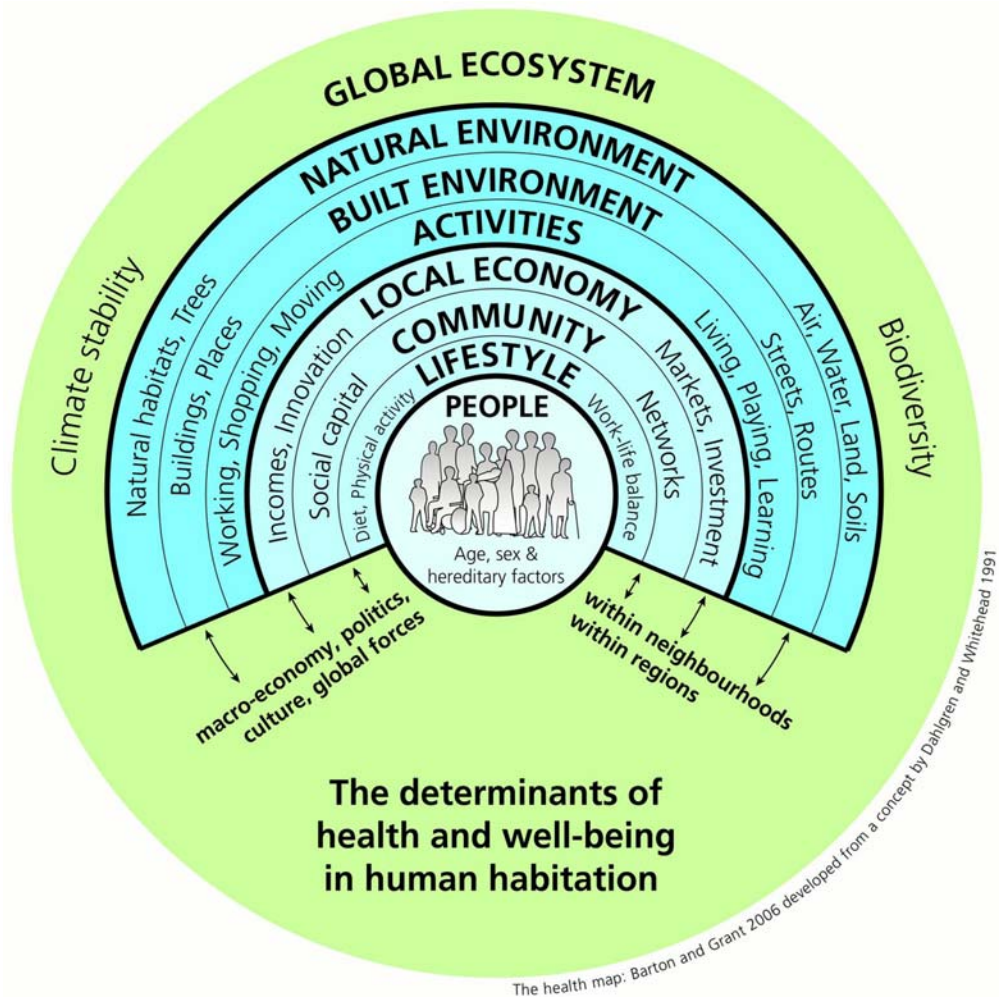

Figure 1.3 Ecosystem model of health determinants.[36,37]

public spaces, green areas, slopes and stairs, urban furniture, community severance, and density-related externalities (pollution, noise, litter). The service environment corresponds to the presence, accessibility, and affordability of services such as food and other businesses, banking, postal and administrative services, health services (medical stations, clinics, health centers, community pharmacies), and public transport. The social environment is more difficult to measure. It can rely on objective data such as neighborhood deprivation levels[44] or other income-related indicators, the level of crime, the social mix (measured by the percentage of various social or ethnic groups), or the age-homogeneity of the neighborhood (high presence or concentration of older people). Measured subjectively, the social environment may involve dimensions such as sense

of community, neighborliness, social cohesion, collective efficacy, perceived crime, and social problems.[45,46]

These three attributes of the environment, the physical, social, and service environment, can be measured from either an external point of view (measured by the observer, which does not necessarily mean "objective") or through a subjective point of view (measured by the subject themselves). The distinction between external and subjective assessment is important. For example, the literature suggests that in the case of observational data such as population density or distance to green spaces, the subjective perception of residents may diverge significantly from the external data.[47–49]

The residential environment plays a complex role that can influence the health of older people. This role manifests into two intertwined mechanisms, which can be cumulatively positive or negative: exposure to externalities directly affecting the health of individuals, and exposure to elements that promote or hinder health-related behaviors.[50] These mechanisms, and their interpersonal and spatial variability, not only affect older people. They also correspond to the accumulation of lifelong experiences, habits, and problems.

This view is captured in the life course approach to health,[51] whereby people's functional capacity changes through their life course and the quality of the local environment will affect the degree of independence or dependence, especially amongst lower socioeconomic groups (Fig. 1.4). Using a life-course approach, the habits and life skills we acquire as children, such as active living and healthy eating, are determinants of healthy aging.

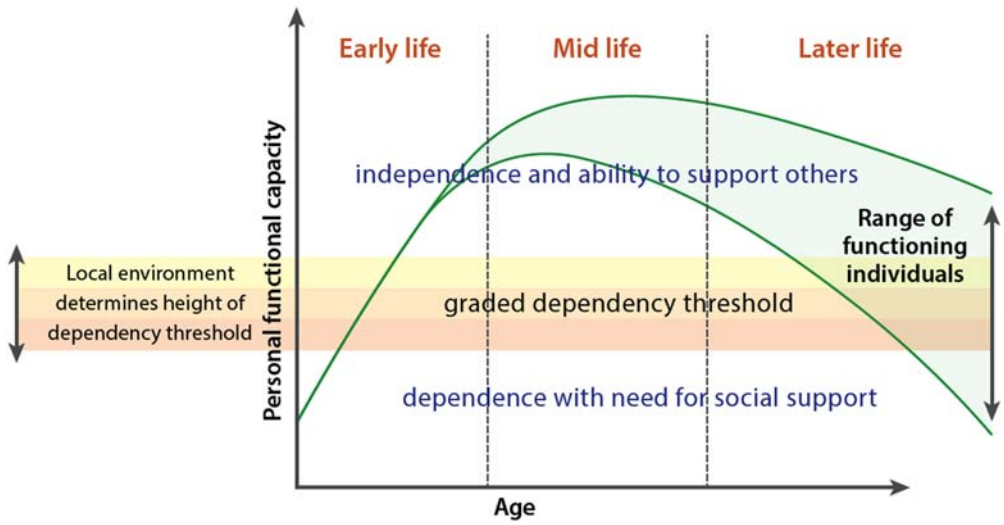

Figure 1.4 The degree of individual independence through the life course.[52, 53] *Adapted from: Kalache A, Kickbusch I. A global strategy for healthy ageing.* World Health *1997;50:4–5.*

Lifetime exposure to a wide range of externalities with direct impacts on morbidity and mortality also contributes to healthy aging. Among older adults, there is substantial evidence for an association between the presence of fine particles in the air, and a deterioration of cognitive,[54,55] respiratory and cardiovascular functions,[56–59] and an increase in obesity[60] and mortality.[61,62] Older people are at higher risk of motor vehicle accidents,[63,64] and it is estimated that one-third of older people fall at least once each year.[65,66] Among these, nearly one-third are environment-related.[67] Hygrothermal discomfort has also been associated with respiratory and mental disease,[68–71] and evidence of associations between noise exposure and cognitive issues are growing.[55,72,73]

Residential environments provide seniors with support for activities of daily living, opportunities for social interaction, and to relax. These three categories correspond to Musselwhite and Haddad's utilitarian, affective, and esthetic needs.[74] These three needs are associated in a variety of ways, with the older people's physical and mental health. Within this framework, the environment may contribute to older adults' choices, and influence health-related behaviors.[75] For example, noise and poor air quality can reduce a person's propensity to go outside, which has both positive and negative implications. On the one hand, by staying at home they are exposed to fewer pollutants, but on the other hand, it reinforces a sedentary lifestyle and reduces social interactions, which can contribute to higher levels of depressive symptoms.[76] Physical activity and active mobility are one of the most studied behaviors, and the lack thereof has given rise to the concept of an obesogenic environment.[77,78] These behaviors increase with the degree of walkability of the residential environment, the care given to public spaces, and the presence and accessibility of local services, such as coffeeshops,[79] public benches,[80] green areas,[81,82] bluespaces,[83] and shade trees.[84,85] Quality of diet is also influenced by the characteristics of the food environment, as defined by the presence, accessibility, affordability, acceptability, and accommodation of food retail shops.[86,87] Even habits such as alcohol and tobacco consumption, traditionally considered social factors, are influenced by the environment.[88] Finally, low geographical access to healthcare (health centers, community pharmacies)[89] reduces health service utilization and medication adherence.[90] This is important as these health-related behaviors have been associated with negative health outcomes, including chronic disease,[91] mobility limitations,[92] depression,[93] and dementia.[94,95]

There is also a role for socio-spatial inequalities.[96] Both exposure to externalities, such as unhealthy behavior, and suboptimal environments are often positively correlated and fueled by the characteristics of the real estate market.[97,98] Literature from the environmental justice field has shown that the most vulnerable social groups are often exposed to suboptimal environmental conditions.[99] The result of this is that low-income neighborhoods show higher potential for poor health outcomes.[44,100–102] Several studies have found an association between poverty level and access to health services,[103–105] partly due to the distance decay effect.[90,106] Spatial inequalities can

also have a countervailing effect in some cases and according to the positive care law[107] public policies can counteract market-driven trends.

Additional policy attention should be focused on the so-called "oldest old" group, those over 80 years of age. A recent phenomenological study of community-dwelling individuals aged 80 years and older indicated new avenues for policy and research. They found that age-friendly developments for this group should highlight functional ability rather than chronological age, informal social community support, and design that recognizes and integrates structures to support their specific needs. They also found that socially-isolated older adults might be difficult to reach, so innovative strategies are required to ensure their unique needs are met.[108]

1.3.3 The subjective experience of place: place attachment and residential normalcy

In recognizing the fundamental aspect of an individual with their place of residence, more qualitative approaches have been adopted to study the subjective experience of place. In the fields of health geography, environmental health, and geographical and environmental gerontology, such qualitative approaches are employed to better understand the mechanisms linking people to their physical and social environment. Those links can then be used to understand the contribution of residence to an individual's health and well-being. Each of us develops a unique relationship to our environment, to the place where we live and to the places we visit. The notion of place attachment has long been studied by the social sciences,[30,109,110] and is now increasingly recognized in healthcare research.[111,112] Defined as the symbolic, cognitive, or emotional connection between an individual and a particular setting or milieu,[113] higher place attachment is associated with higher life satisfaction and survival among older adults.[114,115]

Current inquiries tend to converge on the idea that older adults show, more than other age groups, a particular attachment to their place of residence.[109] They spend more time there than others and rely more on local resources, making them more sensitive to their attributes.[110] Younger adults more often have frequent residential mobility, which results in fewer close relationships with their neighbors and local small traders.[116] However, older adults more often have longevity in their neighborhood, which results in more meaningful memories associated with the various people and elements of their neighborhood.[117] The prospect of aging in place tends to be desirable and associated with a better assessment of their quality of life and well-being. Staying in a familiar neighborhood facilitates social connections, and helps to maintain a sense of environmental mastery since the places, schedules, and routines are known.[118,119] This does not necessarily mean that aging must be accompanied by a simple continuation of preexisting activities, as people can also rebuild their activities.[24] However, such a reconstruction is more easily facilitated when the life frame does not

change, and when a dimension of continuity of the emotional or physical environment persists.

However, the significance of living space for older people poses a number of challenges, not only for themselves, but also for their families and public institutions. These challenges are mainly attributable to the possible discrepancy between the material and social conditions of the place of residence and the attachment to the place demonstrated by the person.

Links within the local community may have a compensatory effect on individual health and well-being. Strong ties with neighbors and the local community helps to strengthen a sense of self, identity, and self-esteem, which help to avoid or alleviate psychological problems. For some older people, age homogeneity in the local community facilitates good connections with peers, and a sense of social identity within a community that shares their daily issues and expectations.[33,120,121] This is manifest in the preference for retirement communities or self-contained residences.[122,123] For others, intergenerationality gives older people a social role, while avoiding what could be interpreted as an exclusionary process from the rest of the population.[79,124,125] This is particularly evident in certain social or ethnic group contexts. Strong local ties and peer-to-peer solidarity can sometimes produce a compensating effect on health and well-being. For example, the contextual effects of aging on mental health can be reduced by recognizing the role that older people can play in the community.[126,127] This can also be achieved by increasing access to local resources, through small informal exchanges of services or goods,[125] or through active civic participation to influence local land-use decisions.

However, this compensatory effect complicates the sole objective of physical health and can lead to situations where an individual prefers to live for a shorter period of time with a worse health status instead of moving to a place without emotional connection. Therefore, it is important to assess how older people cope with adverse living conditions, and how their desired residence may impact their overall health. Golant's[24,128] conceptual model of residential normalcy provides a powerful instrument to assess such individual strategies. The principle of this model is that the place of residence elicits feelings and emotional experiences of both residential comfort and mastery. Older people may find themselves in or out of their residential comfort zone or mastery zone, and it is common for one of the two dimensions to be unsatisfactory. Maintaining a comfortable and independent life frequently requires an uncertain balance between the two.[24] An incongruent residential environment may lead to depressive symptoms or to an individual strategy to adapt to what is perceived as not congruent, either because the person does not want to move or because he/she does not have the opportunity to do so. To cope with adverse living conditions, they implement strategies to either accommodate existing problems, by minimizing or ignoring them, or to adapt proactively, by trying to change their environment.[24]

Ultimately, older people sometimes move to environments they feel are better adapted to their needs and lifestyle. This move presents two major challenges. First, there is the question of who should make the decision to relocate. When the family insists, which is a common case,[129,130] there is a risk of ageism, defined as the stereotyping, prejudice, and discrimination against people based on their age[131–133] and infantilization by exclusion from the decision-making process. This can have mental health consequences, corresponding to a perceived lack of autonomy. Second, the choice often reflects the desire to preserve residential comfort, which comes at the expense of residential mastery. For example, older adults who have moved out of their homes often feel they no longer have access to the previous routines and social interactions that filled their lives.[125]

1.4 Global stressors in a changing world

The last two decades seem to be marked by a weakening of the democratic impetus.[134,135] Concerns are emerging about the change in geostrategic relations, the rise in prominence of non-state actors, the role of the private sector in the production of residential environments, and advances of extremism and populism. While these questions affect all societies, they specifically raise questions about the impact on older people. These changes, whether real or expected, challenge the coping capacities of older people. At least four stressors operate at a global scale and strongly influence the local conditions within which older adults live: (1) the prominence of neoliberalism and the welfare state retrenchment; (2) climate change; (3) the sharp increase in migration, both nationally and internationally; and (4) discriminations.

1.4.1 Welfare state and neoliberalism

The predominant discourse on the welfare state, and its role and margins of maneuver in social and health action, emphasizes the pressures exerted by both demographic aging and the decrease in the working age population. According to this view, this double pressure of aging is at the core of the difficulties of financing pensions, social support, and long-term care, which would consequently slow down economic growth and worsen the debt of future generations.[136,137] It is postulated that public pension expenditure and total age-related expenditures tend to increase because of spending on healthcare and long-term care.[138,139] However, it is likely that these statements are largely unfounded. First, the influence of demographic aging on poor economic performance is not proven,[140] and second, the amount of public spending does not depend on aging directly but on other factors, like the cost of healthcare.[141]

These claims have, however, led to two types of adjustments that pose a challenge for the health of older people. The first is based on the extension of the number of years of work and on the development of unconventional retirement paths.[142]

This trend has resulted in a complexification of individual trajectories, such as non-retirement, phased retirement, and part-time work. This choice may reinforce social and health inequalities, by keeping people with low qualifications, limited resources, and accumulated health problems in their workplace.[141] Furthermore, the profound labor and technological transformations of recent years have outpaced many older workers, and led to the stereotyping of older workers as "incompetent but warm."[143] This results in difficulties obtaining a job for older adults, which can also lead to mental and physical health problems over time.[144]

The second type of adjustment is based on the privatization of pension funds and the reforms toward marketization of social and healthcare programs.[141] This trend toward welfare state retrenchment[145] increases individual financial risks in times of crisis,[146,147] emphasizing individual responsibility for care and resources.[148] This is of particular concern at a time when the number of older people living alone is increasing. The living arrangement and the availability of family support are directly associated with mental and physical health outcomes, especially in the case of older people with low education and health issues.[149] However, different welfare state regimes give rise to differentiated aging and health outcomes.[142,150] In countries where the level of decommodification of labor is higher, the probability of comorbidity in old age is lowest. On the other hand, in more liberal countries where the level of decommodification of labor is lower, the probability of comorbidity in old age is higher.[142] Public spending on health is also associated with a reduction in health inequalities among older adults.[150]

At the local level, residential environments are also under pressure in the context of globalization and the neoliberal approach to urban production methods.[151] The majority of local governments now embrace an entrepreneurial management approach[152] embedded in the logic of enhancing urban spaces.[153] Urban regeneration operations, though supported by public authorities, are also increasingly financed by private actors who transform the territory into financial assets. The enhancement and transformation of urban spaces leads in many cases to the eviction of lower socioeconomic groups, and among them many older people.[154,155] Eviction is both tangible, when the person has to move, and symbolic, when the environment is transformed to the point of eliminating the usual landmarks. In both instances, this leads to a loss of social interactions and routines often anchored for several decades, with significant consequences in terms of mental and physical health.[156] Low participation of older people in urban development decision-making processes[157] further adds to the difficulties of taking them into account in urban regeneration operations.

1.4.2 Climate change and health in old age

Emerging during the 1970s in a context of criticism of the dominant economic model, the discourse on the preservation of biodiversity gradually turned into a growing

concern about climate change. Evidence of human contribution to climate change has accumulated in recent decades, accompanied by an increasingly refined assessment on its impacts on society.[158,159] Ongoing climate change is causing a series of effects that include: greater climate instability,[160] marked by an increase in the frequency of heat waves; a greater variability of tides and an increase in sea level, precipitation, storm surges and flooding episodes in an urban context;[161,162] and a greater frequency of wildfires, particularly in Mediterranean and hot climates.[163] Climate change also has indirect effects linked to increased migrations[164] and crop failures,[165] with short-term consequences of poor diets and starvation and long-term consequences for health, such as cardiovascular disease and frailty.

Older people are a particularly vulnerable group due to various factors such as reduced mobility, increased vision and hearing problems, and greater risk of heat stress.[166] For example, people aged 75 and older represented nearly 83% of excess mortality in France during the 2003 heat wave.[167] The medium- to long-term health consequences of natural disasters and major climatic events are varied, and they include worsening of preexisting health conditions and psychological trauma, for example during forced relocations.

However, climate change should not be seen only as an external event affecting older people, representing an intrinsic and individual inability to adapt. Rather, viewed through the socio-ecological model and social determinants of health, the adverse effects of climate change can be viewed from a perspective that minimizes direct causation. The increased vulnerability of older people, when it exists, is mainly the result of a physical, economic, and/or social environment that is unfavorable to their resilience. Thus, the health impacts of various climatic events should be interpreted as the result, not of the climatic event, but of loneliness, housing conditions, and social inequities.[168] These inequities, which make it necessary to recognize the heterogeneity of the older population, have several consequences that play a cumulative role in the intrinsic vulnerability of age. These include: housing hygrothermal conditions are socially patterned; body temperature regulation and mobility are negatively impacted by having more medical conditions, more medications, and greater socio-spatial inequalities; poverty often results in low resources and low resilience; and inequalities of vulnerability among older people are aggravated by differences in literacy, economic resources, and access to digital tools and information.

1.4.3 Migrations and health in old age

Migration has historically been analyzed using theories based on the push-and-pull factors of networks or national policies. The contemporary theory of climate-migration[164] focuses on the environmental conditions of push-and-pull factors that influence international migration. From this point of view, it is likely that migration

will increase in the coming decades. The number of international migrants worldwide has already increased sharply in recent decades. While there were around 153 million migrants in 1990 (2.9% of the world population) and nearly 192 million in 2005 (2.9%), their number now exceeds 270 million in 2019 (3.5%).[169] Among them, the 32 million older migrants accounted for 11.8% of the total in 2019.[169] Their profile shows great heterogeneity because the condition of the older migrants can fall into three different categories: a foreign-born person who migrates from one country to another at the age of 65 or over (retirement migration); the person who, having migrated in the past, is aging in the place where he/she has settled; the person whose migration is the result of a natural disaster or conflict, and who is either aging in place or has migrated after the age of 65.

While retirement migrations most often concern people with more than average financial assets and moving to countries or regions with a more lenient climate,[10,170] they can also concern the return of migrants to their country of origin.[171] In the latter case, returning migrants may experience problems of re-adaptation to a context that has changed profoundly since their first migration. These problems sometimes translate into a feeling of unbelonging, loneliness, and difficulty finding one's bearings in the functioning of the country's services and institutions.[171,172] This ultimately can reduce their access to healthcare or lead to unhealthy behaviors.

The challenge is immense in the case of older people whose migration results from conflict or natural disasters. While the number of people of concern to the United Nations High Commissioner for Refugees rose from 36 million people in 2009 to over 86 million people in 2019[173] of which nearly 8.5% are aged 65 or over, the number of people affected by natural disasters is now approaching 100 million.[174] Seniors are generally the most reluctant to leave their place of residence, which places them at a higher risk of injury or death during a conflict or natural disaster. They are also vulnerable because of the disintegration of their support system in a crisis context.[175] At the same time, while the affected populations are increasingly urban, the burden of noncommunicable diseases is increasing sharply and marks a notable epidemiological change.[176–178] Adjustments to this development have not been fully realized, as priority is still given to the treatment of communicable diseases and immediate emergencies.[176,179]

For the older persons who have migrated several years ago and are aging in the country where they have settled, the impact of migration on their life expectancy and health status is complicated. Their health status is generally better than that of people in their native country, and worse than that of people in their receiving country. This is observed in spite of the healthy immigrant effect,[180] which is related to the fact that healthier people are more likely to migrate. After several years spent in the receiving country, immigrant health status worsens, due to labor and housing conditions, stressful events, unequal access to healthcare, and language barriers. Older immigrants eventually experience higher rates of comorbidity and disability,[181] which may be aggravated in the

case of undocumented migrants.[182] However, health status is often an important factor in the decision to return home,[183–185] which can give a false image of good health in immigrant populations.[183]

1.4.4 Discrimination as a health issue for older persons

Older people are subject, like their younger counterparts, to various discriminations due to attributes such as gender, sexual orientation, race, ethnicity, religion, and weight.[186–190] Discrimination does not affect all older people in the same way. Minorities tend to be the most affected, raising important health equity issues. In addition to these forms of discrimination, older people also frequently suffer from ageism.[191] In a scoping review of the literature, Wilson et al. found that the prevalence of experienced ageism varied from 48% to 91%.[192] These discriminations and stereotypes occur more frequently in Western societies, due to cultural differences linked to norms and values related to respect, support, and care for the older people in a family setting.[193] Recent literature suggests a potential shift in this cultural paradigm, with different manifestations of ageism.[143,194]

All forms of discrimination can negatively impact older people's mental health. These stressors can decrease self-esteem, social identity, and feelings of self-worth.[188,195,196] This may lead to internalization of negative stereotypes and discriminations, according to the Stereotype Embodiment Theory.[197,198] These stressors can thus lead to a negative assessment of old age and life satisfaction,[186] increased anxiety states and depressive symptoms,[195,199] and even suicidal ideation.[200,201]

The negative impact of discrimination on mental health has additional consequences for the physical health of older people, based on two mechanisms. First, negative mental consequences frequently induce the following impacts: avoidance behaviors, such as reduced activities and social interactions, reduced physical activities, such as walking or frequenting public places, and the non-use of certain services and shops. These behaviors are generally associated with adverse health outcomes.[92,95,202] Second, the negative experiences associated with discriminations can produce physiological responses, as described by the Stress Process Model.[203,204] These responses can include increased blood pressure,[205] inflammation,[206] increased risk of dementia,[207] and early mortality.[208] Perceived age discrimination has been associated with adverse health outcomes,[206] poor self-rated health, and with a higher prevalence of comorbidities.[209]

1.5 Conclusion

With the aging global population, the relationship between older people and their residential environments is increasingly important. This relationship is based on the match between the individual characteristics of a person, their needs and expectations, and the characteristics of their environment. By creating access to various health

improvement factors and exposure to various risk factors, the conditions under which an individual ages can be modified. This helps to accelerate or decelerate the process of incapacitation that individuals undergo as they age. This can also reduce or reinforce socio-spatial inequalities, which underlie the preponderant role of territory and spatial policies in the prevention and promotion of healthy aging.

Exploration of these relationships and a growing number of themes related to the health of older adults point to several avenues for future research. It is increasingly necessary to examine certain groups, like those aged 80 and older, migrants, people suffering from chronic diseases, and people suffering from cognitive conditions. In methodological terms, future research efforts should also focus on the development of mixed methods that are able to explore the experiences of older people in their environments in more detail.

The aging of the world's population is accelerating and the challenges that this entails will affect all continents in the coming decades. Increasingly, people will age in urban areas, often characterized by significant socio-spatial inequalities. In a world that is increasingly interconnected, mobile, globalized, and marked by new geopolitical stressors, it will at the same time be increasingly important to take into account the multiple impacts on health in later life.

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CHAPTER 2

Flagship initiatives for healthy living and active aging in Europe: the European Innovation Partnership on Active and Healthy Ageing and the Reference Sites

João O. Malva[1], Daan Bultje[2], Vicente Traver-Salcedo[3], Ana Maria Carriazo[4], Maddalena Illario[5], Vicenzo De Luca[5], Lucyna A. Wozniak[6], Jean Bousquet[7], Nick Guldemond[8,9], Inês Costa[1], Helena Canhão[10], Elísio Costa[11] and John Farrel[12]

[1]Coimbra Institute for Clinical and Biomedical Research (iCBR), CIBB - Center for Innovative Biomedicine and Biotechnology (CIBB), Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[2]Healthy Ageing Network Northern Netherlands (HANNN), Groningen, The Netherlands
[3]ITACA, Universitat Politecnica de Valencia, Valencia, Spain
[4]Andalusia Reference Site, Regional Ministry of Health and Families of Andalusia, Seville, Spain
[5]Dipartimento di Sanità Pubblica, Università degli Studi di Napoli Federico II, Naples, Italy
[6]Lodz EIP AHA Reference Site, Medical University of Lodz, Lodz, Poland
[7]MACVIA-France, Montpellier, France
[8]Department of Public Health and Primary Care, Leiden University Medical Center, Leiden, The Netherlands
[9]Faculty of Preventive Medicine, Department of Public Health and Healthcare NA Semashko, IM Sechenov First Moscow State Medical University, Moscow, Russia
[10]Lisbon AHA Reference Site, CHRC, NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal
[11]UCIBIO REQUIMTE, Faculty of Pharmacy and Competence Center on Active and Healthy Ageing (Porto4Ageing Reference Site), University of Porto, Porto, Portugal
[12]Reference Site Collaborative Network (RSCN), Brussels, Belgium

2.1 Demographic changes and aging

Demographic changes and population aging have been recognized as key priorities to ensure sustainability of global health and social care systems in the next decades. The WHO and the European Commission (EC) clearly defined the priority to support healthy living and active aging as key elements of the EC Horizon 2020, Horizon Europe and the United Nations (UN) announced the decade of healthy aging (2020–30). The aging report, annually published by EC, forecasts major demographic imbalances in the vast majority of European Union (EU) countries, with important consequences concerning social and health care support including social inequalities, poverty, loneliness, and migrations among others. In spite of the expected overall decrease in the population, in the EU, the old-age dependency ratio (ratio between people ages 65 and over to those aged 20–64) is expected to rise sharply in the coming decades.[1]

Aging
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Life expectancy at birth in males is expected to increase 7.4 years, whereas life expectancy at birth in females is expected to increase 6.1 years, in a forecast for 2019–70. Taken together, these numbers indicate that the expected lifetime for men will increase from 78.7 to 86.1 years, whereas the expected lifetime for women will increase from 84.2 to 90.3 years.[1] This demographic scenario is especially challenging when considering the current prevalence of lifestyle and age-related chronic diseases and disability leading to functional dependence.[2] The forecasted socioeconomic scenarios will impose major tensions related to the expected impact and sustainability related to pensions, healthcare, long-term care and aging-related education, which are the most affected areas.

With the successful launching of the European Innovation Partnership on Active and Healthy Ageing (EIP on AHA), the EC offered a pan-European partnership made of quadruple helix-based innovation networks to develop, implement, adopt and scale at large, groundbreaking solutions to support healthy living and active aging and good practices across the European Regions.[3]

2.2 The European Innovation Partnership on Active and Healthy Ageing

In 2012 the EIP on AHA was established to tackle the challenge of an aging population. It set a target of increasing the healthy lifespan of EU citizens by 2 years by 2020, and aimed to pursue a triple win for Europe by: (1) Enabling EU citizens to lead healthy, active and independent lives while aging; (2) Improving the sustainability and efficiency of social and healthcare systems; (3) Boosting and improving the competitiveness of markets for innovative products and services, responding to the aging challenge at both the EU and global level, thus creating new opportunities for businesses.

EIP on AHA brought together public and private stakeholders to accelerate the deployment of major innovations by committing them to undertaking supply-and-demand-side measures across sectors and the entire innovation system. EIP on AHA was neither a new funding program or instrument, nor a new legal entity and did not replace existing decision-making processes. However, it was recognized that regional commitments to EIP on AHA could influence and inform policy decisions and service delivery models, and therefore identify opportunities, and potential partners, under a range of funding programs for the development of evidence-based innovative solutions.

EIP on AHA was therefore a distinctive opportunity to help deliver on the policy objectives of the Europe 2020 flagships: the Innovation Union, Digital Agenda for Europe, New Skills for New Jobs and the European Platform against Poverty and Social Exclusion. Its objectives and approach were also in line with the principles and

goals of the EU Health Strategy, "Together for Health" and the EIP on AHA represented a significant contribution from the EU for achieving the objectives of the European Year for Active Ageing and Solidarity between Generations in 2012.

The EIP on AHA stands on two main pillars: Reference Sites (RSs) and Action Groups. There were also three cross-cutting initiatives that contributed to EIP on AHA: "The Blueprint,"[4] "Innovation to Market (I2M),"[5] and "MAFEIP."[6]

RSs: an alliance or partnership of stakeholders within a region or major metropolitan area that can implement or develop EIP on AHA topics. They should be able to demonstrate they have adopted, or are working towards the adoption, of a "Quadruple Helix" model to ensure all stakeholders have a common understanding of the organizational, technical and financial challenges facing the region or area within health and active and healthy aging and are working collaboratively to define and implement innovative solutions and possibilities for economic growth. The Quadruple Helix comprises Health and Care Providers/ Public Authorities, Academia, small and medium-sized enterprises (SMEs), and Civil Society. The appropriate lead authority for health and social care in the region or area is expected to be a fundamental stakeholder in any RS partnership or alliance. Following the 3rd Call for RSs in 2019 there were 103 regions and major metropolitan areas awarded with Reference Site Status.

To be recognized as an RS, applicants were required to demonstrate the following key features which were developed in assessment criteria for the evaluation of applications. The key features were: (1) Comprehensive strategies were in place, or under development, which directed and guided policies and practices in the region, including supporting an active and healthy aging population; e.g., Innovation Strategies, R&D Strategies, Smart Specialization Strategies, Older People Strategies, Education and Training Strategies, Economic Strategies, Regional Development Strategies; (2) Responses to health, societal, and economic challenges were through a strategic "whole system approach" to deliver against the EIP on AHA triple win objectives; (3) The degree of their alignment with EIP on AHA through both contributions to the 3 EIP on AHA Horizontal initiatives (Blueprint, Innovation to Market and MAFEIP), and commitments to adopt the relevant elements of the EIP on AHA Action Plans developed by the various Action Groups; (4) The degree they have developed, or are willing to develop, partnerships with other Regions for the transfer and exchange of good practice, and/or joint working on projects to support health and care, including active and healthy aging; (5) Commitment to supporting the Digital Transformation of Health and Care and be able to demonstrate programmes and actions underway, or planned, in the areas of (a) citizens' secure access to their health data, including across borders; (b) personalized medicine through shared European data infrastructure; and (c) citizen empowerment with digital tools for user feedback and person-centered care; (6) Commitment to contributing to the European evidence base demonstrating impact on outcomes for patients and service users; effectiveness of developed solutions

in meeting need; and how provider organizations have adapted to deliver new services and service models; (7) Examples and evidenced impact of good practices and the degree that RSs have scaled up or that they are working to scale up smart health and care solutions for active and healthy aging.

Action groups: communities of partners committed to working on specific issues related to active and healthy aging through (1) peer-to-peer sharing of knowledge and expertise, (2) increasing the added-value of their national and local experience, and (3) identifying gaps to be addressed at European level. The EIP on AHA Strategic Implementation Plan proposed 6 Action Groups involving stakeholders from academia, public authorities, large industry and SMEs, health and care organizations, investors and innovators, and end users and patients' associations. The 6 Action Groups were: (1) Adherence to prescription; (2) Falls prevention; (3) Lifespan Health Promotion and Prevention of age-related frailty and disease; (4) Integrated care; (5) Independent living solutions; (6) Age friendly environments (Fig. 2.1).

2.2.1 European Innovation Partnership on Active and Healthy Ageing cross-cutting initiatives

The *Blueprint* aims to innovate health and care in Europe and is the follow-up of the EIP on AHA *Scaling Up Strategy*. It reflects the policy vision of the EIP on AHA partners and is the channel of the EIP on AHA partners for providing and receiving policy inputs; an iterative process operated between the EC and stakeholders (policymakers and other key opinion leaders) to evolve, update and implement the Blueprint.

I2M targets the scale-up of digital health and care solutions in a cross-border context. This horizontal action is part of the EC strategy on digital transformation of health and care in the Digital Single Market.

MAFEIP is the Monitoring and Assessment Framework initially developed in response to the EIP on AHA-specific monitoring needs. It is to be used as an impact assessment tool to support an evidence-based decision-making process for all institutions and users in the health and care sectors.

2.3 Reference sites—case studies

2.3.1 Ageing@Coimbra Reference Site

Ageing@Coimbra was formally created in January 2013 as a response of the Center Region of Portugal to the first call for the EIP on AHA RSs, to develop and implement innovative good practices to support healthy living and active aging. Ageing@Coimbra is an informal network of 5 founders and 90 associated partners that built a "quadruple helix," aligning: (1) public institutions (e.g., municipalities, health and social care authorities), (2) academia (e.g., universities and research institutions),

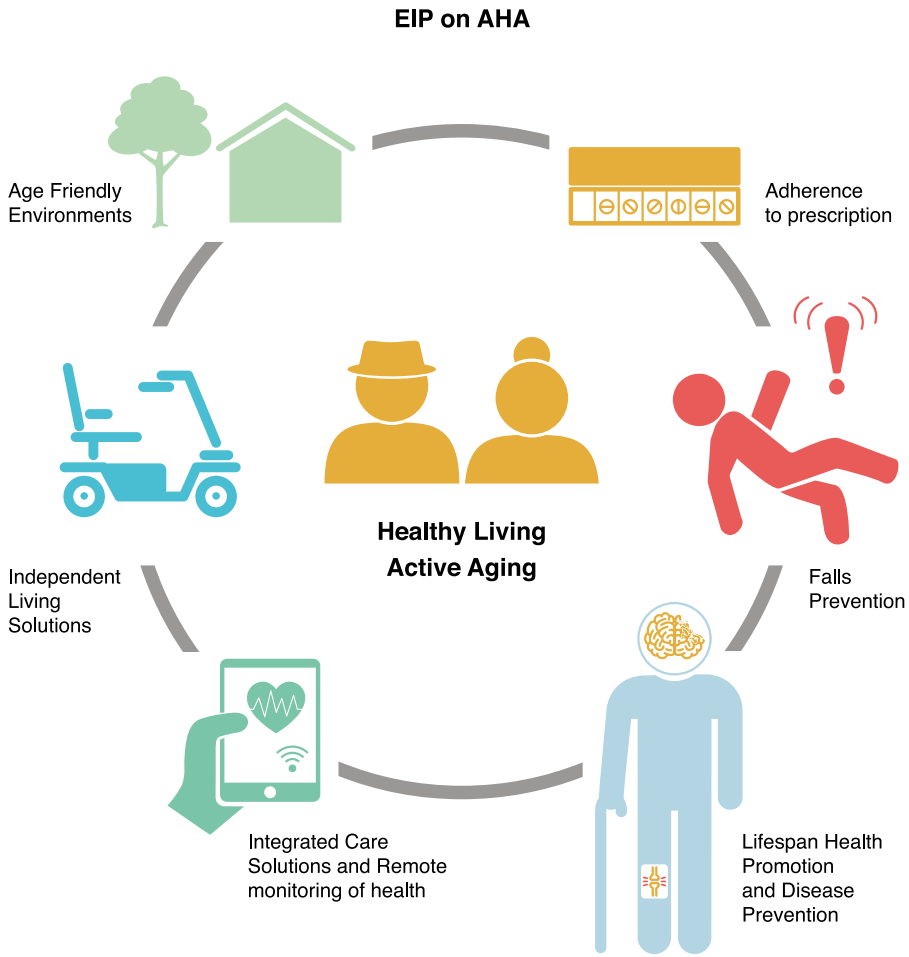

Figure 2.1 Graphical representation of European Innovation Partnership on Active and Healthy Ageing action groups.

(3) industry partners (e.g., business companies, business incubators, and clusters), and (4) civil society organizations (e.g., universities of the third age, cultural, and civil organizations). The Administrative Authority of the Center Region of Portugal ("Comissão de Coordenação e Desenvolvimento Regional do Centro," CCDRC) has been a major supporter and driver for the success of Ageing@Coimbra, actively involved in the implementation of activities, international visibility and inclusion of aging-related key priorities in the Smart Specialization Strategy (RIS3) of the Center Region of Portugal. Ageing@Coimbra has been awarded as the first EIP on AHA RS in Portugal and is included in the first group of Reference Sites in Europe (first award 2013).

The innovative formula and lean governance structure created an inclusive partnership with agility, rapid implementation, and growth potential.

Currently, Ageing@Coimbra is implementing a successful flagship project to concentrate excellence while avoiding the dispersion of scientific resources and to consolidate an aging research ecosystem as the pillar of regional development. The Multidisciplinary Institute of Ageing (MIA-Portugal) (https://www.uc.pt/en/mia) was approved by the EC as a H2020 Teaming project with the support of Newcastle University, University Medical Center Groningen, Copenhagen University and the Mayo Clinic, and it will be installed in the Health Campus of the University of Coimbra, with support of the Ageing@Coimbra network. Three founding members of Ageing@Coimbra, namely the University of Coimbra, the Hospital Center of the University of Coimbra and the Institute Pedro Nunes have been key players of the Knowledge Innovation Community EIT Health. Taken together, the consortium attracted >60 million € of competitive European funding and created >100 high qualifications jobs. MIA-Portugal is also supported by the pan-European school, EIT Health Ageing PhD School (https://eithealth.eu/project/eit-health-ageing-phd-school/) to deliver international and cross-sector training of PhD students in aging with strong innovation and entrepreneurship skills.

2.3.2 The Healthy Ageing Network Northern Netherlands

The Healthy Ageing Network Northern Netherlands (HANNN) is an independent foundation that was founded in 2009 by knowledge institutions in the three northern provinces of the Netherlands: Drenthe, Fryslân and Groningen. The founding members, University Medical Center Groningen, University of Groningen, Hanze University of Applied Sciences and NHL Stenden University of Applied Sciences, realized the importance of translating knowledge about an aging society into initiatives with societal and economic impact. In 2009, the Swedish Presidency of the European Union was also marked by the Lund Declaration,[7] which stated that Europe must focus on the grand challenges of our time: "The European Knowledge Society must tackle these through the best analysis, powerful actions and increased resources. Challenges must turn into sustainable solutions in areas such as global warming, tightening supplies of energy, water and food, ageing societies, public health, pandemics and security."

This declaration was an important encouragement for regional cooperation in the Northern Netherlands regarding an aging society. Especially because this region is the forefront of an aging Dutch society and needed to find ways to tackle the challenges that go with this. In the Netherlands, this is mainly a local issue because municipalities are responsible for ensuring that people are able to live independently and participate in society for as long as possible. But the regional level proved to be appropriate for mobilizing entrepreneurs, thinkers and decision-makers who could contribute to innovative

solutions for these challenges. HANNN organized the interaction between the healthy aging knowledge institutions, policymakers and society. It developed into a quadruple helix ecosystem with all the major stakeholders in the field of active and healthy ageing in the Northern Netherlands among its membership, focusing on the life course of people and promoting active and healthy aging in the broadest possible sense.

HANNN works together with local communities to offer an environment that stimulates a healthy lifestyle. Charting local networks and through them, simultaneously promoting active citizenship, accessible green spaces, active lifestyle, healthy mobility, healthy living and healthy food. The network provides these local communities with knowledge and (digital) infrastructures that support a healthy environment. An important resource for ageing research is Lifelines (https://www.lifelines.nl/), a large, multigenerational, prospective cohort study that includes over 167,000 participants, 10% from the northern population of the Netherlands. Following this population over a period of 30 years will provide many opportunities for studies worldwide, unraveling the cause of multifactorial diseases focusing on multifactor risk factors.

The Northern Netherlands has been recognized as a RS for the EIP on AHA three times. It stands out as a RS not driven by a government, but by a coalition of knowledge institutions. In 2019 HANNN was awarded the "Certificate of Excellence" by the European Commission in recognition of the work in driving regional innovation in active and healthy aging, improving the quality of life of the aging population, making health and social care delivery more sustainable and stimulating economic growth and competitiveness.

2.3.3 Valencia region Reference Site

In the Valencia region of Spain, there is a very active working ecosystem that is fully aligned with the goal of the EIP on AHA under the quadruple innovation helix. It is led by INCLIVA-Hospital Clinic, with the involvement of other healthcare providers and research institutions, public health authorities, healthcare associations, universities, municipalities, SMEs and multinationals, jointly with citizens from patients' associations that, in many cases, cooperate also under other umbrellas like the European Connected Health Alliance (ECHAlliance).

A success story within this region is the Healthy Loneliness Initiative, intended to empower citizens in a lonely situation through formative and informative activities that help them to understand, focus and visualize the most positive point of view of their situation, thus promoting their physical and mental health status. Funded by EIT Health under the Campus pillar, and cooperating with other regions, most of which are also involved in EIP on AHA (Coimbra, Lodz, and Paris). The inception of the idea came from an existing need to tackle loneliness. This has been enhanced by raising the innovation lab of the municipality of Valencia, supported by the Universitat Politècnica de Valencia, to cocreate

with the different stakeholders in the city, share knowledge and ideas with other cities, run different interventions with a scalable and sustainable approach (channeled through primary care centers and neighborhood associations) and generate evidence and methodologies to support scaling this initiative to the European level.

To address this scaling up initiative and to raise awareness among municipalities and policymakers on how to tackle the loneliness issue, the RS Collaborative Network (RSCN) led in a cooperative way, the edition of a Manifesto for Addressing and Reducing Older Adults Loneliness In Europe, which recognizes the impact loneliness has on the health and mental or psychological well-being of older citizens and the vulnerable and made a call to transform health, care services and intervention programs to be more proactive and responsive.

This experience in the Valencia region concludes that there is not a unique recipe to successfully address isolation. Different lessons that were learned have been extracted and even shared with other regions: (1) The municipality context is key to guarantee the success and sustainability of the interventions, especially using a "train the trainers" approach, engaging close entities to the elderly as primary care centers and neighborhood associations; (2) Those that prescribe the educational activities to the elderly to tackle loneliness have to belong to already known and confident entities, like primary care centers in Valencia; (3) The best common solution agreed upon in the different sites to minimize impact of loneliness on health outcomes is on the importance of being active and socially involved through leisure and cultural activities in group, having networks of friends and family, and also continuing having personal projects and dreams.

2.3.4 Andalusia Reference Site

Andalusia, located in the south of Spain, is one of the largest regions of Europe, with a population of 8.5 million inhabitants and an area of 87,597 km^2. Life expectancy is 84.5 years for women and 79.2 for men. The population over 65 years exceeds 1.3 million and its growth represents both a challenge and an opportunity for the region, like other territories in Europe. Andalusia has a formal policy commitment that sets active and healthy aging as a strategic priority and as such, is included in its Smart Specialization Strategy.

The Regional Government of Andalusia (Junta de Andalucía) is responsible for public health, health policy and healthcare provision and management as well as social welfare and social care policies. **The Regional Ministry of Health and Families and** the Regional Ministry of Equality, Social Policies and Conciliation work together in the consolidation and maintenance of these fundamental social welfare pillars, particularly in the field of active and healthy aging. In addition to these public authorities and their institutions, a wide range of partners from academia, research institutions, private and voluntary sectors, as well as civil society are contributing to foster innovation to boost personal autonomy, prevent dependency, improve sustainability and

efficiency of health and care services, and support entrepreneurship created employment in the field of active and healthy aging.

Andalusia has been actively involved in the EIP on AHA since its inception and in 2013 was recognized as a 3-stars RS thanks to its Active Aging and eHealth Strategies. In 2016, Andalusia was awarded as a 4-stars RS due to its excellence at adopting the quadruple helix approach which seeks synergies that design knowledge ecosystems with a commitment of exchange and collaboration, going beyond its borders. In 2019, Andalusia also achieved the highest recognition as 4-stars RS with special recognition for excellence, continuing the quadruple helix approach and contributing to the digital transformation of health and care.

Some of the entities involved are: Andalusian Public Health System, Andalusian Health Service, Andalusian Agency for Social Services and Dependency, public universities in Andalusia (Universities of Granada, Seville, Malaga, Cordoba and Cadiz, with medical schools, and University of Jaen), Andalusian Council of Official Colleges of Pharmacists, Andalusian School of Public Health, local social services, pharmacies, scientific societies (Family and Community Medicine, Nursing), technology enterprises (Indra-Minsait, Fujitsu, Phillips, Everis, Tunstall, and others), centers of active participation, and elderly and patients' associations, among others.

Several strategies such as the Andalusian Health Plan or the Andalusian Plan for Promotion of Personal Autonomy and Prevention of Dependency, as well as several integrated plans (care plan, integrated care for patients with multiple chronic diseases, diabetes, oncology, palliative care, and others) contribute to this recognition; together with a well implemented digital health strategy, including a corporate electronic health system -Diraya-, ClicSalud +, Receta XXI or the EnBuenaEdad platform (http://www.enbuenaedad.es) and links with other innovative systems and initiatives as well as the involvement in European projects, such as Vigour, mHealthHub, or several joint actions (Advantage, Chrodis, Chrodis Plus, Jadecare).

As a founding member of the RSCN, Andalusia RS allows for a fruitful exchange of innovative ideas and experiences as well as building collaborative initiatives of common interest. The EIP on AHA has positively contributed to share knowledge, continue learning and improve collaborations in this field, based on ongoing activities, assets and potentials, while adapting to different environments.

Andalusia represents the RSCN in the eHealth Stakeholders Group of the European Commission, contributing to its works and discussions, and facilitating the adoption of digital innovative solutions in the field of health and social care.

2.3.5 The Lodz4Generations Reference Site

The Lodz4Generations partnership invests efforts and resources to work on population aging-related issues. It is a joint initiative of the City of Lodz and the Lodzkie Region

with the Medical University of Lodz (MUL) (https://en.umed.pl) under the University leadership. This initiative in Poland's heart (Lodz is located in the very center of the country) functions in an education-innovation-business in the health ecosystem. Compared to other cities in Poland, Lodz and the Lodzkie Region have the highest percentage of elderly citizens. In 2035, senior (60 +) citizens will form the majority of the city's population.

The most impactful in generating knowledge, novel education skills and experience, and sharing good practices relevant to alignment with EIP on AHA initiative include:

1. EIT HEALTH (https://www.eithealth.eu) is a top EU initiative, which brings together over 150 leading healthcare partners across multiple industry sectors, public and private research centers, and top universities, with a clear mission of accelerating entrepreneurship and innovation in healthy living and active aging. It provides Europe's most talented with new opportunities and resources for the benefit of all citizens. Only within EIT Health, MUL collaborates with a total of 79 partners from 19 countries.
2. Through our researchers' participation in Action Groups, a living link between EIT Health and EIP on AHA is established, allowing for feeding of good practices into the Good Practices Repository.
3. Highly relevant to developing, sharing, and scaling a good practices project is being implemented under 3rd Health Programme: Evidence-based guidance to scale-up evidence-based integrated care in Europe VIGOUR (https://vigour-integratedcare.eu/about/project.html). The partnership of 17 institutions, MUL included, brings together 15 regions from 7 EU member states. The project aims to support health care authorities in progressing the transformation of health and care systems to provide sustainable models for integrated care, facilitating the identification of good practices and scaling-up.
4. Within ERASMUS + MUL and Lodz4Generations an educational project has been implemented that increases medical professionals' capacity to manage patient adherence and polytherapy in the elderly, called SKILLS4ADHERENCE (http://skills4adherence.eu). The project aims to improve healthcare professionals' ability to effectively manage adherence and polypharmacy in the elderly through an innovative vocational training program and dedicated online tools. The project is considered a good candidate for scaling from the regional to national level.
5. The Lodzkie Region, together with MUL, implement an INTERREG CENTRAL EUROPE project, an innovative ecosystem for smart care of older people: iCARE-SMART (https://bruksela.lodzkie.pl/nasze-projekty/i-care-smart-lodzkie/).

MUL is also a partner in Joint Programming Initiative on Antimicrobial Resistance (JPI AMR) ImpresU, improving rational prescribing for urinary tract infections (UTI) in frail elderly (https://www.era-learn.eu/network-information/networks/jpiamr/fifth-joint-call-of-jpiamr/improving-rational-prescribing-for-uti-in-frail-elderly), which addresses an increasingly important issue of reducing antibiotic overuse in the frail elderly population receiving care at home or in care homes, through the implementation of a new algorithm,

developed by an international Delphi. The results will help to strengthen Antibiotic Steward Interventions in this group of patients.

The MUL Interdisciplinary PhD program on the Biopsychosocial Model of Human Functioning in the Social Environment (InterDok) remains valid, concerning delivering good practices to PhD educational programs and complements the activity of the International PhD School.

2.3.6 Campania Reference Site (ProMIS network)

Campania is a region in southern Italy with a total population of about 6 million inhabitants. Demographic changes are modifying the epidemiological scenario of the region, which requires the reorganization of health service provision according to the changing needs for care. The increase in the older adult population and COVID-19 pandemic have led to an increase in chronic diseases, multimorbidity, social isolation, and lack of self-sufficient conditions. These changes were accompanied by dynamic progress in the technological and therapeutic field, social changes, and the increasing absorption of resources from the health and care sector.

The Research and Development Unit of Federico II University/Hospital received the recognition of RS from the EC for the first time in 2012, as the coordinator of the Campania cluster. Campania Region recognized and shared the importance of achieving the objectives of the EIP on AHA and then set up a specific coordination group. In 2016, Federico II University/Hospital resubmitted the application to the EC, obtaining the recognition of "3 stars RS." In 2019, Federico II University/Hospital obtained the "4 stars RS" recognition.

During its eight years of activity, Campania RS worked on the development, validation and scaleing of innovative solutions for AHA. Campania RS was involved in activities that generated small-scale successes, which could be scaled to all European regions, and are currently leading the effort to raise awareness on the opportunities that aging offers, to change our approach to health and turn aging into an opportunity for sustainable development.[8]

The participation of regional stakeholders in the EIP on AHA action groups has been leading to the development of collaborative research activities aimed at designing and testing innovative good practices for AHA. Campania RS contributed to integrate its local regional innovation ecosystem into ongoing EU activities aimed at providing new insights into all determinants of health (social, environmental, molecular, physiological, pathological, etc.) hinder active and healthy aging at every age. The involvement of universities enabled and facilitated multidisciplinary collaborations, which fosters the development and adoption of innovations.

Twinning activities with more mature RS from other European regions contributed to the transfer of available good practices into the local system, allowing solutions to be tailored to the needs of the local organizational, technological and IT literacy specifications.

Campania RS has involved IT suppliers in the identification of standards and gaps. Campania RS stakeholders have contributed to the development of new ready-to-market solutions by joining several innovative procurement consortia. This allowed the RS organizations to express their need for innovation, and the IT suppliers to provide appropriate and sustainable solutions.[9]

Programma Mattone Internazionale Salute (ProMIS), the Italian Ministry of Health network for the internationalization of regional health systems (RHS), was an enabling factor to strengthen synergies among Italian RS, and to address the current and emerging health needs of the Italian population, sharing and exploiting at the national level, the opportunities offered by the innovative practices of the EIP on AHA.[10]

2.3.7 MACVIA-France Reference Site

MASK, the Phase 3 ARIA (Allergic Rhinitis and its Impact on Asthma) initiative is a Good Practice of DG Santé for digitally-enabled, patient-centered care.[11] It has been developed from the MASK-air app (https://www.mask-air.com) to a flexible e-platform for allergic diseases and asthma in order to improve the management of asthma and rhinitis in a patient-centered approach and to facilitate shared decision-making.

MASK includes: (1) a freely available app (MASK-air, formerly the Allergy Diary, Android and iOS)[12], (2) tools to support health care professionals in shared decision-making through an interoperable electronic decision support system (e-CDSS)[13], (3) a web-based interoperable questionnaire for physicians[14], (4) a questionnaire on asthma and rhinitis (CARAT) for screening allergic diseases and assessing their control, and (5) a sentinel network for air quality and pollen seasons. MASK-air is validated and follows the General Data Protection Regulation (GDPR) for the processing of personal data in the European Union (EU).

MASK-air is an app centered around the patient[15], operational in 27 countries and 18 languages.

2.4 Reference Site Collaborative Network

The RSCN was founded in 2013 as a direct response from a number of newly accredited EIP on AHA RSs. Its aim was to provide a forum for exchanging knowledge and learning on health and care policies, health and care pathways, and evidence-based good practices and innovation solutions that could be adopted at scale. It would also facilitate RS regions in collaborating on projects to address active and healthy aging priorities and needs across European regions.

The RSCN seeks to help its members accelerate the development, deployment and adoption of innovative health and social care solutions, including proven AHA delivery models and digital solutions that create a real impact for patients and citizens, and contribute towards sustainability of services.

Membership is made from EIP on AHA RSs, and candidate RSs, accredited by the European Commission following publicly-announced Calls for RSs; along with other organizations and individuals that support the principles of EIP on AHA.

The RSCN supports RSs in Europe, and beyond, to: (1) Promote active and healthy aging by supporting and developing strategic collaborations across government, health and care providers, universities and researchers, industry, and citizens to implement creative and multidimensional approaches to prevent disability, frailty and age-related diseases during the entire life course, and to support independent living; (2) Accelerate the deployment of major innovations which will improve health and care outcomes, increase the sustainability of health and care systems, and create economic growth and jobs.

This is achieved by: (1) Facilitating members to develop, share and adopt good practices and innovative solutions and technologies at scale; (2) Facilitating the scaling of evidence-based solutions and technologies through the alignment of policies at regional, national, and international levels; (3) Influencing and providing strategic input to bodies such as the EC, and WHO, building on the knowledge and expertise of its regional members representing organizations within the Quadruple Helix ecosystem; (4) Providing thought leadership through expert working groups; (5) Offering a range of advisory and management services to members.

It is widely recognized that many benefits have been gained both by regions and for those who were actively engaged in contributing to the initiative. Aging remains one of the demographic challenges for Europe and there are opportunities for the EIP on AHA principles to continue to evolve within a changing environment. At the time of writing the European Commission is consulting on its Green Paper on Ageing, which considers not just health and social care challenges but also the wider challenges of delivering the range of public services.

Other relevant drivers for change include: European Commission Report on Demographic Change, Digital Europe Programme, Resilience and Recovery Fund to combat COVID-19, future growth of Digital Innovation Hubs, the need for closer alignment of EIP on AHA, Active and Assisted Living (AAL), the Joint Programming Initiative on More Years Better Lives (JPI-MYBL), and the European Partnership under Horizon Europe—transforming health and care systems.

Through their experience of contributing to EIP on AHA, regions are ideally placed to contribute to addressing the issues and challenges that may emerge from these areas. RSs through their Quadruple Helix ecosystems can ensure both the supply-and-demand sides within the health and care field are engaged in understanding the challenges, codeveloping solutions, and creating and scaling digital innovation.

The RSCN has identified the following health and care challenges and has brought together experts from across Europe to consider and make recommendations: (1) Defining resilience: what that means in terms of person and of population; (2) Integration of social and health services, starting from data standardization, sharing, and

secondary use of data; (3) Defining well-being (feeling healthy) vs. staying healthy: implications for food and nutrition, cultural and environmental dimensions, etc.; (4) Participation in society, health, happiness, well-being, and active living; (5) Addressing the social and mental health needs of rural areas especially for chronic disease prevention and palliative care for noncommunicable diseases; (6) Secondary use of data.

2.5 Transition from Horizon 2020 to Horizon Europe—the role of IN-4-Active and Healthy Ageing

The green paper on aging[16] points out the need to ensure proper planning by the EU member states to tackle the challenges of demographic change by promoting AHA, based on healthy lifestyles, personal accountability, adequate public policies, aided by digital health and personalized medicine. This approach should be based on the concept of lifelong learning (most effective when implemented early), that can take form in acquiring and updating skills to keep people employable (health care and other types of workers). Extending the working lives of EU citizens is especially important due to the shrinking of working age populations, but it also helps ensure adequate, fair, and sustainable pension systems, and help avoid old age poverty. Flanking measures such as reducing early retirement and advertising supplementary pension schemes should also be debated. Nevertheless, old age inevitably leads to a shift in demand for products and services that reflect the specific needs and preferences of older people, but also leads to more people needing long-term care, whenever they become too frail, or the community-based health care systems (preferable, because they allow for patients to live in their own homes) capabilities are surpassed. Hence, more job openings are created in the fields of tourism, smart homes that support independent living, assistive technologies, accessible products and services, service robotics, wellness, cosmetics and fashion, safety, culture, personal and automated mobility, and banking. This is an opportunity for productivity and employment gains, and investing in these gains by fostering the development and uptake of innovations can also improve the sustainability and efficiency of health and care systems.

In this discussion, the territorial- and infrastructure-related issues must not be forgotten, in the sense that working environments, mobility (transportation schemes), urban and home (ex: smart homes) environments need to be addressed, because they are key factors in allowing people to remain independent and productive longer.

Finally, social issues such as old age loneliness, intergenerational solidarity, mental health, informal care reform, women's dual labor journey, and legal migration as another solution to help reduce labor shortages, also need to be accounted for. The same goes for regional disparities between member states, and within member states, rural and urban areas.

These dimensions are analyzed in detail in the discussion paper long-term vision for rural areas.[17] Health care accessibility is still an issue in some of these areas, for

which e-health services and digitalization could serve as potential solutions. Furthermore, the potential for economic and job growth related to innovation implementation, could also benefit these areas. Taking all these ideas into account, a new European Partnership, under Horizon Europe has now been drafted. This time, it was based on the need to transform health and care systems, to be able cope with the foreseen challenges. It called for cross-border, multistakeholder, and multinetwork action, to increase the capacity of health and care systems in terms of better use of their infrastructures, workers and materials, fostering implementation research, technological development, and biomedical innovation to bolster the transformation of healthcare systems towards proactivity (not only treatment) and prevention.

This transformation should also consider climate change, efficient people-centered and accessible health care, adopting digital technologies, exploring new ways of providing care and building health system resilience.

The main takeaway is the demand for cooperation between networks closely related European partnerships, mainly: Active & Assisted Living Programme (AAL Programme),[18] Joint Programme Initiative "More Years, Better Lives" (JPI-MYBL),[19] To-Reach Coordination and Support Action, and EIP on AHA. These networks are complementary to each other, inferring that partnering would enhance their overall impacts. In a sense, TO-Reach and JPI MYBL have delivered evidence-based research for policymakers, and the AAL program is linked with the innovation drive of the health and care service delivery through public and private enterprises/entities. Finally, the EIP on AHA's aims to integrate learning into regional ecosystems. It is important for EIP on AHA to continue providing the connections that allow these networks to flourish, by offering tools to help implement innovation in real life settings, based on accurate data, allowing healthcare systems to adjust and prepare responses for upcoming challenges. Supporting the integration of these networks is also among the tasks of the IN-4-AHA consortium, aligning this objective with the need to adjust the action groups to these contemporary questions, and engage former and current stakeholders to bring their knowledge and expertise to this new partnership.

2.6 Future perspectives

The COVID-19 pandemic has gravely shown the weaknesses of health and social care systems across the globe. Governments have been forewarned about disruptive health threats for many years. This applies to sudden threats as pandemics as well as gradually developing one, such as a rise of chronic conditions due to an aging society. The COVID-19 pandemic also shows the interrelatedness of health and social care system performance, economic resilience and the importance of political leadership. Despite this knowledge, recommendations and solutions available, almost no government realized the necessary changes to improve health and social care

systems in order to anticipate emerging and sudden health threats. It is therefore valuable to look back and see what lessons can be learned over the last 10 years for the near future.

Transformation of health and social care systems appears to be very complex and difficult, given the enthusiastic attempts made by governments and authorities which mostly resulted in disappointing outcomes. Standalone initiatives without consideration of system aspects such as financing, regulations and incentives are unlikely to be effective in the long-term. Successful implementation and upscaling of digitally-enabled care concepts require a cross-sectorial system redesign rather than single innovation projects. Regional and local settings seem to be the most appropriate context for system redesign and the remodeling of health and social care services. It is where people's needs are best translated into integrated care services with all the relevant actors involved.

The EIP on AHA is a program which connects regions and countries on a Pan-European level with the ambition to improve health and social care services. This unique initiative allows local settings to learn mutually from methodologies and practices across Europe as well as find useful networks for collaboration and funding. Despite the challenges of this program, the EIP on AHA proved to be successful in mobilizing and empowering localities in a geographically multilevel network for the implementation and upscaling of technology-enabled health and social care services.

To make Europe more resilient with better health and social care systems, these region-oriented Pan-European networks should be further strengthened for efficient transformation and in post-COVID-19 recovery. A shared vision should create a hopeful perspective and ingredients for a concrete roadmap with actions. Leadership and alignment of coordination on European, national and the local level is essential to make actions effective while balancing the interests of participating stakeholders (public and private). Streamlining process implementation, adoption and upscaling of solutions from start-ups and industry will support economies and facilitate the innovative strength of Europe.

The positive promise for the future lies in building on existing collaborations and successful initiatives, while better alignment and leadership is needed to generate a European-wide movement for impactful transformation of health and social care systems focusing on people needs.

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CHAPTER 3

Aging in Africa, challenges and opportunities—the particular case of Cabo Verde

Fernando J. Regateiro[1,*], António Correia e Silva[2,*], António M.D. Brehm[3,*], Dario D. Reis[4,*], Deisa S.R.C. Semedo[2,*], Edith Pereira[5,*], Hélder Spínola[3,*], Jorge N. Barreto[6,*], Judite M. Nascimento[7,*], Maria L. Lima Mendonça[8,*] and Maria Natalina L. Silva[6,*]

[1]Institute of Medical Genetics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[2]Faculty of Social Sciences, Humanities and Arts, University of Cabo Verde, Praia, Cabo Verde
[3]Human Genetics Laboratory, University of Madeira, Funchal, Portugal
[4]Private Medical Practice, Praia, Cabo Verde
[5]IHMT, Universidade Nova de Lisboa, Lisbon, Portugal
[6]Directorate National of Health, Ministry of Health and Social Security, Praia, Cabo Verde
[7]Faculty of Science and Technology, University of Cabo Verde, Praia, Cabo Verde
[8]National Institute of Public Health, Praia, Cabo Verde

3.1 Aging in Africa and West African Region

In Africa, the population is projected to reach 147 million by 2050, compared to 64 million observed by 2015, most of which is in sub-Sharan Africa.[1]

Healthy life expectancy at birth in Africa was 45.8 years for both sexes, by 2000, and 56 years in 2019, the lowest compared to the rest of the world.[2]

The epidemiological profile in Africa is characterized by the burden of communicable diseases, but also with an increasing prevalence of emergent noncommunicable diseases. The risk of dying from communicable diseases such as HIV, malaria, hepatitis and neglected tropical diseases, is highest in Africa. By 2020, noncommunicable diseases such as heart disease, cancer and diabetes will be among the main causes of mortality in Africa.[2] In West Africa, the most prevalent causes of mortality are stroke, septic shock, malaria, and cancer.[2]

Nutrition transition is real in most West African countries, with the greatest pace being in Cabo Verde, Ghana, and Senegal, leading to consequences such as obesity, high blood pressure and diabetes.[3]

Chronic poverty is a major risk factor for the well-being of older people in Africa. Older people also have less information on healthy aging and must compete for essential health services with all other age groups. Elderly and gender discrimination are a

* All authors contributed equally to this chapter.

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major concern in these areas, often leading to disempowerment and poor health outcomes, victimization and even death, for elderly women in Africa.[2]

According to the Regional Strategic Plan 2020–24 for Healthy Ageing in West African Region, among risk factors for healthy aging are unhealthy lifestyle, absence of palliative care, insufficient structures for health promotion and adequate diseases treatment, and absence of a vaccination policy for elderly. The same document also notes that aging is somehow neglected in this region by public policies not conducive to healthy aging. Specialists in gerontology and geriatrics are insufficient and older people's care is mostly carried out by specialists from other medical fields.[1]

African people spend most of their lives in the family environment, which can be considered a favorable for passing on knowledge between generations and strengthening family ties, but also as a strength for healthy aging.

More than half of the countries of the West African Region have a strategic plan to guide healthy aging policies (Senegal, Niger, Guinea Conakry, Burkina Faso, Ghana, Benin, Togo, Nigeria, and Cabo Verde).[1]

Despite this, attention on healthy aging through life, in the West Africa Region, needs strong reinforcement, namely the implementation of inclusive policies intended to "leave no one behind." Four priority areas for actions recommended by WHO,[4] can help to achieve this goal:

1. Aligning health systems to the older populations they now serve
2. Developing systems of long-term care
3. Creating age-friendly environments
4. Improving measurement, monitoring, and understanding

3.2 Geography and climate of Cabo Verde

Cabo Verde is an archipelagic country, located about 500 km^2 off the West African coast. The islands are located between latitude 14° 48' and 17° 12' North and longitude 22° 40' and 25° 22' West. Cabo Verde has an emerged surface of 4033 km^2 and an exclusive economic zone of more than 700,000 km^2.

Depending on the position in relation to trade winds, the Archipelago is divided into two groups of islands: Barlavento (downwind) islands (Santo Antão, São Vicente, Santa Luzia (uninhabited), São Nicolau, Sal and Boavista); and Sotavento (upwind) islands (Maio, Santiago, Fogo and Brava).

Cabo Verde is geographically integrated in the Sahel, a semi-arid climate zone. Cabo Verde's climate is strongly influenced by the Harmattan, which is a fresh, dry and dusty wind, coming from the Sahara, which blows from Northeast to East between December to mid-March across West Africa, including Cabo Verde, where it is called *Lestada* or *Bruma Seca*. Characteristics of Harmattan, influence morbidity in

Cabo Verde, because it is an enhancer of respiratory diseases, with a greater incidence in children and the elderly population.

Cabo Verde is framed in the long strip between the centers of high subtropical pressures in the North Atlantic (the anticyclone of Azores) and the Intertropical Convergence Zone (ZCIT). Given Cabo Verde's marginal position in that area, it's not always hit by rains, causing cyclical droughts, with negative impacts on productivity in rural areas.

3.3 Cabo Verde, the historical healthy islands

The first feeling of Portuguese navigators about Cabo Verde islands they just found in 1460 was of a healthy space. The islands seemed able to provide a healthy and prolonged life, by standards of that time, quite different from the reality they had experienced at an equivalent latitude, on the neighboring Sub-Saharan African continent. There, malaria and other infections lashed crews of ships. Oceanic localization of islands, the distance from the coast and the uninhabited condition prevented the access of pathogens circulating on the continent.

The perception of a positive health differential is likely one of the reasons why the Portuguese decided to settle in Cabo Verde. In the early years of colonization, these islands were famous through western Christendom, as a healthy place inhabited by many turtles, whose blood was made into an ointment that cured leprosy patients coming from Europe. Several travelers left testimonies in this regard. The Belgian merchant and navigator Eustache de la Fosse, who stopped at Santiago and BoaVista in 1480, was one of them. Another was Fernando Colombo, son and companion of Christopher Columbus during his third trip to the Americas (1498). However, the islands' fame as a curative place lasted a short time!

3.4 Epidemiology of slave society

The main motivation for the settlement of these tropical and semi-arid islands by the Portuguese was the establishment of trading between the Guinea Coast and Iberian world. Therefore, they put pressure on the king and obtained legal prerogatives to trade in slaves, using local production of horses and cotton as a means of payment. In a short time, African slaves imported from Rios de Guiné on a more permanent and large scale, became most of the island population.

Thus, their initial healthiness was profoundly altered. Valentim Fernandes, a Moravian typographer, wrote in 1506 that "islands were healthy at first, but the situation changed profoundly with the arrival of rescued blacks on the Guinea Coast, because they made the airs corrupted".[5]

European slave ships established on the island became privileged vehicles for the import of viruses, bacteria and parasites commonly circulating in the African continent. Overflowing boatholds and lack of hygienic conditions on board created the right atmosphere for contagion. Syphilis and smallpox expanded during sea crossing and landed on the islands together with slaves, sometimes starting local chains of contagion and causing outbreaks. On slave ships, also traveled the vector of the African variant of *P. falciparum*, the parasite responsible for malaria. The vector found ideal conditions to multiply around Ribeira Grande, then the capital and trading post.

Malaria, known as "the disease from the land," started to have a high prevalence and the greatest impact on mortality and morbidity. Europeans were much more vulnerable than slaves to *P. falciparum*, but Africans were far from immune. Should Europeans escape from first infection, they still would be prone to prolonged morbidity. According to the Accountant of the Royal Treasury, the accounting of tax revenues for the years 1513 to 1515 hadn't yet been closed in 1518: "the account was delayed in this way, due to a lot of illness, the land and the disposition of officials."[6]

Clearly, poor hygienic conditions of slave transport and trading placed limits on an extended lifespan, for traffickers and trafficked people.

Slavery imposed severe living conditions to Africans, compared to members of other social groups. One of them is the uprooting and disintegration of social solidarity networks. In Cabo Verde, the few slaves who escaped illness and reached advanced ages were often abandoned by their masters, under the false pretext of manumission, invoking religious piety. Sometimes the church denounced this perverse form of piety—slaveholders were really avoiding costs of inactivity resulting from the aging of slaves.

Unlike what happened in traditional African societies, where the elderly had a prominent status in kinship networks, in Atlantic and colonial societies, aged slaves were seen as a burden. Hence, they were doomed to abandonment and marginalization. In Cabo Verde, this was not the case with slave landowners, whose assets and prerogative of testing heritage guaranteed them social protection in elderly. It was the case to say: "tell me the position you occupy, and I'll tell you how the elderly will be."

3.5 Cabo Verde Famines

Famines resulting from cyclical droughts were another factor limiting prospects for a long life of Cabo Verdeans. From 1460 to 1583, when the first famine occurred, causing massive mortality, droughts had little demographic impact. Commercial prosperity allowed the local society to import food from both Africa and Europe, compensating for the sharp but conjunctural drop in agricultural production, associated with drought or irregular rains.[7]

With Cabo Verde's disintegration from transatlantic trade routes, it ceased the import of food to compensate for the agricultural drop and a long cycle of epidemic famines, not yet well mapped, which began in 1583 and only ended in 1950. Deserving special attention was the violence of the following years: 1609–11, 1719–20, 1748–50, 1773–75, 1831–33, 1864–66, 1903–05, 1922–24, 1940–42, 1947–49.[8,9]

From wide and multidimensional repercussions of famines, we will only focus on the demographic effects, and therefore on society. Installation of a famine is gradual. Lost crops degrade diet, especially of families directly or indirectly depending on rainfed agriculture. The population resorts to poorer and/or highly toxic foods or herbs of low nutritional value. Children and elderly people are the first victims of food impoverishment and associated diseases. Working age population will be later victims.

The risk of hunger and disease is socially different among groups: life expectancy was greater for those who had irrigated land, public employment, or other forms of income than for peasantry-dependent on rainfed agriculture. Average life expectancy at birth and other global indicators ends up hiding situations of deep inequality within countries, a valid warning sign when the tendency is to compare inequality between countries.

3.6 Cabo Verdean population genetics

Cabo Verdeans possess their one genetic profile, distinct from the nearby West African populations. Human leukocyte antigens (HLA) allele frequencies, both for neighbor-joining (NJ) and principal coordinates analysis (PCoA), cluster Cabo Verde in an intermediate position between Sub-Saharan African, nearby Guinea-Bissau, and Caucasian populations from North Africa and Europe. For HLA haplotypes, Cabo Verdeans show an important genetic contribution from the Portuguese population.[10,11] The percentage of common HLA haplotypes with the Portuguese population (9.3%) is higher than the nearby population of Guiné-Bissau (2.2%). When considering all the haplotypes of Sub-Saharan origin, its presence in the Cabo Verde population reaches 15% in total, indicating a mixed African influence.[11]

Similar results, even revealing a stronger admixture from Caucasian, were obtained when studying other autosomal markers, namely red cell enzymes, plasma proteins and short tandem repeat (STR) polymorphisms, showing mean proportions of European ancestry ranging from 36% to 54%, depending on the markers and methods used.[12–14] When autosomal STR polymorphisms were used for NJ and PCoA analysis, results were similar to those obtained with HLA loci, showing that Cabo Verde is an atypical Sub-Saharan population, positioned between Africans and Caucasians.

Genetic studies of polymorphisms linked to susceptibility for some diseases, also carry evidence for this strong admixture. Mutations on the protease inhibitor (PI) locus associated to alpha-1-antitrypsin deficiency, show a lower prevalence in African populations compared to European populations. However, genotypes associated with the disease (PI*ZZ, PI*SS, and PI*SZ) were estimated to be in Cabo Verdeans as one of the highest in sub-Saharans overall (15/1000). This could be explained by the strong admixture with the Portuguese gene pool, where this prevalence is even higher.[15] Concerning interleukin 4 (IL4) cytokine polymorphisms, Cabo Verdeans appear again in an intermediate position between Sub-Saharans and Caucasians.[16] The same happens when considering the prevalence of single nucleotides polymorphisms (SNP) across candidate loci for skin and eye color,[14] or the CCR5-delta32 mutation, which is absent in sub-Saharans but reaches a prevalence of 3.9% in Cabo Verdeans, and up to 10.3% in Portuguese populations.[17]

Contributions for the Cabo Verdeans admixture coming from Europe and Africa are shown to be sex-biased. The fact that almost European settlers were males, is clearly seen on the Y-chromosome polymorphisms. According to an admixture analysis with 21 non-recombinant Y-chromosome (NRY) markers,[18] most male genetic contributions came from the Iberian Peninsula (68%), followed by West Africa (27%). Similar results were obtained with the identification of 16 Y-chromosome SNPs haplogroups,[19] from which 53.5% are of Caucasian origin and 26.5% are of West African origin. This higher genetic contribution from Caucasian males is explained not only by their intermarriage with female West Africans but also due to their social dominance and associated reproductive success during the settlement process of Cabo Verde. This admixture involving predominantly, European men and African women, has also "the other side of the coin," a stronger West African contribution from the female lineage, seen on studies conducted with mitochondrial DNA (mtDNA) markers. Analysis of the mtDNA hypervariable segment I (HVS-I) control region, and the characterization of its haplogroups, showed that 93% are clearly from Sub-Saharan African origin,[20] a result confirmed by others.[21]

The levels of admixture and European male and African female influences, vary along the different islands. A panel of autosomal ancestry informative markers (AIMs) reveals that Southern islands have higher levels of West African ancestry, especially Santiago (65%), with the Northern islands, Santo Antão, São Vicente and São Nicolau, showing the lower values (56%), and Boavista, geographically located in the middle, having an intermediate value (59%).[18] As so, there is an evident South to North decline progression on the African ancestry, with Fogo as an exception. Despite being located on the south, near Santiago, Fogo shows the lowest values (53%). This admixture progression along Cabo Verde Archipelago is explained by the settlement process. Santiago was the first to have a population, mainly African slaves. The northern islands were populated later with free admixed people that moved upward from

southern islands.[22] As mentioned, despite the geographic proximity and settlement contributions from Santiago, Fogo is an exception to this South to North cline. This exception should be the result of the lower dimension of the island, founder effect and its particular social organization. Additionally, the harsh labor conditions should have its contributions through the higher mortality among African rural slaves than among Europeans and their offspring with domestic slave women.[18] This pattern of differential admixture along the Archipelago, was previously reported for autosomal STR polymorphisms,[13] and even for HLA allele and haplotypes frequencies.[10] The prevalence of West African ancestry is also variable across the archipelago, showing a lower prevalence in northern islands.[19] The highest level of West African ancestry, evaluated through NRY markers, is found in Santiago island (57%), and lower values in northern islands of the archipelago, as Boavista (5%), São Nicolau (10%), Santo Antão (36%) and São Vicente (21%). Again, Fogo displays an exception in this South-North polarity, revealing one of the lowest values of West African ancestry (9%).[18] Hegemonic West African female lineage ancestry mentioned above, shows also some differences between Cabo Verde islands, with its highest value found in Santiago (76%) and lower in Boavista (62%), Fogo (63%), São Nicolau (64%), São Vicente (67%) and Santo Antão (70%).[18,20]

3.7 Age pyramid of Cabo Verdean population

According to Census 2010, the resident population of Cabo Verde was 491.875 inhabitants, distributed among the nine inhabited islands.

The age pyramid of the Cabo Verdean population (Fig. 3.1) demonstrates a demographic transition phase. The base of the pyramid, up to 19 years old, is progressively narrower. There is a widening of the pyramid between 20 and 24 years old, but then the trend towards narrowing is resumed, which represents a transition to a pyramid with a tendency for an aging population.

Analysis of Cabo Verde age pyramid clearly show a strangulation between ages 60 and 70, which can be explained by the effects of the great drought of the 1940s, with the greatest impact between 1947 and 1954, when many people died victims of hunger. In addition to famine, there was an intensification of migrations to big cities (Praia and Mindelo), but also out of the country, especially to São Tomé and Príncipe, to work in the large fields. For ages 70 to 74 years, the number of people increased, compared to ages 60 to 69 years, which can be attributed to an increase in birth rates and a reduction in mortality in the second half of the century.

3.8 Urbanization of Cabo Verdean population

Cabo Verde has two levels of strategic management: national and municipal. Governors, at both levels, are elected by universal suffrage. The country is divided into 22

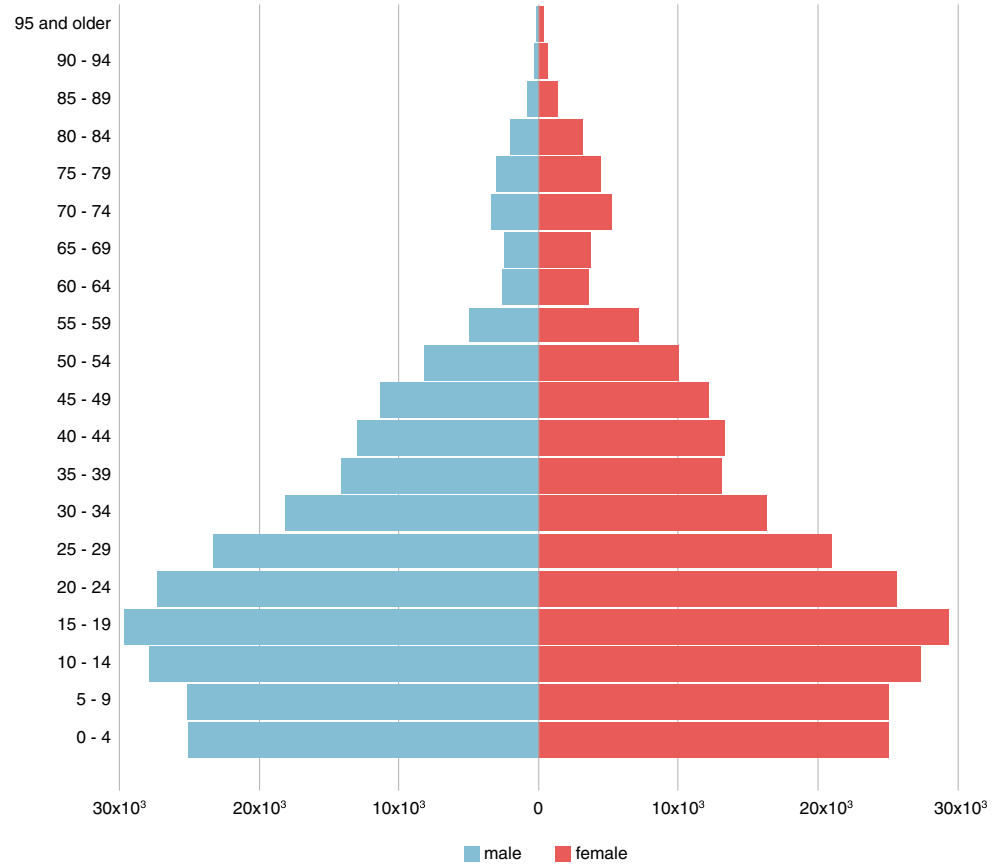

Figure 3.1 Population living in Cabo Verde, by sex and age groups (Census 2010).

municipalities, whose borders, in five of them, coincide with the island's limits: São Vicente, Sal, Boavista, Maio and Brava. Cabo Verde also has an uninhabited island, Santa Luzia and eight islets, also uninhabited, that constitute Cabo Verde's nature reserves.

The population of Cabo Verde is becoming more and more urban, as result of rural exodus, but also under the influence of urbanization of rural areas. The irregularity of rains has caused an intense rural exodus, affecting young and working age population, which can result in aging of the rural population, especially in the most remote municipalities.

Census 2010 registered 62% of the population residing in an urban environment. Rural exodus, as it mainly affects young and adult populations, has caused the aging of the rural population, in a global way, but with greatest significance in the most remote villages of some municipalities such as Porto Novo and Ribeira Grande on Santo Antão, Tarrafal and Ribeira Brava on São Nicolau and on Brava.

People migrate to main cities of islands, but also to the largest cities in the country, Praia and Mindelo, looking for opportunities to improve living conditions. With the development of higher education, a large number of young people have moved to urban centers, where higher education institutions exist. In some municipalities, there are villages already abandoned, or almost abandoned and mainly inhabited by elderly people. But these elderly people are not abandoned; family members living in the same island visit them and support their subsistence. Even family members emigrated to other islands or abroad, send remittances for their subsistence and visit them.

The great challenge for authorities is equitable regional development, for the attachment of young people in villages of origin. In Cabo Verde, it is traditional to care for elderly people in the family. Placing elderly in institutions is a families' last resort. Young people are educated to see care for elderly in the family as a responsibility. Intergenerational solidarity still exists in Cabo Verde, with more intensity in rural areas.

3.9 Aging and the emergence of a new demographic model

Evidence of the conditioning role the climate and diseases have had on Cabo Verde demography since settlement, are scattered and inaccurate. However, from the XIX century, figures have reliability, on the ravages produced by droughts and epidemics.[23,24] For example, hunger and diarrhea caused 30,000 deaths during the 1864 famine; in the 1940–50s, 65% of the workforce of the largest and most populous island perished from starvation,[23] due to the last drought to plague the archipelago.

Cyclical famines that plagued Cabo Verde since the XVI century, ended by 1949. Authorities could no longer allow a famine to occur, facing the existence of United Nations, the influence of its decolonization committee and the emergence of independence movements following Bandung Conference. Portugal, as colonial state, until then minimalist in its social functions, assumed the fight against effects of drought and endemic diseases, namely through Fomento Plans. Thus, historically and demographically, 1950 is an outstanding milestone and a dividing date in Cabo Verdean society. Previously, for most people, life was short and aging and healthy aging were a privilege of a few. Thereafter, increased lifespan was observed, and a long period of uninterrupted population growth began, due to a fall in mortality in general and maintenance of the traditional high birth rates. The cohort of individuals born in the 50s and 60s of last century will be the first to integrate a Cabo Verdean population where sociodemographic weight of elderly population will be significant.

Human societies, from different regions of the globe and with different degrees of socioeconomic development, are aging at different speeds (Table 3.1).[25]

Several external factors have a significant role in population aging: economic and social development, environmental sanitation, medical advances, good health

Table 3.1 Percentage of general population aged 65 years and over (2020).[25]

Africa and middle east					European Union and Japan	
Cabo Verde	Uganda	South Africa	North Africa and Middle East	Sub-Saharan Africa	European Union	Japan
5	2	6	5	3	21	28

organization, and good governance. These factors contain within themselves, the seeds of population aging and the driving forces conducive to the demographic, epidemiological, economic and social transition.

By 1975, Cabo Verde integrated the platoon of the least developed countries, with high rates of child mortality (108/1,000 newborns), high prevalence of infectious and parasitic diseases, high fertility rate (6.8/woman), a modest average life expectancy (57.63 years at birth; 59.12 for women and 56.00 for men) and a negligible GDP per capita (USA, $300). By 2000, life expectancy at birth raised until 66 years for men and 75 years for women. In 2019, it became 73 years for men, and 80 years for women. The aging index was 20.3% in 2000, 21.4%, in 2010, and 29.4% in 2020.[26] Reduction of mortality from infectious diseases and reduction of synthetic fertility index are other relevant causes of population aging.[27]

In the last 30 years, Cabo Verde reached the platoon of a developing country[23,26,28,29]:

- GDP increased to 7000 US dollars (2017)
- Child mortality dropped to 13.0/1,000 (2018)
- The fertility rate dropped to 2.2/woman
- The prevalence of infectious and parasitic diseases decreased significantly
- Chronic degenerative diseases increased being now the prevalent causes of morbidity and mortality
- Life expectancy rose to 73 years for men and 80 for women.

3.10 Elderly in Cabo Verde

Elderly is an economically and socially heterogeneous group: different at literacy level, in financial capacity, in family responsibilities, but similar in diseases (multi-morbidities, hearing, visual and motor deficits, sexual dysfunction, depressive syndromes, dementia) and the painful sensation of human finitude.

Regarding elderly people in Cape Verde, it should be noted[30]:

- They constitute 5.9% of the total population, women being 60.7%;
- 66.1% are family heads, 14.9% live alone and only 10.8% live with their consort;

- 87.7% are inactive, while 11.9% are still working;
- 59.3% are beneficiaries of social security;
- 44.3% are poor and 14.4% very poor;
- 63.9% are illiterate, women being 76.4% and men 46.5%;
- 2.6% have higher education;
- 30.6% have a mobile phone;
- 4.4% use a computer and 6.0% use the internet;
- 2.2% practice physical activity

Roughly speaking, it can be said that about six out of 10 elderly people are illiterate, poor or very poor, family heads, receive a social pension, have no occupation and never used any information and/or communication technology. Almost nine out of ten are sedentary.

3.11 Health and national health service—philosophy, structures, and budget

The right to health is constitutionally enshrined in Cabo Verde. The state must create conditions for universal access to health, through an adequate healthcare delivery network, the National Health Service (NHS). NHS philosophy is to offer health services to all individuals and communities, as close as possible to their areas of residence and at a minimal cost.

In addition to the central level comprising services to support the Ministry of Health, the NHS is structured into three levels of care: primary care based on health centers, secondary care based on regional hospitals and tertiary care based on central hospitals.

Care provided to the elderly by primary care network contemplates the improvement of access conditions to NHS, adequacy of specific space for care, follow-up of elderly people, and implementation of activities intended to promote and/or improve the independence of elderly people. Secondary and tertiary care networks still don't have geriatrics or palliative care services.

The functioning of the NHS is financed by the State Budget, Social Security, and direct contributions from users.

In the first ten years following independence, the percentage of the state budget allocated to health was low (Table 3.2). In the last five years, percentage has fluctuated between 8% and 10.6%.

Table 3.2 Percentage of state budget allocated to health.

1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	...[a]	2016	2017	2018	2019	2020
5.91%	4.37%	3.14%	2.87%	2.65%	1.95%	2.42%	1.46%	1.65%	1.72%	...	8.8%	8%	10.6%	9.6%	9.5%

[a]Data between 1986 and 2015 were not collected—timelined evolution of budgetary allocations to health is not the purpose of this chapter.

3.12 Healthy and active aging policies

In 1998, WHO already drew attention to the importance of countries implementing "active aging" policies to improve health, participation, and safety of aged citizens.In Cabo Verde, elderly rights are constitutionally safeguarded, through special protection by the family, society, and public authorities, and operationalized by the National Policy Charter for Elderly.

With a specific chapter alluding to priorities, integrated in the National Health Development Plan, 2012–16 (NHDP), the issue of elderly health has become even more pertinent.

In 2015, a National Health Program for Elderly was approved. For the period 2017–21, a National Strategic Plan for Active Aging and Healthy Elderly was in action, with five priority areas: multidisciplinary and network work, promotion of health and active aging, continued care, coordinated management, and aging under the perspective of intersectorality.

NHDP 2012–16 already forecasted the elaboration and implementation of protocols on healthcare for elderly, a goal put into action with the elaboration of the Protocol for Integrated Health Care for Elderly 2018–23.

Current Government's program (2021–26) states to have the creation of a care system for dependent elderly people, financial support for social solidarity institutions dedicated to activities aimed to elderly, support to hosting centers managed by municipalities, all complemented with improvement of social services and medical and medication assistance for elderly.

NHS has been working to promote active and healthy aging through activities carried out in collaboration with churches and development partners, including nongovernmental organizations. For better operationalization of activities, health structures have a focal point intended towards elderly health, in turn articulated with central level structures.

To facilitate the elderly's access to health care, there is a policy of exemption from user fees for consultations, medicines, and auxiliary diagnostic tests. For provision of integrated continuous care, training of informal caregivers is foreseen.

3.13 Aging and poverty

The response to social and economic implications of aging and increased life expectancy, must consider, as an emerging aspect, the heterogeneous nature of the elderly, namely in developing countries.

Poverty, as a deprivation or lack of access to goods and services beyond subsistence, affecting human beings in their existential dimension, violates human rights.

How is aging in Cabo Verde, where a fringe of the elderly population lives in poverty? Around 23.7% of Cabo Verdeans live in extreme poverty, with less than US

$1.90 a day. Extreme poverty mainly affects rural populations, where 18.8% of the affected population are aged 65 years or older.[31]

United Nations defines the elderly, for developed countries as persons aged 65 years or over, and for developing countries, such as Cabo Verde, as persons aged 60 years or over.[32] In Cabo Verde, age retirement is 60 years for women and 65 years for men.

Within the scope of policies to combat poverty, social promotion of the vulnerable population, in which elderly people are included, is legally contemplated in Cabo Verde, through adequate provisions in terms of food aid, medical and drug assistance in public health establishments and allocation of a monthly social pension (US$47.54), to all Cabo Verdeans aged 60 years and over, who have no monthly income.

Cabo Verde's National Policy Charter for Elderly People puts in action social objectives, in an integrated and articulated approach to problems affecting this aged group. This Charter aims at specific responses for elderly, emphasizing integration and attention to families, especially in the presence of special needs, and improving achievements in the fight against poverty.

The eradication of extreme poverty, in all its forms and places, is a priority of sustainable development goals (SDGs) to be achieved by 2030, together with falling by half, the proportion of men, women and children, of all ages, living in poverty. Cabo Verde assumed this commitment. At the national level, the Government assumed, for the period 2016–21, the reduction of relative poverty to a single digit and the eradication of hunger and extreme poverty in the country, within the framework of promoting inclusive economic growth.

Getting older as a poor person entails a lack of access to basic resources and services that guarantee well-being of older people, and puts quality of life at risk, thus affecting aged people at the physical, mental, social, cultural, environmental, and spiritual levels.

Understanding the effect of poverty on aging requires knowing paths of life and Cabo Verdean reality. At the genesis of poverty are irregular and late deductions for Social Security, low pay during the professional career and the burden of subsistence for extended families, a quite common situation in Cabo Verde. Although Cabo Verde is considered a medium development country, from the point of view of economic sustainability, it is one of the most vulnerable countries in the world.[31]

3.14 Aging and gender

Worldwide and currently, due to gender gap in longevity, older women, in particular, those aged 80 years or older, outnumber older men across the age range. In 2019, for every 100 women aged 65 years or older, there were 81 men, and for every 100 women aged 80 years or older there were only 63 men. By 2050, elderly women are expected to represent 54% of the world's population aged 65 years or over.[32] As previously described, women also have a longer life expectancy than men, in Cabo Verde.

Although women live longer, they experience a worse quality of life compared to men, mainly related to the gender effect. Gender structures the entire life cycle and influences access to resources and opportunities. Difficulties associated with aging women are not always known, as most studies on aging doesn't consider specific characteristics of men and women.[33]

In Cabo Verdean, the population that has never attended an educational establishment, 10.9% are women, which correspond to double that of men, with 4.5%. The literacy rate in the population aged 15 years or over was 87.7% in 2018, with 92.6% being men and 83% women.[31]

Poor households have, on average, 3.8 years of schooling, compared to 7 years of schooling recorded for non-poor households. Representatives of poor households are mostly women (61%), compared to 51% for non-poor households.[34]

Females (53%) are the majority of poor people in Cabo Verde.[34] Cabo Verdean aged people was born and raised in a period when access to education was difficult, mainly in rural areas. For cultural and social reasons, parents gave priority to their sons, leaving daughters to help with domestic activities, so they could not study.

Clearly, gender is a determining variable in living and aging of individuals, with direct implications on health, well-being and quality of life. However, social customs change. What will be the best sense to give to evolution of customs to reduce and eliminate the negative weight gender now provokes on women's lives and aging?

Today, women continue to accumulate disadvantages throughout life, such as violence, discrimination, lower wages than men, double shifts, low education, loneliness due to widowhood, greater risk of poverty and greater dependence on external resources.[35]

Cabo Verdean authorities have shown concern with social and gender inequalities throughout life cycle, so specific measures are expected to correct social distortions, while respecting biological and psychological specificities of old-aged women.

3.15 To a healthy living and active aging in Cabo Verde—the future

Approaches to best solutions to aging in Cabo Verde should be framed in the context of the SDGs, namely in points 2 (Zero hunger), 3 (Good health and well-being), 10 (Reduced inequalities) and 11 (Sustainable cities and communities).

This requires integrated strategies, going from a healthy prenatal period of life to healthy and active aging and social interactive participation of older people, always ensuring conditions proper to human dignity.

Recommendations for public policies intended for the elderly should consider these main principles:

1. The recognition of the elderly as a social asset
2. The spreading of the idea that it is never too late to promote health
3. Health care equity

4. Empowering of autonomy and self-control
5. The respect and value of diversity

Cabo Verde's first Strategic Plan for Active Aging was elaborated in 2017. It defines, as main values for health care provision: equity, respect for culture, integrality and continuity. The Plan (1) encourages intergenerational dialog and cooperation, (2) establish health conditions, and the reform and update of health, social, economic and infrastructural policies as main strategic axes to support elderly people, and (3) defines measures intended for health prevention and promotion, the control, rehabilitation and management of health conditions, the efficient control of chronic diseases, the offer of rehabilitation, integrated continuous healthcare and palliative care, the training of informal caregivers, the education, social promotion and autonomy of the elderly, the housing and accessibility to buildings and the implementation of community centers and long-term care beds.

The Strategic Plan for Healthy Aging will be updated for the period 2021–25. Strategies to adopt should consider: involvement of society, addressing social transformations in family and society and their impact on lives of elderly people, promotion of social balance and cohesion, reinforcement of continuous training of health professionals and informal caregivers, effective creation of an integrated continuous healthcare network, creation of elderly statute defining specific rights and obligations of elderly people, creation of an association of elderly people.

Furthermore, special attention should be paid to sustainability of Social Security: increasing beneficiaries and reducing taxpayers due to reduction of young labor, will put it in stress in the medium term.

When thinking about population aging, we should be thinking about the past, the present and the future of Cabo Verdean society.

Public policies were designed and dimensioned for a young society in a demographic explosion. The next challenge will be adapting public policies to this new historical cycle, marked by an increasing percentage of elderly people.

Current and future elderly people deserve a new paradigm of public policies intended to create a balanced social protection plan and programs to supporting their rights, needs, expectations and duties, allowing them the right to make plans, create new possibilities for their life, be interested in the future, enjoy an active and healthy aging, and to live their one life entirely and feel a living part of society, leaving no one behind!

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CHAPTER 4

Flagship initiatives to prevent and treat diabetes as a burden of western societies

Peter Goulden[1] **and Eugenia Carvalho**[2,3]
[1]Icahn School of Medicine at Mount Sinai, Division of Endocrinology, Diabetes & Bone Disease, Department of Medicine, Mount Sinai Morningside & West, New York, NY, United States
[2]CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[3]IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal

4.1 Introduction

One of the biggest challenges facing Western society is the human and economic costs associated with the rise seen in diabetes cases, particularly over the last two decades. The massive increase in diabetes has been called a tsunami. The twin drivers of obesity and an aging demographic in most Western countries have underpinned the changes seen. The underlying reasons for the massive rise in obesity cases are multifactorial, however, it is clear this demographic shift has driven the enormous rise in diabetes incidence seen in recent years. This increase in incidence requires access to providers who possess the appropriate expertise both in primary and specialist care.

As the trends continue, there is a growing need to develop strategies that measure the risks and address the challenges. Data is the key to driving this change, coupled with the need to prove cost-effectiveness for any intervention made. Initiatives need to be accessible, standardized, and sensitive to local conditions. A rural community may have a very different need compared to a more urban area.

The COVID-19 pandemic has accelerated the pace of technology change in healthcare, increasing telephone and video visits to provide diabetes prevention and care. The use of video to deliver group classes has also provided much-needed support.

We will outline the evident, behaviorally-focused initiatives in diabetes prevention and treatment within North America. To best tackle the rise in obesity and diabetes, it is clear that projects, which target and encourage behavioral change are essential. We will outline specific evidence of projects, which have provided benefit, and how a longer-term multidisciplinary team joint approach, is key to addressing this public health challenge.

Aging
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4.1.1 Demographics of diabetes and prediabetes

The US center for disease control and prevention (CDC) 2020 report outlined the current scale of the prevalence of diabetes and prediabetes in the US.[1] The crude estimates of the prevalence of diabetes in the US for 2018 in this report currently was 34.1 million adults or a total percentage of 13.0% (12.0−14.1) comprising 10.2% (9.3−11.2) diagnosed and 2.8% (2.4−3.3) undiagnosed cases. It is projected that this number may reach 64 million by 2050,[2] putting a significant health and financial burden on both to the individual and the larger population. Looking at this predicted prevalence rise, it is critical to ensure that prevention and treatment initiatives are responsive and reduce the overall number of cases and thus the cost to the individual and society regarding diabetes complications. It is noteworthy that diabetes is one of the most common causes of blindness in people of working-age.

The prevalence of prediabetes is also striking; comprising 88 million people, age 18 years or older or 34.5% of the adult US population. Part of this group comprises 24.2 million adults who are 65 years or older. This is a demographic challenge similarly faced by many Western nations, and the twin drivers of obesity and an aging population have underpinned these trends over time.

The term syndemic aptly describes the overlapping of the challenges of obesity, undernutrition, and climate change.[3] These challenges have become particularly acute given the COVID-19 pandemic faced by the global population.[4]

One other area which can prove a challenge is food insecurity. Worldwide, it is estimated that more than 800 million people live everyday not having access to sufficient food, or food of adequate quality, to meet their basic needs. The United States Department of Agriculture (USDA) estimated that in 2019, 10.5% or 13.7 million were food insecure at some stage. This was a significant reduction from 11.1% in 2018.

Also, access to medical care is not guaranteed. Access to healthcare includes "the timely use of personal health services to achieve the best health outcomes.[5]" This may be impacted by the physician-to-patient ratios depending on local geography, financial challenges faced, transport, and other barriers to care. Access is critical to ensuring the individual can reach or be reached by the appropriate treatment program.

It makes sense that the target of interventions should be those who perhaps carry the highest risk; the presence of prediabetes or diabetes in association with obesity provides such a target for intervention.

4.1.2 Healthcare literacy and numeracy

In 2014, the Institute of Medicine[6] convened a roundtable that recognized that health literacy might be defined as: "the product of the interaction between individual's capacities and the health literacy-related demands and complexities of the healthcare system." This is an important area in any chronic disease management program and

particularly for those patients with prediabetes and diabetes. The ability to navigate any healthcare system does impact access for those individuals. Health numeracy as part of literacy is also a vital component of any diabetes education model. The patient newly diagnosed with diabetes is suddenly thrust into a world of numbers: HbA1c, serum glucose, blood pressure, cholesterol, etc. This can be daunting to the individual who may struggle in this area and ensuring that the individual who needs support, receives it. Health numeracy may be critical for interpreting everyday areas which impact glucose management, such as following a diet or exercise plan. They must also understand goals for glucose, calories, carbohydrates, physical activity, or choosing the correct treatment dose, such as insulin.

The roundtable looked at the measurement of numeracy and focused on the national assessment of health literacy (NAAL). The assessment includes the proportion of adult Americans who fell into four quantitative literacy and numeracy levels. The report noted that 22% of the population falls into a below introductory class of quantitative performance. This is important given that assumptions are often made when teaching diabetes management and prevention.

4.1.3 Food insecurity and dietary quality

Hanson and Connor[6] have defined food insecurity as having four components. Namely quantity, quality, feelings of deprivation, and disrupted eating. In their 2014 review, they noted that in 2012, about 20% of American households with children were considered food insecure at some point during the previous year. In 10% of households, children experience food insecurity, impacting both physical and mental health.

This review noted that food insecurity impacted dietary quality in adults, specifically the intake of nutrient-rich vegetables, fruit, and dairy. It was also pointed out that the association was less commonly related with reduced dietary quality in children suggesting that parents were attempting to minimize the impact of food insecurity on their children. Options to address this challenge have included addressing food insecurity at either food distribution sites or alternatively at healthcare provider visits. This highlights the importance of taking a proactive approach to managing this challenge and importantly destigmatizing the subject so it can be addressed proactively

Given the impact of obesity on prediabetes and diabetes, it is clear that initiatives that address this area are also important moving forwards.

4.1.4 Community engagement

A community-based participatory research (CBPR) has been shown to provide a bridge between researchers and the community, which offers an opportunity to introduce improvements in diabetes and prediabetes. One example of this work is with the

Arkansas's Marshallese Pacific Islander community, a population with a high prevalence of prediabetes and diabetes and facing difficulties with access to care historically. Work done by McElfish et al. on adapting the diabetes self-management education (DSME) curriculum to this population using a CBPR approach has been able to demonstrate a significant improvement in glycemic control in this population.[7] One of the critical pieces was engaging the community in designing the program around the population mapped to community priorities and needs. This approach utilized a model which included the family of the subject in work done.[8] The advantage of this approach was that when discussing behavioral change, the individual, instead of being seen in isolation, was educated together with the family group on DSME.

This approach can be applied to any group which may struggle to access healthcare or suffer a disproportionate burden of diabetes. Similar work to adapt to the requirements of other cultures is required to ensure equity of outcomes.

4.2 Impact of research: prediabetes

The two pivotal trials which have focused on diabetes prevention comprise the diabetes prevention program (DPP)[9] and the Finnish Diabetes Prevention Study.[10] These studies have concentrated on lifestyle intervention around physical activity and diet and have proven key in providing an evidence base for approaches to diabetes prevention. The information gleaned from this work has provided a foundation for the work done to this day.

4.2.1 The diabetes prevention program study

The DPP comprised a group of 3234 subjects with an average body mass index (BMI) of 34 kg/m^2 with an average age of 51 years and fasting and/or two-hour plasma glucose concentrations in the prediabetes range. Subjects were randomized in to one of three groups.

The first group comprised intensive lifestyle intervention with a weight loss goal of 7%, including an exercise program of 150 min per week and a low-fat diet focusing on behavioral change. The second group included metformin 850 mg twice a day plus general diet and exercise information. The final group received a placebo plus information on diet and exercise.

The DPP lifestyle intervention provides a clear guide on what works, given the impact with a 58% reduction in the incidence rate of diabetes. It has provided a blueprint for interventions that succeed. In 2002, the DPP research group published a detailed description of the lifestyle intervention.[11] The program's key features included an individually-assigned case manager or "lifestyle coach," frequent contact, and a core 16 session program that taught self-management approaches for physical activity and weight loss. All exercise sessions were supervised. The maintenance program was

flexible, including group and one-on-one sessions, motivational techniques, and "restarts" if needed. There was a focus on individualization and adherence. Notably, the materials used were adjusted to be inclusive of ethnic diversity. In addition, there was a network of training, feedback, and clinical support.

It is clear from the success of this research program that targeted interventions worked exceptionally well. For example, the lifestyle arm of the DPP study was almost twice as effective as the metformin arm, which showed a 31% reduction in diabetes cumulative incidence compared with the 58% seen in the lifestyle arm.

A follow-up of the DPP cohort, 15 years later showed the benefits persisted, albeit to a lesser extent, with a reduction of 27% of diabetes cases in the lifestyle group.[12] For those patients who did go on to develop diabetes, the participants delayed the onset of the disease by around four years. An important question is the cost-effectiveness of such an intervention. Metformin as a low-cost therapy has been proven to be cost-saving.[13]

4.2.2 The finnish diabetes prevention study

This study included 522 patients with impaired glucose tolerance, a mean BMI of 33.2 kg/m^2, and a mean age of 55 years to one of two groups. There was a control group and a weight reduction/exercise program implemented with the other group.[10]

At the end of two years, the intervention group had achieved a mean weight loss of 3.5 kg versus 0.8 kg in the control group. This translated into a cumulative incidence of diabetes of 23% in the control group and 11% in the intervention group at the end of four years; and an effective 50% reduction.

The benefits of the intervention lasted three years after with some attrition of the effect from a 58% reduction in diabetes incidence during the trial and a 36% reduction in the three-year follow-up.

4.3 Lifestyle interventions

4.3.1 The national diabetes prevention program

The national diabetes prevention program (national DPP) builds on the DPP blueprint to create a program that partners with organizations from all sectors to prevent type 2 diabetes. This highlights the potential for private-public sector partnerships. Organizations that form part of this partnership are diverse and include federal agencies, state and local health departments, national and community organizations, employers, public and private insurers, healthcare professionals, universities, and businesses that focus on wellness. The National DPP was authorized by congress in 2020 based on the DPP trial data. One of the critical drivers for the program was the evidence supporting cost-effectiveness. A systematic review of literature was performed on the cost-effectiveness

of diabetes interventions between January 1985 and May 2008 based on the cost per life-year gained (LYD) or quality-adjusted life year.[14] Intensive lifestyle interventions to prevent type 2 diabetes in people with impaired glucose tolerance were shown to be very cost-effective, defined as a cost of < = $25,000 per life-year gained.

Consequently, support was provided via grants from the C.D.C. to set up programs across the US. This has resulted in a registry of programs that deliver evidence-based type 2 diabetes prevention programs in communities across the US.

4.3.2 Exercise

Initiatives that target exercise have been shown to impact diabetes risk. One key question has been on duration and quantity. The DPP provides a blueprint with a goal set of at least 700 kcal per week from physical activity. This equates to 150 mins per week of moderate physical activities such as brisk walking. The evidence base available supports the long-term effectiveness and durability of 150 min per week exercise programs. This is important given the need for longer-term consistency to maintain any benefit. The DPP study encouraged the distribution of physical activity over at least three sessions. Also, it indicates that up to 75 mins of strength training could count towards this goal. While brisk walking was seen as a standard activity, other options included aerobic dancing, bicycle riding, skating, and swimming.

A metaanalysis of 28 prospective studies published by Smith et al.[15] showed that for individuals exercising 150 min per week; there was a risk reduction of 26% (95% CI 20%, 31%) for type 2 diabetes for those who achieved 11.25 MET h/week which is equal to 150 min a week of moderate activity. At double this amount of training, namely 5 h per week, there was a risk reduction of 36% (95% CI 27%, 46%). There was evidence that benefits of exercise continue at levels significantly higher than those recommended in current guidelines and that higher intensity physical activity provided greater benefit than low-intensity training.

4.3.3 Pharmacologic therapy in prediabetes

For patients who are unsuccessful with lifestyle intervention, medication may provide benefits. DPP clearly showed evidence of the ability of metformin to prevent the onset of type 2 diabetes. This has provided another helpful tool to help patients at increased risk. Patients in the DPP study received metformin 850 mg twice a day alongside information on diet and exercise. This resulted in a 31% reduction in diabetes cases diagnosed by fasting glucose and a 2-h post-glucose tolerance load. In the follow-up study over ten years, diabetes prevention program outcomes study (DPPOS) showed that ten years after the original trial, there was an 18% reduction in the incidence of diabetes.

4.4 Impact of research: diabetes

The four cornerstones of diabetes care include nutrition, physical activity, monitoring, and medication adherence. There has been much evidence on the role of medical nutrition therapy (MNT), which is essentially a nutrition prescription based on individual needs and unique environment. The focus is often around total calories, quality of calories, carbohydrate intake, and MNT, a component of the broader area of DSME. The approach may include weight loss, carbohydrate consistency or counting, meal timing, and the quality of calories consumed. Tailoring the approach to the individual is vital; however, group education has also been used. In such cases, seeking community engagement in designing and delivering the program may help.

4.4.1 Diabetes self-management education

Any patient with newly diagnosed diabetes or established diabetes should participate in a diabetes self-management education program (DSMEP). This is defined as a program that provides the individual with the self-efficacy to manage their diabetes effectively. DSME may include a focus on dietary adherence, MNT, physical activity, medication adherence, and/or complication prevention. DSME may take many forms, however, all forms should cover the key areas of diabetes management to empower the patient to manage their condition.

There is good evidence of how impactful DSME may be. Programs aim to provide a core curriculum. The American Diabetes Association (ADA) and American Association of Diabetes Educators (AADE) use the accreditation standards established by the National Standards for Diabetes Self-Management Education Support (NSDSMES).[16] The national standards as outlined are:

1. Internal structure. The provider(s) of DSME will document an organizational structure, mission statement, and goals. For those providers working within a larger organization, that organization will recognize and support quality DSME as an integral component of diabetes care.
2. External input. The provider(s) of DSME will seek ongoing input from external stakeholders and experts to promote program quality.
3. Access. The provider(s) of DSME will determine who to serve, how best to deliver diabetes education to that population, and what resources can provide ongoing support for that population.
4. Program coordination. A coordinator will be designated to oversee the DSME program. The coordinator will have oversight responsibility for the planning, implementation, and evaluation of education services.
5. Instructional Staff. One or more instructors will provide DSME and, when applicable, DSMS. At least one of the instructors responsible for designing and planning DSME and DSMS will be a registered nurse, registered dietitian, or pharmacist

with training and experience pertinent to DSME, or another professional with certification in diabetes care and education such as a CDE or individual Board Certified in Advanced Diabetes Management (BC-ADM). Other health workers can contribute to DSME and provide DSMS with appropriate training in diabetes and with supervision and support.

6. Curriculum. A written curriculum reflecting current evidence and practice guidelines, with criteria for evaluating outcomes, will serve as the framework for the provision of DSME. The needs of the individual participant will determine which parts of the curriculum will be provided to that individual.
7. Individualization. The diabetes self-management, education, and support needs of each participant will be assessed by one or more instructors. The participant and instructor(s) will then together develop an individualized education and support plan focused on behavior change.
8. Ongoing support. The participant and instructor(s) will together develop a personalized follow-up plan for ongoing self-management support. The participant's outcomes, goals and the plan for ongoing self-management support, will be communicated to other members of the healthcare team.
9. Patient progress. The provider(s) of DSME and DSMS will monitor whether participants are achieving their personal diabetes self-management goals and other outcome(s) as a way to evaluate the effectiveness of the educational intervention (s), using appropriate measurement techniques.
10. Quality improvement. The provider(s) of DSME will measure the effectiveness of the education and support and look for ways to improve any identified gaps in services or service quality using a systematic review of process and outcome data.

These standards provide a framework for diabetes education that is comprehensive, and takes into account both the patient and microcosm and macrocosm surrounding the individual.

4.5 Summary and conclusions

The challenges faced to address the rising numbers of cases of prediabetes and diabetes in the US are acute. The human and financial costs are profoundly concerning, so there must be a focus on an intervention that will make a difference and prevent the human cost. These challenges have been brought into sharp focus by the COVID-19 pandemic. As outlined above, the predicted rise in the number of cases needs to be matched by a plan to have initiatives that manage both the significant rise in numbers of instances of prediabetes as well as diabetes.

To have maximal impact, any approach must consider both the individual's needs and the impact of their environment. There is a need to be able to adapt strategies to local needs and optimize local resources. The options around technology have also grown markedly in recent years, and this also provides an exciting opportunity,

tempered perhaps only by the need to recognize that this may vary depending on location and socioeconomic resources.

For the patient with prediabetes, one challenge is to ensure an awareness of the diagnosis, and take action focused on lifestyle changes. It may be that this drive to modify behavior needs to be initiated by a conversation between the primary care provider and patient and then ensuring access to an appropriate program that incorporates aspects of the DPP.

Diabetes self-management education and programs such as the diabetes prevention program are at the heart of this plan. Ensuring a robust approach to providing education to individuals newly diagnosed with diabetes is critical. Education that focuses on the essential areas of medication management, nutrition, monitoring, and physical activity is vital.

It is clear, that partnership between the umbrella organization, provider, and the patient is vital and that the resources required to do this should be self-sustaining.

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CHAPTER 5

Determining factors on active aging in Asia and Oceania: a systematic review

Yuezhong Liu[1], **Rakhi Verma**[2] **and Yin-Leng Theng**[1]
[1]Ageing Research Institute for Society and Education (ARISE), Nanyang Technological University, Singapore
[2]Nanyang Business School, Nanyang Technological University, Singapore

5.1 Introduction

Population aging has significant social, economic and health implications, which strains existing models of social support, social insurance and pension systems and the health sector. On the other hand, it also represents a significant opportunity for society to accumulate more experience, knowledge and wisdom with a long lifetime.[1] Globally, there are more than 700 million people aged 65 and over, with more than 240 million of them living in Asia and Oceania.[2] The Asia and Oceania region represents about 46% of the world's population, which also is one of the largest and fastest-growing older populations in the world.

Meanwhile, healthy living and active aging are about creating the environments and opportunities that enable people to be and do what they value throughout their lives, which emphasizes the need for action across multiple sectors and enables older people to remain a resource to their families, communities and economies.[3] Encouraging physical activity and social participation are the key aspects of healthy active aging, which reduces the risk of a range of ailments, for example, type 2 diabetes, stroke, depression, etc. Countries and areas in the Asia and Oceania region should take related action to prepare for the needs of an aging population. The coronavirus disease 2019 (COVID-19) pandemic has served as a reminder of the importance of digital solutions for the future. One of the important objectives in public health today is to promote and maintain the health and well-being of the rapidly growing aging population. Technology has been viewed as a tool to improve the independence of older adults. In this chapter, we have identified and evaluated major active aging-related technologies that are designed to assist older adults throughout the aging process.

In summary, with a focus on Asia and Oceania, this chapter aims to review the important indicators of active aging digital solutions that motivate older adults towards mindset and behavioral changes in the literature in the last five years. Through keyword frequency analysis, we defined several important determining factors often

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studied by researchers. Next, we will review the promising technologies, programs and digital solutions (e.g., exergaming, wearable and smartphones, etc.) designed to enhance active aging; and recommend ways of advancing research to improve access to effective technologies for older adults.

5.2 Methodology

5.2.1 Population aging in Asia and Oceania

The Asia and Oceania region constitutes: Eastern Asia, Central Asia, Southern Asia, South-Eastern Asia, and Oceania.[2] Table 5.1 shows the Percentage of the Population Aged 60 and Over in Asia and Oceania from 2000 to 2050. Sagaza[4] divided Asia and Oceania into three groups of countries and regions according to the level of population aging. Here, they also categorized them accordingly, with the first group with the most advanced aged areas such as Japan (28%), Australia (15.9%), and New Zealand (16.0%) whose percentage of the older adults aged 65 and over is more than 15% in 2019.[2] The second group consists of Korea (15.1%), Taiwan (15.1%), Thailand (12.4%), Singapore (12.4%), and China (11.5%) whose percentage of the elderly is between 10% and 15%. The third group is the remaining countries in this region whose percentage is less than 10%, such as India (6.4%), Vietnam (7.6%), Indonesia (6.1%), etc.

5.2.2 Data sources and search strategies

The scope of this systematic review focused on studying the determining factors motivating older adults to exercise and engage in social participation through digital solutions in Asia and Oceania. A literature search was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (http://www.prisma-statement.org). We systematically searched the bibliographic databases PubMed and Web of Science from 1 Jan 2016 to 19 Mar 2021. As there were many synonymous terms to "active aging" in both practice and research, this study included all synonyms of active aging in the search strategy referred to in Cosco

Table 5.1 Percentage of the population aged 60 and over in Asia and Oceania from 2000 to 2050.

Country/region	Percentage of the population aged 60 and over (%)			
	2000	2015	2030	2050
East Asia	11.3	16.8	26.2	35.7
Central Asia	8.0	7.8	11.9	18.0
Southern Asia	6.7	8.4	12.0	18.9
South-Eastern Asia	7.4	9.3	14.6	21.0
Oceania	13.4	16.5	20.2	23.3

Table 5.2 Terms used to search relevant literature.

SI	Search terms
1	active aging OR successful aging OR healthy aging OR aging well OR productive aging OR vital aging OR positive aging OR optimal aging
2	1st Practical idea: 1 AND exergaming OR active video games; 2nd Practical idea: 1 AND wearables OR activity trackers; 3rd Practical idea: 1 AND smartphones OR mobile OR mHealth; 4th Practical idea: 1 AND web-based OR internet OR web;

et al.[5] Hence, the specific search strategy included "active aging" along with seven synonyms: "successful aging," "healthy aging," "aging well," "productive aging," "vital aging," "positive aging," and "optimal aging." These phrases were used with both "aging" and "ageing" spelling conventions, "Asia" and "Oceania" as the selected region and linked via the Boolean operator "OR." We connected the mentioned string with four practical ideas using the Boolean operator "AND." The keyword used to search in the titles and abstracts of the articles are listed in Table 5.2.

5.2.3 Study selection

Studies were included if the: (1) interventions were related in full or in part to older adults (around 65 years and above); (2) interventions were intended to promote healthy and active lifestyles in older participants in full or in part; (3) articles recorded some form of outcome measures with or without process measures; (4) articles had at least two measures (pre- and post-test). We excluded studies if they: (1) were reviews/comments; (2) focused on the clinical study (i.e. not via social engagement program); (3) performed prediction analyses or (4) were specific publication types that do not report original scientific research (editorials, letters, legal cases and interviews).

5.2.4 Bias assessment

Following Cochrane's method (Cochrane Collaboration's tool) for assessing the risk of bias, two reviewers were involved in the assessment of the bias of each study.[6] Six individual types of risk of bias were considered: (1) sequence generation, (2) allocation concealment, (3) blinding of participants and personnel, (4) blinding of outcome assessors, (5) incomplete outcome data, and (6) selection outcome reporting. The six domains were given one of three ratings: "Low risk of bias," "Unclear risk of bias" or "High risk of bias." A study was rated as "Low risk of bias" when it received six strong ratings and no weak ratings. A study was rated as moderate if it received one weak rating and less than four strong ratings. Finally, a study was rated weak if it received two or more weak ratings. Study quality was assessed in terms of the reported association.

5.2.5 Data extraction

One paper reviewer performed data extraction, according to a standard protocol, including measures of study design, technology type, demographics, and key findings. Data extraction was appraised by a second reviewer for a random subsample of the included studies. Fig. 5.1 shows the inclusion process of this proposed systematic review.

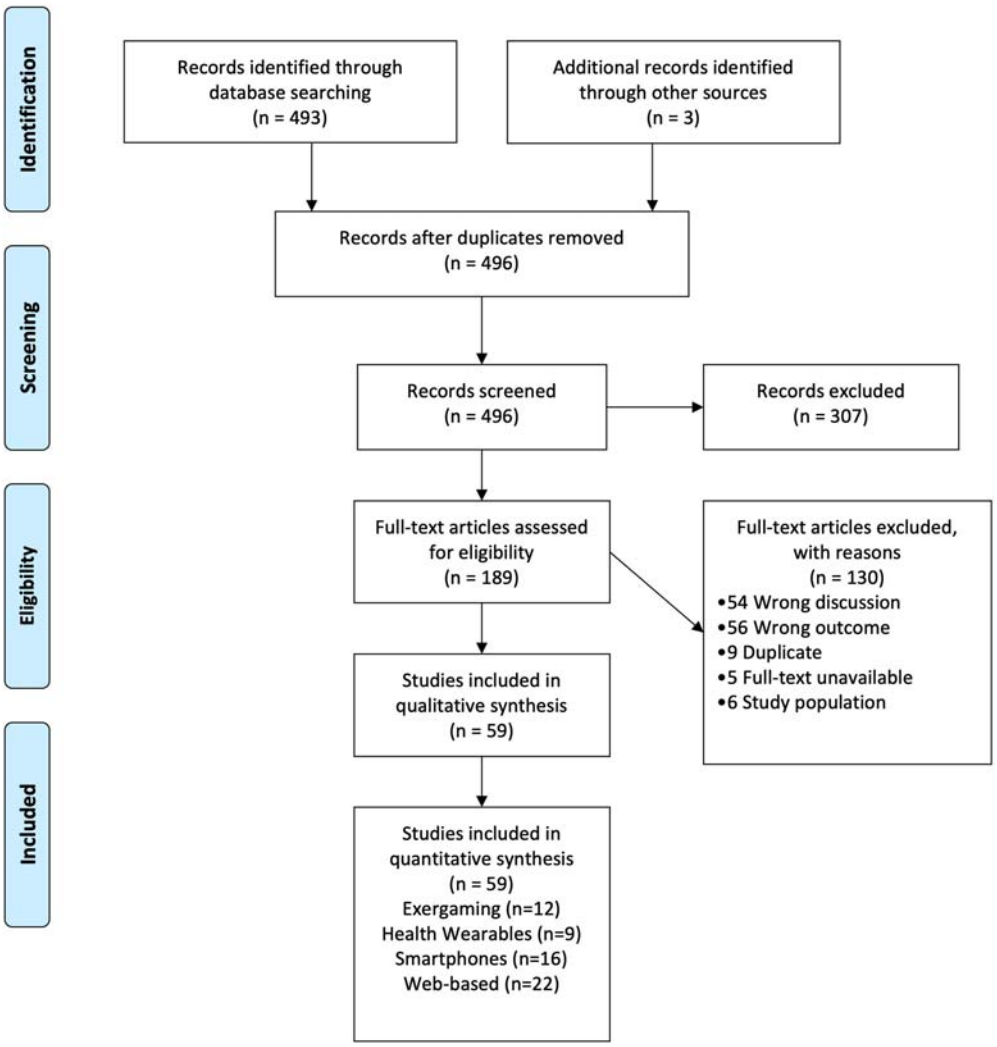

Figure 5.1 An overview of the inclusion process.

5.3 Results

The current selected research presents the ideas for healthy active aging practice and raises questions for future research about reasons older adults do not exercise and whether or not the mindset of behavior change can be sustained. Here are the four practical categories for implementing digital solutions in the Asia and Oceania region to help motivate older adults to partake in physical activity and social participation.[7]

5.3.1 Exergaming/active video games

In general, exergames/active video games benefit older adults, which is a feasible alternative to more traditional aerobic exercise.[8] Table 5.3 lists all the reviewed papers for Exergaming/Active Video Games from 2016 to 2021 in the world. Exergames make it possible for older adults to physically interact via arm, leg or whole-body movements with images onscreen in a variety of activities and sports, including boxing, dancing, tennis, skiing, soccer and walking.[9,14,19,21] Research has focused on how these types of exergames can be used to encourage physical activity and improve wellness and well-being of individuals. Researchers from Taiwan[10,11] reported exergaming benefits in delaying disability and improving frailty status and the frailty phenotype in older adults. The study from Australia[20] evaluated the effectiveness of group-based active video game programs by Nintendo Wii training on physical activity levels. In Singapore, researchers developed an exergaming platform as "International - Singapore Intergenerational Games (I-SING)" encouraging senior citizens to exercise but also enabled intergenerational bonding between youth and old adults through fun and interactive activities.[19]

In summary, exergames/active video games are a new emerging form of exercise that has become popular among older adults in the Asia and Oceania region including Taiwan, Australia and Singapore (Table 5.3). However, findings from the research were not strong enough to warrant recommendations due to the small sample sizes and heterogeneity in the study participants or exergaming platforms. Further research is needed with larger sample sizes and less heterogeneity to adequately explore the true effects of exergaming on the mindset of behavior change of older adults. From the keyword frequency analyses, we identified the technology development determining factors on exergaming for older adults were in the field of physical, cognition and gait.

The reviewed journal articles in this chapter confirmed that exergaming or active video gaming had been used to improve physical activity levels and also can improve the overall physical health of older adults (Table 5.3). Research has shown that exergames or active gaming are a safe, fun, and valuable means of promoting physical activity in older adults. It promotes physical activity engagement and fostering social cohesion with peers, thus mitigating loneliness among older adults.

Table 5.3 Publications related to exergaming/active video games from 2016 to 2021 in the world.

ID	Title and authors	Country/region	Year	Exergames type	Participants size (n)	Age group	Key findings
1	Effects of Exergaming on Cognition and Gait in Older Adults at Risk for Falling.[9]	United States	2020	8-week exergaming program, gait	35	≥ 65	An 8-week exergaming (EG) program for older adults at risk for falls contributed to modest improvements in many cognitive measures and single-task but limited improvements in dual-task gait measures, compared with a traditional physical exercise program.
2	Effects of Kinect-based exergaming on frailty status and physical performance in prefrail and frail elderly: A randomized controlled trial.[10]	Taiwan	2019	12 weeks with 36 exergaming sessions	52	≥ 70	Three out of 5 physical characteristics of the frailty phenotype, namely, weakness, slow walking speed, and low activity level, were significantly reversed by both exergaming and combined exercise.
3	Effects of a hybrid intervention combining exergaming and physical therapy among older adults in a long-term care facility.[11]	Taiwan	2018	12 weeks combining commercial exergaming with physical therapy	17	≥ 65	The hybrid intervention is safe and feasible and could delay disability in older adults.
4	Recalibrating disparities in perceived and actual balance abilities in older adults: A mixed-methods evaluation of a novel exergaming intervention.[12]	United Kingdom	2018	4-week exergaming intervention in which they participated in 8-sessions	26	≥ 70	The results demonstrate that exergaming is possible to recalibrate the perceptions of older adults relating to their balance abilities through targeted, short-term intervention.
5	Assessing dynamic postural control during exergaming in older adults: A probabilistic approach[13]	Netherlands	2018	Six weeks of unsupervised home-exergaming	10	average 77.9	The probabilistic model is suitable for real-time dynamic postural control assessment

6	The effects of functional training, bicycle exercise, and exergaming on walking capacity of elderly patients with Parkinson disease: A pilot randomized controlled single-blinded trial.[14]	Brazil	2017	Eight weeks of exergaming	62	≥ 60	Eight weeks of exergaming can improve the walking capacity of elderly patients with Parkinson disease (PD).
7	Neuropsychological Benefits of neuro-exergaming for older adults: A pilot study of an interactive physical and cognitive exercise system (iPACES).[15]	United States	2017	Neuro-exergaming (physical activity with cognitive training) using an iPACES	30	≥ 50	Results demonstrate feasibility for older adults to use a novel and theoretically-derived neuro-exergame and also provide promising new evidence that neuro-exergaming can yield greater cognitive benefit than either of its component parts.
8	A community-based exergaming physical activity program improves readiness-to-change and self-efficacy among rural-residing older adults.[16]	United States	2017	8-week exergaming program	265	70–89	Results suggest that an exergaming-themed physical activity intervention is effective at increasing physical activity participation and self-efficacy for physical activity among rural-residing older adults.
9	Effects of cycling and exergaming on neurotrophic factors in elderly type 2 diabetic men—a preliminary investigation.[17]	Germany	2017	30-min submaximal cycling with those of exergaming	8	average 71	Acute exercise can increase neurotrophic factors (BDNF, VEGF) in elderly T2DM patients, depending on exercise mode.
10	Moving real exergaming engines on the web: The webFitForAll case study in an active and healthy aging living lab environment.[18]	Greece	2017	FitForAll web exergaming platform	116	N/A	The combines a unified solution for input devices such as Ms Kinect and Wii Balance Board which may seamlessly be exploited through standard physical exercise protocols (American College of Sports Medicine guidelines) and accommodate high detail logging

(*Continued*)

Table 5.3 (Continued)

ID	Title and authors	Country/region	Year	Exergames type	Participants size (n)	Age group	Key findings
11	Improving Psychosocial well-being of older adults through exergaming: the moderation effects of intergenerational communication and age cohorts.[19]	Singapore	2016	Three Kinect exergames sessions were held on every other day within 1 week.	122	average 75	There was a significant decline in social anxiousness and an increase in sociability for young-old participants playing with youths. The sociability improved significantly for old-old participants playing with their peers. There was also a significant decrease in loneliness after exergaming, but little differences were found across different play types or age groups.
12	Investigating innovative means of prompting activity uptake in older adults with type 2 diabetes: A feasibility study of exergaming.[20]	Australia	2016	4 weeks of group Nintendo Wii training	11	N/A	Significant effects occurred on moderate physical activity time, total physical activity, dominant handgrip strength, non-dominant handgrip strength, 30-second chair stand and 400 m walk test. No effect was observed for BMI, quality of life, or balance.

5.3.2 Health wearables and activity trackers

Health wearables and activity trackers offer the opportunity to increase physical activity through continuous monitoring.[22] These devices are instruments generally worn on either the trunk or limbs that measures duration, frequency and intensity of physical activity. The gathered values are recorded and then downloaded to a computer for analysis and interpretation. The primary advantages of activity trackers are that they are small, lightweight and are usually not cumbersome. Moreover, physical activity data can be recorded over prolonged periods, ranging from several days to even weeks.[23,24] In Australia, Alharbi et al.[25] identified that wearable trackers encouraged users to increase their daily level of physical activity and decrease waist circumference, facilitating atrial fibrillation diagnoses and predicting length of stay. Redfern[26] reviewed a gait assessment through the use of wearables and highlighted the need for algorithms to measure it, culminating in the ability to better detect and classify falls. Li et al.[27] from Hong Kong investigated the factors that contribute to the acceptance of such smart wearable models for older adults. They concluded that the perceived usefulness, compatibility, facilitating conditions, and self-reported health status significantly and positively affected older adults' intention to use such technologies. Table 5.4 lists all the reviewed papers for health wearables and activity trackers from 2016 to 2021 in the world.

There were two publications from Asia and Oceania regions, which are from China and Hong Kong (Table 5.4). Studies have shown that older adults identify motivations such as monitoring activity, improving health, receiving social support, engaging in competition, and losing weight as reasons to start using wearable devices. Research on investigating the use of wearable devices and activity trackers is still emerging. User-centered design processes for development and training programs can help older adults engage with digital health technology, however, more robust research is needed to understand its benefits.

In the meantime, there are also many products and initiatives from regional companies or governments to encourage physical activity and social participation for older adults, such as Sony's wearables from Japan including smartwatches, activity trackers, and VR headsets; Samsung's wearable portfolio from South Korea, Xiaomi from China and initiatives like "The National Steps Challenge" in Singapore. Health wearables and activity trackers show the possible results of motivating physical activity of older adults. However, the reasons for older adults who did not join were still unclear. Older adults are not a homogeneous group, future study needs to explore the perception and adoption of wearables by older adults.

5.3.3 Smartphones

The technology of mobile phones has changed dramatically over the past few years. Mobile technology has spread rapidly around the globe. Today, it is estimated that

Table 5.4 Publications related to health wearables and activity trackers from 2016 to 2021 in the world.

ID	Title	Country/region	Year	Wearable type	Participants size (*n*)	Age group	Key findings
1	A social group-based information-motivation-behavior skill intervention to promote acceptability and adoption of wearable activity trackers among middle-aged and older adults: Cluster randomized controlled trial.[28]	China	2020	7-month wrist-worn activity trackers for acceptability and adoption	149	≥ 45	Higher adoption was also observed among participants in the intervention arm, who were twice as likely to have valid daily step account data than their controlled counterparts
2	How are wearable activity trackers adopted in older adults? comparison between subjective adoption attitudes and physical activity performance.[29]	United States	2020	3-month activity trackers	16	65−77	Considering the accuracy of the activity tracker and older adults' athletic ability, moderate and vigorous physical activity times are more likely to be a reliable measure of older adults' long-term use and successful adoption of activity trackers than step counts.
3	Toward using wearables to remotely monitor cognitive frailty in community-living older adults: An observational study.[30]	United States	2020	A pendant sensor to monitor daily physical activities and sleep for 48-h	163	average 75	Results support remote patient monitoring using wearables to determine cognitive frailty.

4	The use of wearable activity trackers among older adults: Focus group study of tracker perceptions, motivators, and barriers in the maintenance stage of behavior change.[22]	United States	2019	Focus group study of activity trackers	48	≥ 65	The activity trackers may be an effective technology to encourage physical activity among older adults, especially those who have never tried it
5	Accuracy of consumer-level and research-grade activity trackers in ambulatory settings in older adults.[31]	Ireland	2019	Six trackers (Fitbit Charge2, Garmin VivoSmart HR + , Philips Health Watch, Withings Pulse Ox, ActiGraph GT9X-BT, Omron HJ-72OITC) for estimating: Steps, traveled distance, and heart-rate measurements	18	65−74	Every tracker showed a decreasing accuracy with slower walking speed, which resulted in a significant step under-counting.
6	Health monitoring through wearable technologies for older adults: Smart wearables acceptance model.[27]	Hong Kong	2019	Survey samples and feedback from activity trackers	146	≥ 60	The results indicated that perceived usefulness, compatibility, facilitating conditions, and self-reported health status significantly and positively affect older adults' intention to use such technologies.
7	Motor planning error: Toward measuring cognitive frailty in older adults using wearables.[32]	United States	2018	an interactive instrumented trail-making task (iTMT) platform	32	≥ 65	The motor planning error could be estimated from a low-cost and simple wearable platform, is able to simultaneously determine cognitive and motor impairments

(*Continued*)

Table 5.4 (Continued)

ID	Title	Country/region	Year	Wearable type	Participants size (*n*)	Age group	Key findings
8	Wearables to support self-management of older adults with chronic diseases: A qualitative study from the perspectives of patients and physicians.[33]	Germany	2018	A semi-structured questionnaire design	14	N/A	The usage of wearable devices could have a positive effect on the course of the disease; however, personality and environmental factors should be taken into account to individually adjust and support the usage of wearable devices.
9	Older Adults' Acceptance of Activity Trackers[34]	United States	2017	28-day field study	16	65−75	The heuristic evaluation revealed usability barriers in system status visibility, error prevention, consistency and standards. The field study revealed additional barriers (e.g., accuracy, format) and acceptance-facilitators (e.g., goal tracking, usefulness, encouragement).

more than five billion people have mobile devices, and over half of these connections are smartphones.[35] Given the rapid growth of mobile phones, their potential as a platform for improving the health of older adults should be explored and there is much research that has been conducted across Asia and Oceania regions (Table 5.5). In Korea, Kwak et al.[52] developed a healthcare application for the elderly who suspect or know they have a hearing loss, named, the Hearing Rehabilitation for Older Adults (HeRO), which is available in a mobile device and used to confirm the probability of acceptance among elderly users. Koo and Vizer[53] described research on mobile technologies that support people with dementia to (1) perform daily activities, (2) maintain social interaction, (3) aid memory, (4) engage in leisure activities, (5) track location, and (6) monitor health. Hoque and Sorwar[54] from Bangladesh developed a theoretical model based on the Unified Theory of Acceptance and Use of Technology and tested it for determining the key factors influencing elderly users' intention to adopt and use the mHealth services. In China, Lin et al.[55] revealed that mobile devices had a significant association with the cognitive function and depressive symptoms of older adults living in residential care homes (RCHs), and thus should be encouraged as a measure to maintain and improve cognition and prevent depression. Zhong and Rau[38] pointed out that a phone app is a health management tool for older adults to self-manage their gait quality and prevent adverse outcomes. Huang et al.[56] from Singapore showed that applications could play an important role in complementing multifaceted diabetes care, but should preferably be regulated, context-specific, and more tailored to users' needs with clear guidance for patients and clinicians about the choices. Ahmad et al.[57] from Malaysia reviewed older adults' willingness, perceived barriers and motivators towards the use of mobile apps to monitor and manage their health.

Hence, in Asia and Oceania region, smartphones were widely available to test and validate how to help older adults in terms of self-management and lifestyle. There were two publications from Korea and Taiwan separately, and one publication from China (Table 5.5). It had been established that the rate of smartphone ownership declined with age. The keyword frequency analyses indicated that smartphone applications used for the older population could help with the engagement with physical activity and reducing frailty (speech recognition, fitness, food, etc.). Smartphones could assist older adults in various aspects of their lives, such as medication adherence, lifelong learning, and survival. The current proliferation of smartphones has resulted in widespread development and availability of digital health interventions with the potential to mitigate many health risks such as cardiovascular disease. Studies demonstrated the effectiveness of text messaging and applications in improving health outcomes as well.

However, less research had been conducted on behavioral changes of older adults. In the future, it would be important to take psychosocial factors such as social support and region into consideration while designing a mobile phone-based physical activity

Table 5.5 Publications related to smartphones from 2016 to 2021 in the world.

ID	Title	Country/region	Year	Smartphones type	Participants size (*n*)	Age group	Key findings
1	Design and usability evaluation of mobile voice-added food reporting for elderly people: Randomized controlled trial.[36]	Taiwan	2020	Two innovative mobile voice-added apps for food intake reporting, namely voice-only reporting (VOR) and voice-button reporting (VBR)	57	≥ 60	As voice-only reporting (VOR) outperformed and voice-button reporting VBR, it suggested that voice-only food input reporting is preferable for elderly users.
2	User-dependent usability and feasibility of a swallowing training mHealth app for older adults: Mixed-methods pilot study.[37]	Korea	2020	Swallowing training apps	11	average 75.7	Qualitative analyses via semi-structured interviews yielded promising outcomes regarding app acceptability, training program utilization, emotional responses, and learning. experience.
3	A mobile phone-based gait assessment app for the elderly: Development and evaluation.[38]	China	2020	A gait-monitoring mobile phone app	148	≥ 60	Older adults identified improvements such as larger font size, the inclusion of reference values for gait parameters, and inclusion of heart rate and blood pressure monitoring.

4	Mobile computing technologies for health and mobility assessment: Research design and results of the timed up and go test in older adults.[39]	Portugal	2020	XIAOMI MI 6	40	≥ 60	These timed-up and go test parameters could be automatically and reliably detected with a mobile device. Moreover, we identified that the time to perform the timed-up and go test increases with age and the presence of diseases related to locomotion.
5	m-SFT: A novel mobile health system to assess the elderly physical condition.[40]	United Kingdom	2020	A comparative study using mobile Senior fitness test (m-SFT) for health evaluation	7	53–61	The m-SFT is a reliable and easy-to-use mHealth system for the evaluation of the elderly's physical condition, and is also useful in intervention programs to follow-up with the patient's evolution.
6	Effect of an mHealth wheelchair skills training program for older adults: A feasibility randomized controlled trial.[41]	Canada	2019	A 4-week monitored home training program with a computer tablet	18	≥ 50	Enhancing participation in the community by improving wheelchair skills participants demonstrated good program adherence and clinical benefits were evident in community participation and wheelchair self-efficacy.

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Table 5.5 (Continued)

ID	Title	Country/region	Year	Smartphones type	Participants size (*n*)	Age group	Key findings
7	Mobile geriatric teams - a cost-effective way of improving patient safety and reducing traditional healthcare utilization among the frail elderly? A randomized controlled trial.[42]	Sweden	2019	Mixed methods to measure the effectiveness and user satisfaction of mobile geriatric team	62	average 84	The Mobile Geriatric Team initiative was clearly appreciated but did not fully achieve the desired reduction in healthcare utilization in this study.
8	Trainer in a pocket—proof-of-concept of mobile, real-time, foot kinematics feedback for gait pattern normalization in individuals after stroke, incomplete spinal cord injury and elderly patients.[43]	Germany	2018	Four weeks post-training a follow-up visit was performed. Visits started with an initial gait analysis (iGA) without feedback, followed by 5 feedback training sessions of 2−3 min and gait analysis at the end.	15	≥ 65	Mobile, real-time, verbalized feedback is feasible and results in a normalization of the feedback gait parameter.
9	Evaluating authentication options for mobile health applications in younger and older adults.[44]	Canada	2018		102	18−30 years (n = 59) 50 years (n = 43)	On mobile devices, PIN and pattern-lock outperformed graphical passwords and swipe-style fingerprints.

10	Use of speech analyses within a mobile application for the assessment of cognitive impairment in elderly people.[45]	France	2018	A mobile application while performing several short vocal cognitive tasks during a regular consultation	165	average 76.2	The potential value of vocal analytics and the use of a mobile application for accurate automatic differentiation between SCI, MCI and AD. This tool can provide the clinician with meaningful information for assessment and monitoring of people with MCI and AD based on a noninvasive, simple and low-cost method.
11	Efficacy of bingocize: A game-centered mobile application to improve physical and cognitive performance in older adults.[46]	United States	2018	Using the app for 1 hour, twice per week, for 10 weeks	85	N/A	The fun and interactive nature of Bingocize engender high levels of adherence to a health-promoting program in a difficult to serve population, serving as a conduit to potentially improve multiple aspects of quality of life for older adults.
12	Speech perception enhancement in elderly hearing aid users using an auditory training program for mobile devices.[47]	Korea	2017	An auditory 4-week training program using a mobile device	20	average 75.6	This result pattern suggests that a moderate amount of auditory training using the mobile device with cost-effective and minimal supervision is useful when it is used to improve the speech understanding of older adults with hearing loss

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Table 5.5 (Continued)

ID	Title	Country/region	Year	Smartphones type	Participants size (*n*)	Age group	Key findings
13	iTrack: Instrumented mobile electrooculography (EOG) eye-tracking in older adults and Parkinson's disease.[48]	United Kingdom	2017	Wireless mobile EOG; Head-mounted Dikablis mobile eye-tracker; IR used a dual-camera system	20	≥ 50	The developed algorithm for saccade detection and measurement in EOG signal can detect saccades in EOG data and agreed well with video inspection and IR algorithm output during static testing.
14	Evaluate the usability of the mobile instant messaging software in the elderly.[49]	Taiwan	2017	Instant messaging (LINE app)	41	≥ 50	The positive acceptance of LINE APP in the elderly refers to the probable similar acceptance for them to use other communication software.
15	Effects of three motivationally targeted mobile device applications on initial physical activity and sedentary behavior change in midlife and older adults: A randomized trial.[50]	United States	2016	An initial 8-week evaluation of three different customized physical activity-sedentary behavior apps	95	≥ 45	The results provide initial support for the use of a smartphone-delivered social frame in the early induction of both physical activity and sedentary behavior changes.

16	A mobile application improves therapy-adherence rates in elderly patients undergoing rehabilitation: A crossover design study comparing documentation via iPad with paper-based control.[51]	Germany	2016	A mobile application Medication Plan, installed on an Apple iPad	24	average 73.8	A mobile app for medication adherence increased objectively and subjectively measured adherence in elderly users undergoing rehabilitation. The findings have promising clinical implications: Digital tools can assist chronic disease patients to achieve adherence to medication and blood pressure measurement.

motivation app. The mindset feedback of the participants would help to design more effective smartphone apps.

5.3.4 Web-based programs

There are more than 2.3 billion internet users, which equates to 50.3% of the world's internet users being within the Asia and Oceania region.[58] Internet users are becoming more representative of the overall population, including older adults. Internet-based interventions that incorporate professional and social support, and provide instructions to change behavior and problem solve in an interactive manner appear to be effective in that they provide a solution to many of the traditional barriers to exercise, including lack of time or money.[59] The Internet is also an important source of health- and fitness-related information for more than half of all users.[60] From China, Chen et al.[61] investigated the knowledge, perceived beliefs, and preventive behaviors towards COVID-19 of older adults in China and determined the factors that influence their practice of preventive behaviors. Chiu[62] showed that older adults with different internet behaviors were associated with distinct sociodemographic and social engagement behaviors in Taiwan. Jo et al.[63] indicated that the elderly acknowledged its necessity and showed a high level of willingness after acquiring enough awareness about the integrated smart home system's benefits. From Australia and New Zealand, research investigated whether an educational training course on using the internet and touchscreen technology (TT) would decrease social isolation[64] and highlighted that internet use can support older adults' well-being.[65] In Singapore, Cao et al.[66] evaluated that online health coaching could provide older adults with knowledge of nutrition and exercise, raised their awareness of well-being in terms of daily meals and regular exercise, and provided an alternative to maintain a healthy lifestyle amidst the global pandemic. Table 5.6 shows the selected publications in the world.

The web-based programs became more popular after the COVID-19 pandemic in Asia and Oceania region. Table 5.6 shows the web-based related research projects from Australia, China, Malaysia and Taiwan. However, it was still too early to conclude the effectiveness of the web-based programs for older adults with limited participants and sampling. The COVID-19 pandemic also gave opportunities for further investigations on internet use and web-based programs to improve healthy active aging. Web-based might be a preferred method for delivering interventions because of its accessibility, especially for older adults, among whom internet usage had been increasing rapidly in recent years. Selected studies showed that online programs had the potential to positively impact the physical activity of sedentary older adult participants. The top three determining factors on the usage of web-based programs for active aging are cognition, physical activities, depression.

Table 5.6 Publications related to smartphones from 2016 to 2021 in the world.

ID	Title	Country/region	Year	Web-based type	Participants Size (*n*)	Age group	Key findings
1	Comparing web-based and classroom-based memory training for older adults: The ACTIVE memory works study.[67]	United States	2021	5−6 weeks web-based and classroom-based memory training, 10 sessions	208	Average 71.1	No significant training effects were found. Demonstrated that a web-based platform is an acceptable and feasible mode to provide memory training to healthy older adults. Further studies are needed to investigate the potential of web-based memory training programs for improving cognition and function in cognitively healthy older adults.
2	A pilot study of health coaching on older adults' personal healthcare and maintenance during the outbreak of COVID-19 in Singapore.[66]	Singapore	2021	online health coaching intervention, training, consisted of four modules (introduction to gerontology, nutrition for older adults, exercise and fitness, and health coaching basics)	18	≥ 55	The health coaching provided older adults with knowledge of nutrition and exercise, raised their awareness of well-being in terms of daily meals and regular exercise, and provided an alternative to maintain a healthy lifestyle amidst the global pandemic.

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Table 5.6 (Continued)

ID	Title	Country/region	Year	Web-based type	Participants Size (*n*)	Age group	Key findings
3	Usability, acceptability, and effectiveness of web-based conversational agents to facilitate problem solving in older adults: Controlled study.[68]	United Kingdom	2021	Automated chatbot interventions	112	Average 69.21	Participants in the 'method of levels approach chatbot' group spend significantly longer time interacting, having a lower problem with distress than humanistic counseling approach/ chatbot.
4	Web-based exercise versus supervised exercise for decreasing visceral adipose tissue in older adults with central obesity: A randomized controlled trial.[69]	Sweden	2020	10 weeks of supervised exercise (SE) and 10 weeks of web-based exercise (WE)	77	≥ 70	Ten weeks of vigorous WE is insufficient to decrease VAT in centrally obese older adults but sufficient to decrease FM while preserving lean body mass (LBM).
5	Knowledge, perceived beliefs, and preventive behaviors related to COVID-19 among Chinese older adults: Cross-sectional web-based survey.[61]	China	2020	a cross-sectional, web-based survey was administered to Chinese older adults	1051	≥ 60	Most older residents had adequate knowledge and positive beliefs regarding COVID-19 and engaged in proactive behaviors to prevent the disease.

6	Participation, retention, and utilization of a web-based chronic disease self-management intervention among older adults.[70]	United States	2019	12 month Web-based self-management (web-based SM) interventions	462	Average 59.43 (range 34 to 76)	Though older adults (>60 of age) were less likely to choose to participate ($F = 57.20$, $P < .001$), a slight majority of participants in the experiment (53%) were over 66 years of age. Enrolled older adults utilized website management tools at a rate equivalent to younger participants.
7	Evaluation of a guided internet-based self-help intervention for older adults after spousal bereavement or separation/ divorce: A randomised controlled trial.[71]	Switzerland	2019	3-month a guided internet-based self-help intervention for prolonged grief symptoms after spousal bereavement or separation/divorce compared to a wait-list control group	110	Average 51	An internet intervention based on models for coping with grief after bereavement was not only beneficial for widowed but also separated or divorced participants
8	Healthy aging through internet counseling in the elderly (HATICE): A multinational, randomised controlled trial.[72]	Netherlands, Finland and France	2019	18 months with an interactive internet intervention stimulating coach-supported self-management or a control platform	2724	≥65	Coach-supported self-management of cardiovascular risk factors using an interactive internet intervention is feasible in an older population and leads to a modest improvement of cardiovascular risk profile.

(*Continued*)

Table 5.6 (Continued)

ID	Title	Country/region	Year	Web-based type	Participants Size (*n*)	Age group	Key findings
9	The effectiveness of a web-based health education tool, WESIHAT 2.0, among older adults: A randomized controlled trial.[73]	Malaysia	2019	The web-based application, WESIHAT 2.0, for improving cognitive function, physical fitness, biochemical indices, and psychosocial variables among older adults	150	≥ 60	Significant intervention effects were observed for self-perception of disability and informational support scores.
10	A randomized controlled trial of internet-delivered cognitive behavior therapy to prevent the development of depressive disorders in older adults with multimorbidity.[74]	Australia	2019	Internet-delivered cognitive-behaviour therapy (iCBT)	302	≥ 65	These results indicate that depressive disorder was prevented in the first six months following iCBT with three times the number of cases of depressive disorder in the control group compared to the treatment group.
11	Efficacy of an individually tailored, internet-mediated physical activity intervention in older adults: A randomized controlled trial.[75]	United States	2019	A 12-week Internet-mediated physical activity intervention for increasing walking behavior in inactive older adults	170	≥ 55	Individually tailored, Internet-mediated PA interventions are an effective way to significantly increase physical activity in older adults.

12	Relationship Between Internet Behaviors and social engagement in middle-aged and older adults in Taiwan.[62]	Taiwan	2019	Telephonic interview data of older internet users from two urban and two rural areas were analyzed.	248	N.A.	Older adults with different internet behaviors were associated with distinct sociodemographic and social engagement behaviors
13	Designing an Internet-Based multidomain intervention for the prevention of cardiovascular disease and cognitive impairment in older adults: The HATICE trial.[76]	3 European countries (Finland, France, and The Netherlands)	2018	18 months multi-domain intervention through a coach-supported, interactive, platform	2725	65 +	Despite differences in cardiovascular disease (CVR) management within the countries considered, it was possible to design and implement the HATICE multidomain intervention. The study can help define preventative strategies for dementia and CVD that are applicable internationally.
14	A web-based multidomain lifestyle intervention with connected devices for older adults: Research protocol of the eMIND pilot randomized controlled trial.[77]	France	2018	A randomized controlled trial (RCT) using a web-based multidomain intervention for older adults	120	≥ 65	There is a high amount of adherers (i.e., >75% compliance to the protocol) to reflect the feasibility.

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Table 5.6 (Continued)

ID	Title	Country/region	Year	Web-based type	Participants Size (*n*)	Age group	Key findings
15	The effectiveness of a web 2.0 physical activity intervention in older adults—a randomised controlled trial.[78]	Australia	2018	Either a paper logbook ($n = 171$), a Web 1.0 ($n = 165$) or a Web 2.0 ($n = 168$) physical activity intervention	504	< 55 ($n = 299$) ≥ 55 ($n = 205$)	Results partially support the use of Web 2.0 features to improve adults over 55s' engagement in and behavior changes from web-based physical activity interventions.
16	Internet cognitive-behavioral therapy for depression in older adults with knee osteoarthritis: A randomized controlled trial.[79]	Australia	2018	A 10-week internet-based cognitive-behavioral therapy (iCBT) program	69	≥ 50	Results support the efficacy of an iCBT program (requiring no face-to-face contact) for depression in individuals with comorbid depression and Osteoarthritis of the knee.
17	Individually tailored internet-based cognitive behavior therapy for older adults with anxiety and depression: A randomised controlled trial.[80]	United States	2018	8-week internet support cognitive behavior therapy (ICBT) program	79	> 60	We conclude that guided, tailored ICBT may be effective for some older adults and that the role of cognitive function needs to be investigated further.
18	Development and evaluation of two web-based interventions for the promotion of physical activity in	Germany	2017	A web-based intervention for 10 weeks allowing them to track their weekly physical activity	228	≥ 65	This study will provide answers regarding the acceptance and effectiveness of web-based interventions promoting uptake and

	older adults: Study protocol for a community-based controlled intervention trial.[81]						maintenance of regular physical activity in persons aged 65–75 years.
19	Development and validation of an interactive internet platform for older people: The healthy aging through internet counseling in the elderly study.[82]	Netherlands	2017	8-week with the interactive internet platform	41	≥ 65	Participants used the interactive features of the platform and appreciated the coaching support.
20	Internet-based vestibular rehabilitation for older adults with chronic dizziness: A randomized controlled trial in primary care.[83]	United Kingdom	2017	A single-center, single-blind randomized controlled trial comparing an Internet-based vestibular rehabilitation intervention (Balance Retraining) with usual primary care in patients	296	Average 67	Internet-based vestibular rehabilitation reduces dizziness and dizziness-related disability in older primary care patients without requiring clinical support.

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Table 5.6 (Continued)

ID	Title	Country/region	Year	Web-based type	Participants Size (*n*)	Age group	Key findings
21	Cognitive cooperation groups mediated by computers and the internet present significant improvement of cognitive status in older adults with memory complaints: A controlled prospective study.[84]	Brasil	2017	A prospective controlled intervention study carried out to estimate the effect of participating in cognitive cooperation groups, mediated by computers and the internet, on the mini-mental state examination per cent variation of outpatients	293	≥ 60	The results suggested that cognitive cooperation groups, mediated by computers and the internet, are associated with cognitive status improvement of older adults in memory clinics
22	An internet-based physical activity intervention to improve quality of life of inactive older adults: A randomized controlled trial[85]	Netherlands	2016	A 3-month Internet program-DirectLife (Philips)-aimed at increasing physical activity using monitoring and feedback by accelerometry and feedback by digital coaching	235	≥ 60	This study shows that an internet-based physical activity program was effective in improving quality of life in 60–70-year-olds after 3 months, particularly in participants that reached their individually targeted increase in daily physical activity.

5.4 Discussion and concluding remarks

Technological devices and systems such as exergaming/Active Video Games, Health Wearables and Activity Trackers, Smartphones, and Web-based Programs hold considerable promise in assisting a growing older population to age in place. These technologies alleviate pain points of traditional healthcare systems, with the potential to increase access to healthcare significantly, while allowing seniors to age in place and receive long-term care in community-based settings. The motivation of participating in these digital solutions in the pre-implementation stage is influenced by multiple factors, like perceived usefulness, compatibility, facilitating conditions and self-reported health status. However, post-implementation research on the mindset of behavior change by community-dwelling older adults is low in recent years. Further research is needed to investigate if and how these digital solutions and determining factors in this review are interrelated for active aging, and how they also relate to a sustainable engagement and mindsets of behavior changes for older adults in the Asia and Oceania region.

The usefulness of active aging gadgets majorly depends on the user's functional, social and behavioral characteristics as well as their ability to adapt to their needs and preferences. As described, exergaming improves physical activity levels mainly focusing on the use scenarios of cognition, walking gait, and falling. In the meantime, the major health wearables are designed to address cognition, motor skills, and monitoring. Smartphones help with speech recognition, fitness and food. And web-based program aims to improve cognition, depression and education. In summary, the requirements from cognition, frailty (walking, monitoring and motor) will be the main determining factors for active aging in Asia and Oceania in the following years. The design of such active aging devices or platforms should be directed to suit these factors for older adults. Hence, we propose enhanced and active research by multidisciplinary teams of healthcare personnel, end-user patients, caregivers, psychologists, social workers, architects and designers, from the first phase of concept development to the final stages of product development.

There are several limitations with active aging technologies research and usage. First, many technologies are in the early stages of development and have only been tested in small, nonrepresentative samples. Second, a multidisciplinary approach is very critical for product development for the needs of the aging population. The research teams typically have expertise in product development and marketing; but, in most cases, they do not have the experience, resources, or motivation to conduct clinical or community-based trials. Third, since there is not much understanding of the sustainability and scaling of technology-enabled programs, the evaluation remains incomplete. Fourth, government and private funding for research development and testing are limited across Asia-Oceania and worldwide. Some of the recommendations include

further research on a clear understanding of the concepts of active aging and the magnitude of its associations with improvement among older adults. Researchers and policymakers need to understand how active aging using technology can intervene to improve the health of an aging population. Along with the aging technologies development, the government can also initiate lifelong learning programs for older adults to facilitate the pervasive adoption of digital and smart technologies for increasing their active aging level. These policies should be integrated into older adult healthcare and easy access for assistive devices. There should be the promotion of healthcare measures such as availability of an exercise center, recreation center, rehabilitation center and facilities for an annual health check-up. Implementation of technology as assisting and monitoring health would help increase active aging among older adults. In turn, it will be helpful with the independent living of aging people and thus increasing the quality of life of the aging population.

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CHAPTER 6

Healthy living and active aging in Latin America and the Caribbean countries: biological, demographic, and epidemiological challenges

João M. Coelho-Filho[1], Reinaldo B. Oriá[2], Karla C. Giacomin[3], Gerly A.C. Brito[4], Marcos Santos[5], Veronica Aran[6], Manoela Heringer[6], Deiziane V.S. Costa[4,7], Ronaldo P. Dias[2] and Vivaldo M. Neto[6]

[1]Department of Clinical Medicine, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil
[2]Laboratory of Biology of Tissue Healing, Ontogeny and Nutrition, Department of Morphology, Center of Biomedicine, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil
[3]International Longevity Centre, Brazil. Centre for Studies in Public Health and Aging, Oswaldo Cruz Foundation, Belo Horizonte, MG, Brazil
[4]Department of Morphology, Faculty of Medicine, Federal University of Ceará, Fortaleza, CE, Brazil
[5]Department of Dental Prosthesis and Materials, School of Dentistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil
[6]Paulo Niemeyer State Brain Institute of the Health Secretariat of the State of Rio de Janeiro, Rio de Janeiro, Brazil
[7]Division of Infectious Diseases and International Health, University of Virginia, Charlottesville, VA, United States

6.1 Introduction

Aging is defined as a dynamic event influenced by biological, physiological, psychological, environmental, behavioral, and social processes intermingled.[1] It can also be considered an age-dependent or age-progressive decline in physiological function, resulting in elevated mortality rate and reduced reproductive function.[2]

The central challenge faced by societies with a rising number of older people is ensuring that individuals grow older actively, socially engaged, and free of disability during most of their lives, characterizing the so-called active aging, which is often used interchangeably with terms such as "healthy," "successful," or "productive aging." The WHO defines active aging as the process of optimizing health, participation, and security opportunities to improve quality of life as people age. The concept of active aging includes six groups of determinants: (1) health and social services (e.g., health promotion and disease prevention, health services, continuous care and mental health care); (2) behavioral (e.g., smoking, physical activity, food intake, oral health, alcohol and medication use); (3) personal (e.g., biological, genetic and psychological factors); (4) physical environment (e.g., friendly environment, safe house, fall prevention, absence of pollution); (5) social (e.g., social support, violence and abuse prevention, education); (6) economic (e.g., wage, social security, work), embedded in a cultural and gender context.[3]

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Even though there is a lack of consensus regarding the definition of healthy aging,[4] the scientific community has tried to identify beneficial factors that enable individuals to age in a physically and mentally healthy way. Several modifiable factors could reduce premature death, prevent morbidity and disability, and improve quality of life and well-being, hence contributing to the likelihood of healthy aging. Compression of morbidity (a reduction over time in the total lifetime days of disability) in old age could be achieved by successful interventions early in life, as many disabilities are the result of the accumulation of a hazardous lifestyle.[5]

High-income countries show some evidence that compression of morbidity is taking place, as noted from trends of functioning and disability status. However, low-income and middle-income countries currently have no reliable evidence of compression, and morbidity might even be expanding, driven by lifestyle risk factors and the increasing prevalence of chronic diseases. The WHO proposed a public health framework for healthy aging across the course of life, which involves developing strategies for health services, long-term care, and environments.[6] This framework also suggests that before shaping policies, continued quantitative/qualitative assessments of healthy aging to help identify the elderly's prioritized health needs are essential. Moreover, to address the challenges of a global aging population, WHO has fostered strategies to encourage the development of community active aging initiatives. According to their key components (i.e., policies, services, and structures related to the communities' physical and social environments), they should be designed to be age-friendly and help all aging adults to live safely, enjoy good health and stay engaged in their communities.[7]

Lu, Pickart, and Sacker[8] conducted a literature review of domains and measures of healthy aging in epidemiological studies. Fifty articles were selected for analysis, with the main outcome being healthy, successful, positive, or active aging. According to their results, psychosocial components are as important as biological ones in healthy aging. Population aging has an impact on insurance, health, and care services. However, evidence on the specific health status of older people is particularly valuable for resource-constrained settings where policymakers are faced with limited budgets and balancing multiple pressing priorities, as in Latin America and the Caribbean States (LAC).

Although the proportion of elderly people is higher in developed countries compared to developing countries, the proportion of older people is increasing globally, but especially in the latter, causing a demographic shift. By 2050, it is expected that 20% of people living in developing countries will be over 60 years of age.[9] The projections indicate that the number of people aged 65 or over will continue to rise in the coming decades, moving from 703 million in 2019 to 1.5 billion in 2050 around the world. The fastest increase of the older population, occurring between 2019 and 2050, is projected to happen in less developed countries.[10] The percentage of the

population aged 65 years or over almost doubled from 6% in 1990 to 11% in 2019 in Eastern and South-Eastern Asia, and from 5% in 1990 to 9% in 2019 in LAC countries. Between 2019 and 2050, the proportion of older individuals in the overall population is projected to at least double in that region.[11,12] The main causes for this shift are a better quality of life, good health care, improvements in preventive medicine, and a decline in fertility rates.[9] The demographic shift will generate big changes in society, including the rise in both public health costs and government spending.

This chapter aims to discuss from a multidisciplinary perspective, the aging process in LAC countries toward the sense of healthy aging, considering social, biological, and epidemiological characteristics of their population. Innovative strategies conducted in LAC countries aimed to promote active aging are also discussed.

6.2 Demographic and epidemiological changes in the Latin America and the Caribbean countries

In 2015, the population of the Americas totaled 992.2 million, which is 13.5% of the total world population: 357.8 million people were living in North America and 634.4 million in LAC (36.1% and 63.9% of the total, respectively). At 207.8 million, Brazil's population accounted for 20.9% of the LAC total; the Andean Area's 137.6 million accounted for 13.9%; Mexico's population of 127 million represented 12.8% of LAC; the 71.4 million in the Southern Cone accounted for 7.2% of the LAC; the 45.7 million in Central America made up 4.6% of the LAC population; and the 44.7 million living in the Caribbean represented 4.5% of the LAC population.[13]

LAC is an extremely diverse region, formed by 33 countries and 15 dependent territories, varying greatly in size and population. Brazil is the largest country, both in territory and population, with 212 million inhabitants. The Federation of Saint Kitts and Nevis is the smallest country in territory and population, with 53 thousand inhabitants.[10] LAC faced noteworthy changes in the age structure of their populations in the last decades. These changes are characterized by the transition from high to low levels of mortality and fertility. At the first stage of its population growth dynamics, a reduction in mortality rates and high fertility rates have been observed, leading to the population growth mainly due to the rise in the number of children and young adults. In the early 1960s, the increase of the population was nearly 2.8 percent per annum. Although a reduction of overall fertility was seen, mortality rates continued to decrease, leading to further growth of the total population, which doubled from 220 to 442 million in 30 years. The current average fertility rate in LAC is two children per women, which is three times fewer than in 1950.[14]

The further reduction of mortality and births led to an increase in the percentage of older persons in the population.[9] In LAC, mortality declined over for the last

century and continues to decline today: from an approximate average of 59 years in the 1965–70 period, life expectancy increased to almost 75 years in the 2010–15 period. The population has gained 16 years of life on average in the last 45 years. Although the life expectancy in the region is currently close to 75 years, in some countries, such as Panama, Chile, and Costa Rica, people can expect to live even more than 80 years, slightly longer than United States residents. There is large variability in life expectancy across cities within each country, sometimes as large a difference as 7–10 years, as is the case in Mexico, Brazil, Colombia, and Peru.[15]

The population in LAC tripled between 1950 and 2000. By then, few countries were still at a pre-transition (Bolivia and Haiti) or incipient transition stage (Belize, Guatemala, Honduras, and Nicaragua). In 2010, these same countries were in the in-progress stage, while the rest was in advanced or very advanced stages of transition. Although LAC populations are still young compared to the USA and Western Europe, the rate of aging is among the highest in the world. This pattern of aging is seen in nearly every country in the region, with a shift from young children to older relatives. Current and expected transformations in the age structure of the LAC are expressed in the population pyramid for 2015 and 2050 (Fig. 6.1).

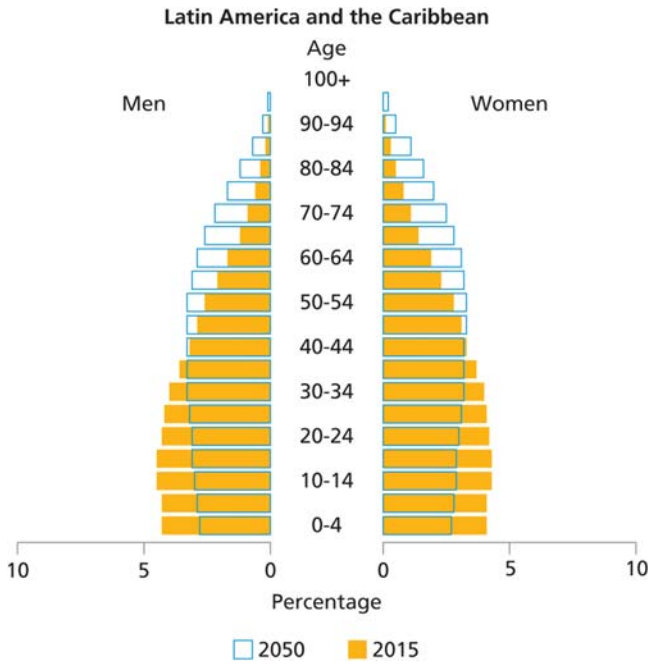

Figure 6.1 Population pyramid in Latin America and the Caribbean, 2015–50.[16] Data from Pan America Health Organization. Health Status of the population: population characteristics and trends. < *https://www.paho.org/salud-en-las-americas-2017/?p = 1864*>; *2017 [accessed 02.05.21].*

Population aging in LAC will not be a uniform process in all countries. In many of them, the young and working-age population will continue to be relevant to population growth even well into the 21st century. This will continue to create demands on societal sectors and pose challenges in public policy. Although the young population (0–19 years) has been the largest segment thus far, by the early 2020s, the young adult population (20–39 years) is expected to be the largest group. By 2045, this group should be replaced by the adult population (40–59 years) and less than 10 years later by the older adult population (60 years and over).

The process of population aging in LAC is different in many aspects from that observed in developed countries. For instance, the speed of the demographic transition in LAC has been regarded as unprecedented. While it took France 115 years to double the proportion of older people in the population from 7% to 14%, this process is projected to occur in Colombia within 19 years and in Brazil in 21 years. Seven countries in LAC, namely Brazil, Chile, Colombia, Costa Rica, Guatemala, Mexico, and Peru, are among the 25 countries with the fastest increase in the population aged 65 and over. The period of demographic transition occurrence varies across this region depending on factors like immigration and social development. Argentina, southern Brazil, Chile, Cuba, and Uruguay, regions with great immigration from European countries during the 19th and early 20th centuries, followed the initial stage of fertility decline of the developed countries, differently from LAC regions which have a greater proportion of the indigenous population, where the fertility decline only started late in the 20th century.[14]

Another unique characteristic of aging in LAC is linked to the "aging of the aged." According to the United Nations, among the world's over-60 population, the proportion of people 80 years old or older increased from 9% to 14% between 1980 and 2015. This scenario is predicted to remain quite stable between 2015 and 2030 and between 2030 and 2050, the percentage of the most elderly is expected to increase from 14% to 20%.[17]

In 2015, the Organization of American States (OAS) issued a remarkable legislation for human rights in the region, during the InterAmerican Convention on Protecting the Human Rights of Older Persons. This document, a point of reference for the world, represented a substantive shift in the understanding of political actions for the older aged, because it recognized the elderly as subjects of rights, who should be recognized and protected by the States that ratify the commitment. Although the Fourth Regional Intergovernmental Conference on Ageing and the Rights of Older Persons, 2017, also ratified this commitment in its Asunción Declaration[18], to date only five LAC countries (Argentina, Bolivia, Chile, Costa Rica, and Uruguay) have ratified it.

Demographic transition is also associated with changes in the health dynamics in a population. The decrease in mortality rate is intricately linked to the control of

infectious diseases and malnutrition, particularly prevalent among children. With the population aging, chronic diseases become more prevalent and emerge as the main cause of disability and mortality. That is the pattern classically observed in developed countries that have completed the demographic transition. However, in developing countries, where the demographic transition occurs in a context of social disadvantage, there would be the coexistence of both infectious and chronic diseases, imposing special challenges for health care. The poorer sector of the population would not only present with higher rates of disease, but of different kinds of diseases.[19]

In a survey (Survey on Health, Well-Being, and Aging in Latin America and the Caribbean-SABE) carried out among people aged between 69.9 and 73.3 years residing in seven cities in LAC (Bridge-town, Barbados; Buenos Aires, Argentina; Havana, Cuba; Mexico, D.F., Mexico; Montevideo, Uruguay; Santiago, Chile, and São Paulo, Brazil), the majority reported at least one chronic disease, ranging from 68.6% in Mexico to 81.6% in Buenos Aires. Hypertension and osteoarthritis were the most frequently reported diseases. The prevalence of a reported disability for performing daily life activity ranged from 33.8% in Montevideo to 64.0% in Sao Paulo. Factors associated with self-reported disability were advanced age, negative self-perception of health, and the incidence of chronic diseases. The SABE survey also showed that seven diseases and chronic conditions disproportionately affect the health status of the aged population, namely hypertension, arthritis, heart disease, diabetes, lung disease, stroke, and cancer.[20]

The concept of active aging is beyond the just the presence or lack of a disease; much of this is unavoidable with aging. More important to evaluating the health status of older people would be the functional capability of an individual, which means the ability to control, cope with, and make personal decisions on a daily basis, according to one's rules and preferences, defined as autonomy. In addition, the ability to perform functions related to daily living, that is the capacity of living independently in the community with no and/or little help from others, the so-called independence/self-sufficiency, is also important. It is possible to attain good functional capacity and quality of life even while coping with disease. For such functional capacity, one needs to have easy access to health assistance, early diagnosis, management, and disease rehabilitation. Dependency required to perform the activities of daily living is an indicator of the burden of chronic diseases and demands for long-term care.[21,22]

Poor health is not an inevitable outcome as people grow older. Policies for controlling risk factors for chronic diseases and promoting healthy behaviors by reducing smoking, alcohol consumption, and a sedentary lifestyle, are necessary to be implemented in the course of life and to prevent/delay disability among older adults. In fact, among the five most significant causes of illness and premature death in LAC are smoking, hypertension, diabetes, obesity, and physical inactivity.[23]

Social determinants of healthy aging also must be considered. This is especially important in low-income countries where the process of population aging occurs in parallel to poverty and income inequality. There are huge differences across LAC countries regarding poverty levels. Considering the national poverty headcount rate, the lowest poverty levels are seen in Southern countries (Chile, Uruguay, and Argentina) and Costa Rica, and the most impoverished settings are found in Nicaragua, Colombia, and Honduras.[24]

Poor fetal health and nutritional disadvantages in early childhood are linked to a greater risk of chronic diseases, such as heart disease and diabetes. Recent findings for LAC populations suggest that early health and early socioeconomic factors contribute to a higher probability of being disabled in older life. Early-life health and socioeconomic experiences encompass a wide array of variables that affect nutritional status *in utero* and perinatally, growth and development in early infancy and childhood, exposure to and acquisition of infectious and parasitic diseases, and other stress-related factors, which shape early-life environments and consequently the lifespan exposome. Understanding the complex interactions of socioeconomic disadvantages, ongoing diseases, and disability in old age is particularly important for LAC countries, where the current cohorts of elderly have survived to old ages much more as a consequence of improvements in health accessibility and medical intervention rather than to amelioration in standards of living.[25]

6.3 Age-related biological changes and diseases in the context of Latin America and the Caribbean countries

Chronic diseases are known to increase with age, thus the understanding of the age-related biological changes and their complex interactions with the onset of pathologic processes is a critical task for healthy aging. Such changes are linked to genetic factors but are also greatly influenced by socioeconomic factors and lifestyles, which vary among LAC countries.

Different studies have addressed genetic and epigenetic aspects of aging. Some examples include studies on longevity and human lifespan variation, especially the ones based on family heritage.[26] For example, when the lifespan of twins was compared, researchers found that approximately 25% of the lifespan variation could be attributed to genetic factors.[27,28] In addition, other reports indicated that not only siblings, but parents and offspring of longevous family members have a significant survival advantage over the general population.[29,30]

Heritable components are frequently a factor in age-related illnesses such as Alzheimer's (58%–79%), cardiovascular (45%–69%), and osteoarthritis (68%) diseases.[31] In contrast, heritable components represent only 5%–10% of all cancer types.[1] Besides genetics, the term epigenetics, first introduced in 1942 by Conrad H.

Waddington, explains heritable changes in gene expression that could occur without changing the DNA sequence. These epigenetic changes can occur by distinct mechanisms, such as DNA methylation, histone modification, and RNA interference.[32,33] One example, is the link between diet patterns and epigenetics. For example, a decrease in caloric intake increased the lifespan of laboratory animals, and a reduction of glucose intake was associated with diabetes and cancer prevention by affecting the transcriptional activity and expression of different genes.[33] Thus, a modifiable environmental factor such as diet could negatively or positively influence the epigenome.

Epigenetic determinants can induce mutations in genes, and these mutations occur in genes that modify the epigenome.[34] Both factors are also affected by the aging process. Healthy young cells maintain a controlled epigenetic state that allows for the formation of a compact chromatin structure, whereas aging cells present modifications in the chromatin landscape, DNA accessibility, and noncoding RNA production, which might compromise genomic integrity leading to a progressive loss of cell viability.[35] Interestingly, the accumulation of cellular damage over time is a common characteristic between the processes of tumorigenesis and aging.[36] Besides cancer, multimorbidity (the cooccurrence of two or more chronic conditions[37]) should also be analyzed when discussing aging. A Canadian study estimated that between 34% to 61% of people above 65 years present multimorbidities, and this could vary according to the population studied.[38] In Brazil, for example, multimorbidity in older adults is a common condition, which appears to be influenced by socioeconomic factors and lifestyle.[39,40] Poor physical activity, smoking, and alcohol abuse were identified as the most frequent behavioral determinants of unhealthy aging. Thus, public health policies need to properly address different factors to plan and manage effective interventions. Preventive strategies should also be designed to reduce disease burden.

The advent of high-throughput technologies has revolutionized medical research and helped shape translational science by improving the connection between basic science and applied research to also provide customized medical care. Different sequencing techniques (i.e., Sanger and Next-generation sequencing) and genotyping arrays allow for large-scale genome-wide association studies (GWAS). The latter comprises studies where a genetic variant is associated with a certain trait, which is performed after careful statistical analysis in large cohorts (several hundred thousand). One example is the studies of nucleotide polymorphisms.[41] GWAS studies have identified several genetic variants associated with aging, including one of the first analyses that found a genetic variant linking age with macular degeneration (a common cause of blindness).[42] Furthermore, GWAS studies identified that various somatic mutations are also drivers of aging and cancer.[43] Small cell and tumor sample studies have also shown that the over-time accumulation of somatic mutations could lead to clonal expansion of the mutated cells.[44]

GWAS helped to uncover germline variants important for glioma susceptibility, including a list of 25 risk polymorphisms.[45] In addition, the analysis of a GWAS by molecular subtype identified 2 new chromosome regions and a candidate-independent region near the gene *RTEL1*, which were associated with certain glioma molecular subtypes.[46] Another study, for example, identified both robust genes and novel hub genes involved in GBM's etiology and tumorgenicity, respectively, which could have therapeutic implications.[47] The clinical applicability of GWAS studies also provides promising opportunities for drug discovery, drug repositioning, and cancer prevention.[48] In addition, genomic science is an important field to understand different pathologies and correlate them with demographics such as ethnicity. In this sense, different LAC countries have been working hard to improve their genomic research by building genomic maps of their population. For instance, the Mexican National Institute of Genomic Medicine (INMEGEN) created a Mexican genomic databank, and researchers at Brazil's Federal University of Minas Gerais have been doing extensive research on the ancestry of Brazilians. Colombia has also been doing the same.[49]

The use of high-throughput technologies is important to predicting different aspects of disease, including diagnosis, disease risk, detection of molecular alterations, and therapy resistance.[50,51] However, the broad implementation of advanced technologies is still a hard task when viewing the scenario of the Brazilian public health system. Many factors are important to achieve an efficient service, such as a sufficient budget, qualified personnel, and infrastructure. Unfortunately, the sequencing tests performed in Brazil were shown to be more expensive than in developed countries, especially due to high taxes, high costs of analysis, and infrastructure.[52] Despite these challenges, better health policies could be implemented to slowly put Brazil in a better global position, which will benefit the population, but particularly the elderly who need more personalized and efficient healthcare.

Fig. 6.2 summarizes the most frequent diseases affecting the elderly population in Latin America. In the following topics, we will discuss different diseases that affect the elderly.

6.3.1 Immunosenescence and infectious diseases

Population habits and medical advances have been changing the epidemiological scenario related to aging and disease worldwide. In developing countries, infectious disease rates, which were the main causes of death in the elderly, are overcome by chronic diseases. Nevertheless, infectious and tropical diseases are still a worrisome burden in LAC countries.[53–55]

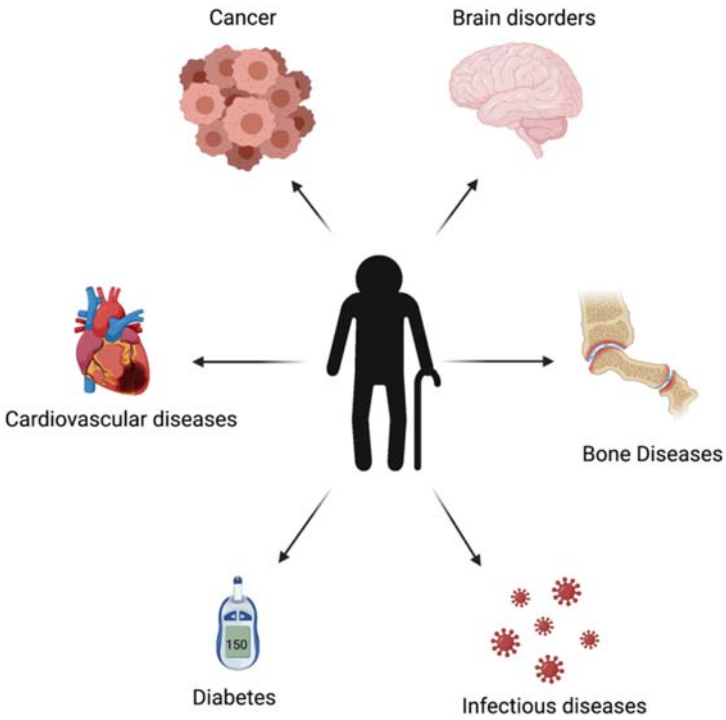

Figure 6.2 Most frequent diseases affecting the elderly population in Latin America. *This figure was created using Biorender.com.*

Aging affects different cellular and molecular aspects that can lead to a progressive decline in homeostasis. However, not all individuals have the same problems, and other external and internal factors can influence the aging process. Loss of cognitive and motor functions are also related to poor immune responses. The elderly is more vulnerable to infectious diseases, as they do not have the same immune response as younger individuals, presenting a more severe disease response, resulting in infection and replication of pathogens.[56,57]

The immune system suffers multifactorial changes during aging, with variability between individuals. The main changes are the disruption of blood cell production (increase in neutrophil:lymphocyte ratio), dysfunction of immune cells, and a constant, chronic inflammatory state. Cells of the innate and adaptive immune response may undergo transformations leading to a greater susceptibility to infectious diseases. Several studies attribute this greater vulnerability in older people, to a process called immunosenescence, which is significantly associated with a decline in immune competence, on average, after 60 years of age.[58,59]

The innate immune response, although preserved in the elderly, can vary in different situations. For example, dendritic cells, an important cell type in the innate immune response, are involved with presenting antigens to T-lymphocytes and their numbers can be decreased in the elderly. Therefore, these individuals may have impaired defenses against a pathogen. In addition to T-lymphocytes, the B-lymphocyte (cells responsible for producing antibodies) population may be significantly decreased in the elderly. The clinical consequence of this decline is less protection against fungi, protozoa and poor responses to vaccination seen in elderly individuals compared to young adults. The quality of the antibodies produced is also compromised, which directly impacts responses to external agents and vaccines.[57,60,61]

Immunosenescence is secondary to hormonal decline, stress, and persistent antigenic stimulation. However, the change in the age-related immune repertoire, especially the decrease in virgin T-lymphocytes and the increase in memory effector clones, seems to be compensated by an adaptation in a nonspecific defense, directing the immune system to make only potentially less inflammatory responses. In other words, immunity in the elderly depends directly on the balance between the beneficial and harmful effects of inflammatory responses. Moreover, the intensity of inflammatory responses that occur throughout life seems to be important in the homeostasis of immune sensitivity.[58,61,62]

Different infectious diseases affect the elderly in LAC, especially in tropical countries where tropical diseases affect millions of people. The world is currently experiencing a pandemic caused by severe acute respiratory coronavirus 2 (SARS-CoV-2), which caused the 2019 coronavirus disease (COVID-19). The pandemic reports documented an increased risk of serious illness with increasing patients' age, thus a higher risk in older adults. The elderly may have severe clinical symptoms, especially those with frailty, and may present chest discomfort or increased sputum production, in addition to tachycardia, tachypnea, delirium, and decreased blood pressure. The risk of dying from COVID-19 increases with age. Although the lethality rate is almost 0.4% for patients under 50, it increases to 3.6% for patients aged 60–69, 8.0% in patients aged 70–79, and up to 14.8% in patients older than 80 years. In addition to advanced age, these elderly patients commonly have comorbidities that can further aggravate COVID-19 infections and lead to poor outcomes.[54,63–66]

Another respiratory infection relevant in the Americas is influenza. The influenza-causing virus appears to be more significant in older adults, varying with the circulating strain, previous exposure to the strain, and the vaccine's effectiveness. The presentation of the disease in the elderly is not typical. They may not have a fever, but chronic obstructive pulmonary disease and exacerbation of congestive heart failure may occur.[67,68]

In LAC, tropical diseases are common, caused by viruses, bacteria, and parasites, affecting up to one billion people worldwide. They occur in tropical and subtropical

countries, where the most vulnerable populations in developing countries are concentrated. The following tropical diseases have deleterious health impacts on the aging population: Chagas disease, malaria, leprosy, tuberculosis, arboviruses, and viral hepatitis among others.[54,69]

Chagas disease is an important contributor of deaths in the elderly in the Americas. It is a tropical disease caused by the *Trypanosoma cruzi* parasite, and it is considered neglected by the WHO. Transmission occurs mainly through the vector popularly known as the "barber." According to the WHO, there are about 6 million infected people in 21 Latin American countries. The average age group of elderly people with Chagas disease varies between 66–69 years. In these patients, there is a predominance of the neurogenic form of Chagas disease, cardiac involvement, and the presence of heart failure due to the association with changes in aging itself and other pathologies related to aging. Thus, it is important to note that the elderly have more morbidities than do other individuals, which can compromise the body's defense against Chagas disease.[70–72]

Another disease, malaria, is caused by protozoa of the genus *Plasmodium* and transmitted by the mosquito bite of the genus *Anopheles*. It is a parasitic disease considered to be a serious public health concern in the Americas, also affecting the elderly. Studies show that most elderly people with malaria are male, probably due to workplaces in the countryside, generally related to male activities, which is still very prevalent in developing countries. The most frequent clinical manifestations are feverish chills and headache, but comorbidities in advanced age may lead to hospitalization.[54,73]

Leprosy is a disease caused by *Mycobacterium leprae*, it is debilitating when left untreated, and potentially devastating for the elderly. Considered a neglected, infectious, and contagious tropical disease, it mainly affects the skin and the peripheral nervous system. It has social and psychological impacts due to deformities and physical disabilities. In the elderly, leprosy can lead to serious physical and psychological problems and chronic disability that can compromise their quality of life. The impairment of the motor system, caused by the infection of the peripheral nervous system may increase neurological dysfunction and greater dependence in this age group.[74–76]

Tuberculosis has *Mycobacterium tuberculosis* as its etiological agent; it is one of the leading causes of death from infectious diseases globally. In recent years, there has been an increase in mortality from pulmonary tuberculosis in the elderly over 60 years of age compared to individuals of other age groups. This can occur due to difficulties in diagnosis and unusual clinical presentations in these individuals. The signs and symptoms of tuberculosis in the elderly are difficult to measure due to their coexistence with other respiratory, cardiovascular, or systemic diseases with similar characteristics. Studies show that the mortality rates associated with tuberculosis in elderly patients can reach approximately 30%.[77–79]

Zika infection is also a health problem to the elderly. The first Zika outbreak occurred in 2007. The Zika virus is transmitted by mosquito bites, mainly of the genus *Aedes*, but other forms of transmission are reported. In most cases, Zika is presented as an uncomfortable disease, but relatively minor, with symptoms such as fever, skin rashes, and conjunctivitis. However, older people, due to a weaker immune system, may require immediate medical attention. These patients are at a higher risk of presenting more severe symptoms and neurological complications.[80–82]

Another tropical disease caused by the bite of the *Aedes* mosquito is chikungunya which is caused by the virus of the same name, chikungunya (CHIKV). The main clinical characteristic of the disease is joint pain even after the acute phase, which can last from months to years, progressing to a chronic phase, characterized by physical limitation, which affects the patient's quality of life. Studies show that the chronic phase may be related to the evolution of inflammatory rheumatic diseases in predisposed individuals. These clinical repercussions mainly impact the elderly. In this age group, there is a higher risk of complications due to previous diseases and a greater propensity for lethality due to the low natural immunological reserve of age. In addition, even in the acute phase of the disease burden, chikungunya can lead to disability and low quality of life in older individuals. Comorbidities and chronic health conditions may be exacerbated during the acute stage of CHIKV infection in elderly patients, such as underlying respiratory disease, hypertension, or chronic heart disorders, which may lead to hospitalization and increased disease severity.[83–86]

Dengue is an acute febrile disease of viral etiology transmitted by *Aedes aegypti* mosquitoes, and it is the most important arbovirus in tropical countries, constituting a serious public health problem. The elderly is more prone to hospitalization due to dengue virus infection and may present severe forms of the infection. They are also at a greater risk of death compared to individuals of other age groups, except for young children. Different studies have reported greater propensity for hospitalization, mortality, and hemorrhagic dengue in old patients. Elderly people over 60 are at twelve times higher risk of death than other age groups. In this age group, dengue may even lead to other complications, such as urinary tract infections. Another important factor to be considered is dehydration, which can lead to severe symptoms in these patients.[1,87–89]

Viral hepatitis is of great concern to the elderly, mainly due to the decline in physiological and immunological capacity, leading to a greater possibility of chronic complications. The etiological agents of viral hepatitis are numerous and with different modes of transmission, such as fecal, oral, parenteral, and sexual. There are several reports of serious complications in elderly patients infected with these viruses, leading to aggravated cirrhosis and death.[90,91]

In summary, due to physiological and immunological aging, effective health care for the elderly in LAC countries is highly necessary. There is a constant need for research, proper diagnosis and adequate management of tropical and neglected diseases, which in most cases do not even have a vaccine or effective preventive policies.

6.3.2 Age-related intestinal microbiota changes: the role in neurodegenerative and metabolic diseases

The microbiota consists of a population of commensal microorganisms present in the intestine, including bacteria, archaea, viruses, fungi, and protozoa, that together have fundamental roles in human health. In turn, the microbiome formed by the microbiota and its habitat changes during life and is directly influenced by genetics, lifestyle, and diet. The aging process is followed by significant changes in the microbiota due to changes in the immune response, diet, reduced mobility, intestinal function, and morphology, as well as infections and drug administration. Studies have shown decreases in the Firmicutes-to-Bacteroidetes ratio and reductions in species that produce short-chain fatty acids (SCFA; primarily butyrate) in the elderly.[92,93] It has also been reported that there is an increased presence of opportunistic bacteria such as *Clostridium perfringens* and *Clostridoides difficile* (*C. difficile*) in the elderly.[94–96]

Recently, most of the studies have been focused on the bidirectional signaling pathway between the intestine and the brain. The normal intestinal microbiota metabolizes the dietary components into amino acids, SCFA, and other metabolites. SCFAs, such as butyrate, are produced during the fermentation of nondigestible carbohydrates by intestinal bacteria and protect the intestinal epithelial barrier, decrease cytokine production by inhibiting NF-κB and, and fortify the blood-brain barrier by regulating microglia function.[94,96] A preclinical study in mice showed that transplantation of aged microbiome into young mice is sufficient to decrease SCFAs in the host and to produce cognitive declines, such as depressive-like behavior and short-term memory impairment.[97] Microbiota-related protein catabolism affects the synthesis of neurotransmitters, such as serotonin and overall brain function.[94,96]

The rapid shift from pre-agricultural diets to processed or ultra-processed foods rich in refined carbohydrates, trans-fats or saturated fatty acids has resulted in a significant change in the intestinal microbiota, a process known as dysbiosis. Dysbiosis alters the integrity of the epithelial barrier, changes immune responses, and disrupts intestinal homeostasis. The complex communication between the intestine and the central nervous system (CNS) includes enteroendocrine cells in the gastrointestinal tract epithelium, the enteric nervous system formed by glial cells and neurons, the autonomic nervous, the vagus nerve, and the immune system.

Food and digestion products, in addition to the microbiota and its metabolites, influence the release of hormones, cytokines, and neurotransmitters that are carried by the circulation or the vagus nerve signal to the CNS, where they alter the integrity of

the blood-brain barrier, the inflammatory response, and consequently brain functioning. A diet rich in saturated fats can cause nuclear factor-kappa B (NF-κB) activation, resulting in the synthesis and secretion of cytokines by immune-inflammatory cells and enteric glial cells (EGC). Thus, the intestinal inflammatory process extends to the blood-brain barrier and can lead to neuroinflammation, contributing to the pathophysiology of depression, memory impairment, stroke, Alzheimer's, Parkinson's disease, and all aging-related diseases.[96]

In Alzheimer's disease, a decrease in bacteria promoting anti-inflammatory responses such as Firmicutes and Bacteroidetes was detected in the intestinal microbiome analysis. A positive correlation between intestinal inflammation and deposition of amyloid-beta plaques in the CNS was suggested by this study, indicating that microbiota may participate in the pathophysiology of Alzheimer's disease. Evidence showed that the microbiota is involved in the microglia maturation and function by modulating phagocytosis of amyloids and tau proteins.[98] In Parkinson's disease, deposition of alpha-synuclein, a pathophysiological marker of this disease, can be found in the gastrointestinal tract before its detection in the brain. Because alpha-synuclein is an inflammation-related protein, it may be that intestinal inflammation caused by dysbiosis may be linked to the pathogenesis of Parkinson's disease.[99] The intestinal microbiota is also involved in the pathophysiology of the stroke, as well as its outcome. Conversely, stroke causes dysbiosis and can lead to changes in gut function. *Lactobacillus*, a bacterium used as a probiotic, was reduced in an experimental stroke model, and its replacement improved cognitive function and mood, and relieved age-related neuroinflammation.[100]

The intestinal microbiome also has a relationship with metabolism and, therefore, with obesity and type 2 diabetes. The intestinal microbiota in the LAC population has been scarcely investigated; however, there is evidence in populations of Latin origin who live on the Mexican border with the United States showing an association between altering the microbiota with obesity and diabetes; a decrease in the diversity of bacteria in the microbiota was associated with obesity, low sensitivity to insulin, and an increase in triglycerides.[101] An increase in the Firmicutes—Bacteroidetes ratio in obese and diabetic patients was reported in some articles; nevertheless, there is still no consensus. The reason for the lack of consensus might involve differences among the techniques used in the investigations.[102]

A systematic review in LAC countries showed an association between healthy food consumption and its availability in the market. On the other hand, this same study found that 55% to 79% of advertisements promoted processed and otherwise unhealthy foods. A positive feature is that the price of traditional diets in LAC (made up of dry foods such as rice and beans and seasonal fruits) is lower than ultra-processed foods.[103] A healthy diet containing fiber and fermented foods is essential for maintaining a normal microbiota. Policies that influence the choice and consumption of

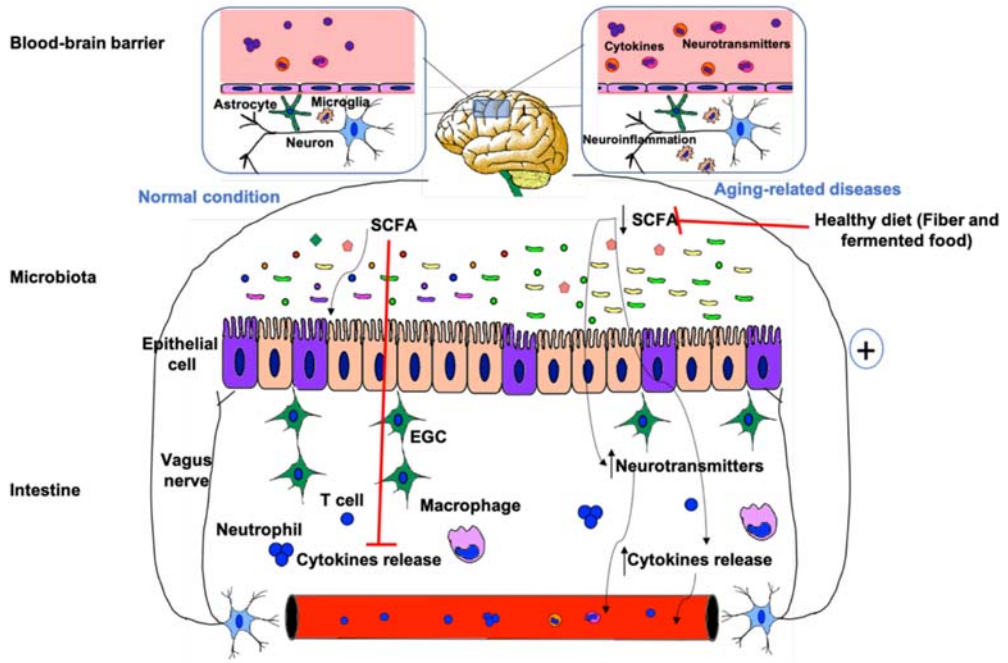

Figure 6.3 Microbiota role in aging-related disease. *EGC:* enteric glial cells. *SCFA:* short-chain fatty acids.

healthy foods are the path to the homeostasis of the microbiota and, consequently, to prevent age-associated diseases, as well as obesity and chronic diseases.

The pathogenesis of aging-related disease is summarized in Fig. 6.3.

6.3.3 Common age-related diseases: cancer, vascular diseases, diabetes and neurodegenerative disorders

6.3.3.1 Cancer

The median age for cancer diagnosis varies; examples include breast cancer (61 years), colorectal cancer (68 years), lung cancer (70 years), prostate cancer (66 years), and glioblastoma (64 years).[1] The Brazilian National Cancer Institute (INCA) estimates for each year of the 2020–22 triennium, 625 thousand new cancer cases (450 thousand, excluding cases of nonmelanoma skin cancer). The cancer type with the highest incidence is nonmelanoma skin cancer, followed by breast cancer and prostate (66 thousand each), colon and rectum (41 thousand), lung (30 thousand), and stomach (21 thousand).[104] Fig. 6.4 shows the highest incidence cancer types worldwide, in LAC, and Brazil affecting the age of 60 years or more, for both sexes. Whereas lung cancer has the highest incidence worldwide, prostate cancer has the highest incidence in Latin America (source: Globocan 2020[105]).

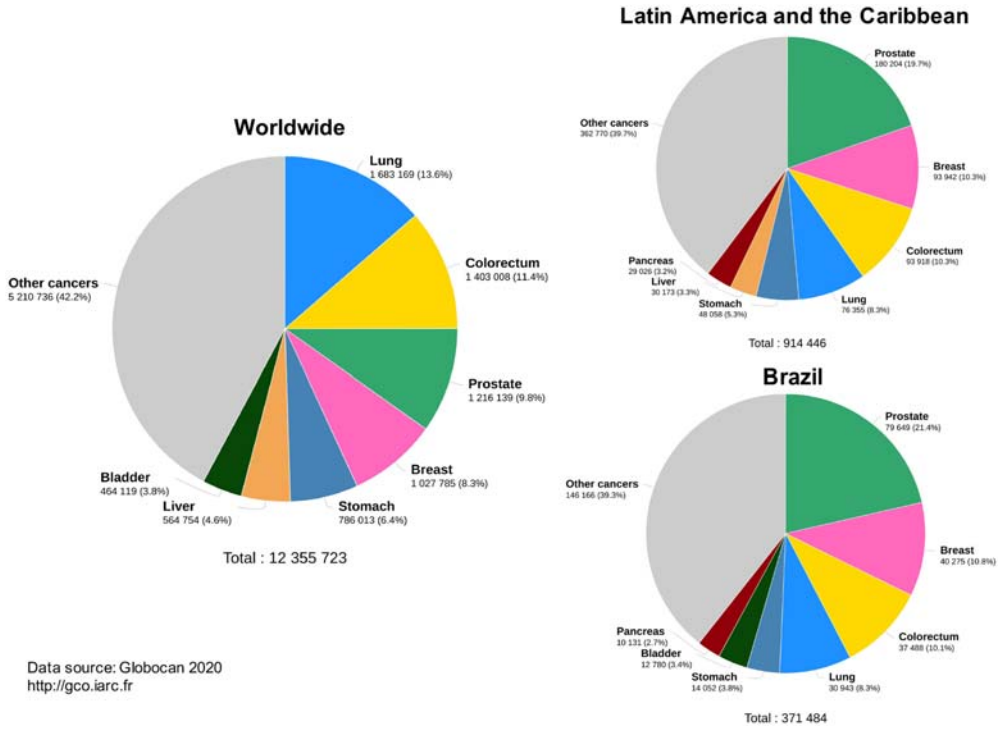

Figure 6.4 Estimated number of new cancer cases in 2020, Worldwide, Latin America and the Caribbean, Brazil, both sexes, age 60 + .[106] Data from International Agency for Research on Cancer. Global cancer observatory. *<https://gco.iarc.fr>; 2020 [accessed 02.05.21]. Globocan 2020 (http://gco.iarc.fr).*

In 2016, the estimated cancer rate in Brazil was 600 thousand, meaning that the numbers are growing, especially in the elderly population (above 65 years).[107] These numbers represent a challenge for the Brazilian public healthcare system since the main geriatric initiatives related to oncology do not cover the whole territory, but occurs more frequently in the southeast Region.[107] In addition, Brazil has ~556,873 medical doctors and less than 2000 geriatricians, which represents a challenge in the context of the rapidly growing elderly population.[108] In the Spanish-speaking countries in LAC, the older population also shows a growing trend, and when they are divided between high- and low-income countries, there is a higher incidence of cancer in the aging populations of high-income countries.[107] In addition, prostate and breast cancer are, overall, the most commonly diagnosed with 50% of the new cancer cases belonging to patients over 65 years.[107] This means that LAC countries need urgent health policies in order to adapt their healthcare systems to the current aging status and disease profile. This could be attained by improving the geriatrics sector, developing new prevention strategies, and offering proper oncology assistance for all.

The CNS is particularly affected by aging, where a functional brain decline occurs alongside impaired neurogenesis.[109] In addition, aging represents an important risk factor for brain cancer, especially glioblastoma (GBM), which is the most prevalent malignant primary brain tumor in adults. Advanced age is a negative prognostic factor for GBM, as aging seems to favor a more permissive microenvironment for tumors.[109] The mutagenic events leading to GBM are not fully understood, since the patients usually go to the clinic when their tumors are at an advanced stage; consequently, there is little information available regarding genetic analysis of early-stage GBM. Some common somatic mutations involve the canonical PI3K/MAPK, Rb, and p53regulatory pathways.[110] Liquid biopsy, which is a noninvasive diagnostic tool able to detect tumors at different disease stages, is a hope to improve our understanding of brain tumors and other cancer types.

6.3.3.2 Cardiovascular diseases

Cardiovascular diseases are the main cause of disability and death worldwide, accounting for about 30% of annual global deaths. Despite these being a problem that significantly affects developed countries, low-income countries and middle-income countries present the highest number of premature deaths related to cardiovascular diseases.[111]

Cardiovascular diseases are responsible for 33.7% of deaths in the Americas.[112] North America has the record of an annual average of deaths (55.3% in total), followed by Latin America (44.3% in total) and the non-Latin Caribbean (0.4% overall). Nonetheless, Latin America and the non-Latin Caribbean have a higher proportion of premature deaths when compared to North America.[111] Ischemic heart disease is the predominant cause of death out of all cardiovascular diseases.

In South America, urbanization of the population initially produced an increase in life expectancy due to a reduction in mortality from infectious diseases. However, the change in habits associated with urbanization, such as increased rate of smoking and stress, lack of physical activity, and poor diets (mainly based on fat and calories), resulted in increased risk factors for cardiovascular diseases such as, obesity, hypertension, dyslipidemia, and diabetes mellitus.[113] In addition, the increase in life expectancy contributed to an increase in the incidence of cardiovascular diseases since aging is an important risk factor for the development of these diseases. The aging of the cardiovascular system promotes several changes at the cellular and physiological levels. Furthermore, a longer life expectancy increases exposure to risk factors.[114]

Low socioeconomic status has been considered one of the strongest predictors of premature mortality as well as morbidity all over the globe. Nevertheless, poor socioeconomic conditions are not considered to be modifiable risk factors in health promotion strategies.[115] In fact, a study conducted in Brazil in 2013 revealed significant social

inequalities in Brazilian adults. The non-white population, people with less education and without access to private health insurance, had a higher prevalence of risk factors for cardiovascular diseases, such as smoking, leisure-time physical inactivity, sedentary lifestyle, heavy alcohol use, and a lower intake of fruits and vegetables.[116] Other studies carried out in the Brazilian population revealed that the prevalence of premature mortality from cardiovascular diseases is 2.6 times higher in poor areas when compared to rich areas. Likewise, the reduction in the risk of death from heart disease is faster in the population living in wealthy areas compared to the population in poorer regions.[111] Another disease that must be considered in this context is diabetes mellitus since it is an important risk factor of cardiovascular diseases.

6.3.3.3 Diabetes mellitus

Diabetes has been reported as one of the ten causes of death around the globe.[117] It has been estimated that more than 465 million people worldwide have type 2 diabetes, the most common form of diabetes. In LAC, it has been estimated that 31.6 million people had diabetes in 2019. By 2030, this number will be around 40.2 million people. In addition, it has been estimated that it will rise to 49.1 million in 2045.[118] The ocurrence of diabetes in LAC has been growing following the epidemiological transition. According to the 2016 Global Burden of Disease Study, the incidence rate increased from 2.74% in 2010 to 3.06% in 2016.[119] It has been estimated that the prevalence of diabetes in LAC is approximately 10%. This prevalence varies across the countries. For example, Mexico has the highest prevalence, followed by Haiti and Puerto Rico, while Colombia, Dominican Republic, Ecuador, Peru, and Uruguay have the lowest diabetes prevalence rates among Latin American countries.[120] Diabetes prevalence increases significantly with age. The elderly population is the age group most affected by the disease and its complications.[118] Furthermore, there are inequalities in the distribution of diabetes by gender, ethnicity, education, and socioeconomic position.

Diabetes causes multi-organ and multi-system impacts. This disease is associated with acute and long-term complications. Diabetes is considered one of the leading causes of death in some LAC countries. In addition, as previously described, it is considered a risk factor for cardiovascular disease. The main cause of death in patients with diabetes is cardiovascular disease, accounting for 44% of deaths in those with type 1 diabetes and 52% in those with type 2 diabetes mellitus.[112] The systematic review published by Blasco-Blasco et al.[121] assessed the barriers and facilitators in type 2 diabetes treatments in LAC. According to the results, the organizational barriers at the level of the health system are related to socioeconomic barriers due to social inequalities. This implies that people with diabetes in LAC have little capacity to act on their disease. Thus, diabetes is often not diagnosed or is poorly treated. As a result, there are high rates of diabetic complications.[121]

6.3.3.4 Neurodegenerative disorders

Parkinson's disease and dementia are two neurodegenerative conditions that must be discussed in the context of Central and South America healthcare systems, mainly due to the great social impact they cause. According to the 2015 Global Burden of Disease, Injury and Risk Factors (GBD) Study, Parkinson's disease was the fastest growing in prevalence, disability, and death among the neurological diseases assessed. Age is the main risk factor for Parkinson's disease, and other factors, such as exposure to industrial chemicals and pollutants (pesticides, solvents, and metals) appear to be correlated with a greater risk of developing the disease. It is expected that with population aging and increased life expectancy, the prevalence of Parkinson's disease will double in the next generation.[122]

According to the same study, some differences across countries could be at least in part explained by inequalities in the socioeconomic and demographic status of each population. With this respect, countries with a higher per capita income have experienced higher increases in the prevalence and mortality associated with Parkinson's disease. For instance, Chile, a country that exhibited a rapid epidemiological transition, marked by an increased life expectancy, showed the highest increase in the prevalence of Parkinson's disease within the period between 1990 and 2016, followed by Paraguay, El Salvador, Honduras, and Guatemala. Conversely, the LAC countries that reported the lowest increase in the prevalence of Parkinson's disease were Argentina, Cuba, and Uruguay. Regarding the number of deaths attributed to Parkinson's, Chile ranks 5th after Paraguay, Haiti, Bolivia, and Honduras. This data is highly relevant since it provides an epidemiological scenario of Parkinson's disease in many LAC countries. Such information is crucial to guide public health policies for the prevention and treatment of different neurodegenerative diseases, which have a strong association with the aging of the population in these countries.[123]

Dementia is related to several diseases that are slow, progressive, evolutionary, and chronic in nature. The Alzheimer's disease subtype corresponds to most of the dementia diagnoses. Dementia is one of the main causes of disability in old age, and its incidence and prevalence have been increasing in LAC. The number of people with dementia is expected to increase fourfold in the period between 2015 and 2050.[124] Despite this, there is scarce information on the incidence and the prevalence of dementia in LAC. These countries face increasing challenges in the diagnosis and treatment of dementia due to lower educational levels, limited access to healthcare, and the rapid aging of the population. In addition, the lack of knowledge and stigmas about the disease also impact the diagnosis and early treatment of dementia.[125] Aging, low cognitive reserve, poor cardiovascular health, and presenting at least one APOE-ϵ4 allele have been identified as risk factors for the development of dementia. However, few studies have evaluated these factors in the LAC population. It has been suggested that the high incidence of dementia in the Latino population may be

associated with low education, socioeconomic problems, poor access to healthcare, and high hypertension and diabetes prevalence.[126]

Several efforts have been made to standardize the diagnosis, promote greater knowledge about dementia for health professionals and the population, and increase research on dementia in this Region. However, most LAC countries still have minimal mental health facilities and do not have specific policies for dementia.[125] In addition, a government effort is needed to promote the prevention of dementia in this Region. Prevention for dementia begins in childhood, promoting access to high-quality education for the population. In addition, preventive measures aimed at risk factors for cardiovascular disease and diabetes should be encouraged. Moreover, measures to reduce social isolation, cognitive inactivity, and depression in the elderly are strongly warranted.[126]

6.4 Health and social initiatives for the promotion of healthy living and active aging

Despite technological and medical advances, people are likely to experience multimorbidity in later life since the risk of disability and noncommunicable chronic diseases increases with age and multiple chronic conditions.[6] The frail older population will possibly lead to higher health and societal costs. In LAC, it is imperative to analyze the aging process and the elements that will enable people to live longer and healthily.

6.4.1 Age-friendly initiatives

An age-friendly city and community are one in which policies, services, and structures related to the physical and social environment are designed to support and enable older people to "age actively"—that is, to live in security, enjoy good health, and continue to participate fully in society.[127]

Two dominant forces are shaping social and economic life in the 21st century: population aging and urbanization. Understanding the relationship between both phenomena has become a major issue for public policy. One significant policy response has come from the WHO through its approach to developing what has been termed "Age-Friendly Cities and Communities" (AFCCs). These have been defined as environments that are supportive of the needs of people as they grow old.

The "age-friendly city" program was introduced in 2005 during the International Association of Gerontology and Geriatrics World Congress of Gerontology and Geriatrics held in Rio de Janeiro, Brazil. The idea was formalized with the launch of the WHO Global Age-Friendly Cities project in 2006 carried out in 33 cities globally in northern and southern hemispheres. This project aimed to identify the core features of an age-friendly city from the perspective of older people, caregivers, and local service providers.[127] A total of 1485 older adults (60 years and over), 250 caregivers, and

Figure 6.5 Themes explored in the WHO Global Age-friendly Cities: A Guide (2007).[127] *From World Health Organization. Global age-friendly cities: a guide. Geneva: World Health Organization; 2007.*

515 service providers (drawn from public and private sectors) took part in 1 of the 158 focus groups conducted in various cities around the world.[128] Findings from the focus groups identified eight domains that needed to be addressed to increase the age-friendliness of cities (Fig. 6.5).[127]

Each of these domains was further defined and presented under the form of a "checklist of core features" in the *WHO Global Age-friendly Cities: A Guide* (2007).[127] This guide has since become one of the most frequently used tools to assess levels of age-friendliness of cities and communities in contrasting environments across the world.[128] To encourage the implementation of recommendations from the 2007 project, the WHO launched the "Global Network of Age-Friendly Cities and Communities" (GNAFCC). Since its launch in 2010, the GNAFCC has had a rapid increase in membership, reaching over 800 cities and communities in the global north and south by 2019. According to their website (http://www.agefriendlyworld.com), in LAC there are too few initiatives: municipalities of Zapopan (Mexico); Coamo (Puerto Rico); Cartago (Costa Rica); Miraflores (Peru); Pato Branco, São José dos Campos, Veranópolis and Esteio (Brazil); Maipu, Santiago, Quillon and Valdivia (Chile); La Plata (Argentina) and Montevideo (Uruguay).

Buffel et al.[129] argued that the age-friendly movement had been weakened by operating separately from other urban projects, with the division between the "healthy" and

"age-friendly" cities programs—both WHO-sponsored. Age-friendly initiatives such as "smart cities," "healthy cities," and "sustainable cities" are needed and should be encouraged to benefit the elderly's accessibility in urban settings.[130,131] According to Buffel et al.[129], a central part of making cities "age-friendly" should be to connect the age-friendly ideas with the "smart" and "sustainable" cities movements around supporting alternatives to cars, increasing energy efficiency and reducing pollution, for example. Engagement with this type of work can produce both further resources for the movement and add to the sustainability of existing projects. Another concern on age-friendly work in policies should challenge social inequality. It means to ensure equal access to the necessities for daily living and the decision-making processes underpinning urban life, explicitly addressing gender, social class, ethnicity, and other inequalities affecting the older population. The age-friendly policies demand a closer engagement with those neighborhoods and groups of older people abandoned in the face of urban change.[132]

Buffel et al.[129] proposed that "optimum environments for aging must be regarded as an interdisciplinary enterprise requiring awareness of the impact of developments such as the changing dynamics of urban poverty on older people; the consequences of urban renewal and regeneration; the influence of transnational migration; and changing relationships between different class, gender, ethnic, and age-based groups" (p. 7). Moreover, at present, discussions around age-friendliness have been disconnected from changes affecting urban environments, where private developers are a dominant influence on the planning and design. According to Harvey (2008, p. 31),[133] the result is that the "quality of urban life has become a commodity, as has the city itself, in a world where consumerism, tourism, cultural, and knowledge-based industries have become major aspects of the urban political economy." That is a necessary closer integration between age-friendly work and developments in disciplines such as urban sociology, urban economics, and human geography.[134]

Another issue is the diversity of health experienced by older people. A relevant question here is: do age-friendly initiatives reach out to people with all types of health conditions, or are they focused predominantly on the "healthy," that is, those involved in different forms of "active aging"?[135] Rather, the approach should acknowledge the variety of groups for whom age-friendly issues are relevant and the need to build environments, which support and reflect the diversity that characterizes an aging world.

6.4.2 Labor force participation and active aging

Health conditions among 50-, 60-, and 70-year-old Latin Americans have improved in recent years[13] and most occupations have become less physically demanding, which could increase older individuals' potential to work in a variety of different jobs.[136] Although declining mortality and improved life expectancy and health are observed in many countries today,[137] unlike their antecedents, nowadays most workers are enjoying a long

period of retirement. In Brazil, for example, the retirement duration for males is predicted to double between 1980 and 2025, rising from 5 years (20% of life expectancy) at age 20 in 1980 to 10 years (10% of life expectancy) in 2025.[138] However, people live longer and enter the labor force later because of increasing educational attainment; they are also leaving the labor force at younger ages.

De Souza, Queiroz, and Skirbekk[136] analyzed the Labor Force Participation Rate (LFPR) in LAC. Defining LFPR as the ratio of the population share of a specific age (from 15 to 65) that are either working or actively seeking employment (concerning a reference period) to the total population of the same age. According to their results, the declining trend in the LFPR of older adult males in Latin America (LA) cannot be explained by health status alone, since as health status (measured by mortality rates) improves, the labor force participation of older workers declines substantially. At a time of important changes in physical work demands and improved worker education, workers in most countries have a significant capacity to continue working into their later years. However, in reality, most years of life gained through mortality decrease are being spent in retirement rather than being divided between work and retirement.[136]

Global Burden of Diseases (GBD) estimates of the number of years lived with disability (YLDs),[139] in 1990 vs. 2015 in a 5-year basis for select younger age groups (45–49 and 60–64) in 1990 to corresponding older age groups (50–54 and 65–69) in 2015, showed that the health conditions of the 50–54 age group in 2015 were very similar to those of the 45–49 age group in 1990. The younger cohorts, particularly, have experienced health improvements. Similarly, by comparing the same age groups in both years, males aged 45–49 lived virtually the same number of years with disabilities in 1990 as 25 years later in 2015, although the LFPR was substantially higher in 1990.[136]

Following decades of health improvements among the population, LFPRs today are lower. Latin American older workers have become healthier yet work less than their counterparts in earlier decades. For Bolivia, Colombia, the Dominican Republic, Ecuador, El Salvador, Nicaragua, and Peru, males with better health conditions in the 2000s/2010s confirm lower LFPRs than their counterparts in the 1990s. Only for Argentina, males with similar YLDs in the 1990s have higher LFPRs than males in the 2000s.[136]

By using mortality rates to proxy health status, health improvements have been demonstrated in many LAC countries (e.g., Brazil, Bolivia, Chile, and Costa Rica), showing that the mortality risk faced by a 65-year-old male worker in the 1970s is not faced by his counterpart in the 2000s/2010s until age 71. At the same time, LFPRs declined steadily across age groups and over time, and given the same mortality level, the labor force participation of older workers is much lower today than in the past.[136]

Most Caribbean countries have not introduced programs for older persons to continue working beyond retirement age, nor have they trained older persons to enter or re-enter the labor market or introduced programs to assist older persons in the

informal economy. In all countries for which data are available, gender inequality in labor force participation extends into later life such that men's labor force participation rates almost double that of women's.[140]

6.5 Selected health issues among older people: evidence from long-term cohorts in Latin America

The aging of the population worldwide will increase at a fast rate, as will the number of families where the elderly live.[141] The population growth projection for the year 2050 estimates that, in some countries of the world, including countries of South and Central America, the number of elderly dependent on care can quadruple.[142]

The need for long-term cohort studies to study how many illness stressors during the lifespan affects aging, has become critical for shaping health policies and governmental strategies to ameliorate the long-term burden and health costs of disease. The burden of endemic tropical diseases (with frequent outbreaks) is of special interest, as many areas of the LAC countries have peculiar climatic and environmental conditions that favor infectious diseases and thus require permanent health attention. Identification of illness-related factors contributing to poor quality of health in aging people and the predictors of morbidity and mortality are key to reduce the burden and frailty in the elderly. Estimates like this drive the development of long-term research to cope with the ever-growing emergence of disabilities and dependence.[142] Long-term cohort research with data collection on aging people can help in planning measures that contribute to the health and well-being of the elderly.

Pirkle and colleagues in 2014, through the longitudinal study, The International Mobility in Aging Study, analyzed self-reports of older women from Latin countries such as Colombia and Brazil and established a relationship between teenage pregnancy, especially for those who were multiparous with more than 2 children, and losses in terms of physical performance. It was found that early pregnancy provided higher rates of chronic lung disease, diabetes, hypertension, and poor physical performance in elderly women.[143]

The identification of predictive factors for disability can potentially contribute to a better understanding of the underlying mechanisms and to direct vulnerable groups to effective prevention and early rehabilitation strategies. In this context, a long-term Brazilian cohort study was conducted aiming at predicting the emergence of the elderly's disability in daily living activities. The group investigated depressive symptoms, emotional support, and their association with the social network. According to this study, the depressive symptoms and low emotional support of caregivers have a strong predictive value for subsequent disabilities in a cohort of elderly with low education and income levels, regardless of other covariates.[144]

There are few studies examining the association between cytokines and chemokines with mortality. A robust 15-year-old Brazilian cohort study included surveillance

of mortality according to standardized criteria. This study analyzed the influence of inflammatory markers defined by baseline serum levels of IL-6, IL1−2, TNF, IL-10, and IL1β and chemokines in Brazilians aged 60 or over. 1191 participants were included in the analysis during the study period, 601 participants died, and the average follow-up period per participant was 12.8 years. The most important finding of this large cohort was that elevated baseline serum IL-6 levels were associated with long-term mortality, regardless of several important covariates. Other biomarkers were not associated or showed weak associations with mortality and the ethnicity did not modify the associations between any inflammatory marker with mortality.[145]

Another study aimed to assess mortality associated with carotid intima-media thickness (CIMT) in participants in the Strategy of Registry of Acute Coronary Syndrome (ERICO) study, an ongoing prospective cohort that involved all consecutive cases of the acute coronary syndrome (ACS) treated at the University Hospital of the University of São Paulo, Brazil (HU-USP). All cases of ACS that survived in the first month after the acute event were subjected to CIMT screening. The study documented that CMIT was mainly influenced by aging, but it was not considered a good predictor of mortality, neither was cardiovascular disease nor coronary disease.[146]

Dialysis treatment has been increasingly lifesaving for the aging population enduring renal failure due to the rise of chronic conditions, such as diabetes and hypertension. Studies have found advantages of peritoneal dialysis (PD), which can be performed in an automated fashion (APD) or a continuous ambulatory approach (CAPD), in relation to hemodialysis. Due to disagreements about the best peritoneum dialysis option for the patient, 762 elderly adults aged 65 years and over were eligible and selected from the prospective multicenter cohort Brazilian Peritoneal Dialysis (BRAZPD). Of these, 413 initiated APD and 349 initiated CAPD and were evaluated for their influence on long-term survival. Up to the 18-month follow-up period, no difference in survival was observed. However, beyond the 18 months, the APD was a protective factor while the CAPD elevated the risk of mortality.[147]

Pharmacotherapeutic treatments have increasingly assumed a prominent place in the health system given the increase in elderly patients worldwide. It is important to consider that the elderly patient often suffers from critical biological changes that influence the pharmacokinetics and pharmacodynamics of drugs. Certain medications and drug interactions are potentially dangerous for this population, gradually creating impaired kidney function. This may require changes in the administration of medications that must adapt to complicating comorbidities, impairment of cognitive function, and social status. The risk-benefit ratio of medication administration being worse among elderly patients on pharmaceutical therapy[148] should be the target of research.

The administration of anticoagulants in the elderly has been underscored, despite the evident benefits, regarding the recurrence of venous thromboembolism and cardioembolic events in atrial fibrillation. Medical misconceptions about bleeding risk estimates are the

reason for this underutilization. A prospective observational study was conducted at the anticoagulation clinic at Hospital Odilon Behrens in Belo Horizonte, Brazil. The inclusion criterion in the study was the use of oral anticoagulation continuously or at least for 90 days, and both those who were beginning anticoagulation and patients already on chronic anticoagulation were admitted. The analysis included a total of 171 patients aged 19–93 years allocated into two groups according to age: elderly, that is, those aged ≥ 60 years (79 patients) and nonelderly (92 patients). The mean follow-up time was 273 ± 84.9 days, equivalent to 127.9 patient-years. The objective was to investigate the quality of anticoagulation control by comparing elderly and younger patients. The dynamics of anticoagulation were similar in the young and elderly, although these two groups had different levels of education, cognitive deficits, chronic diseases, and used various medications.[149]

The fact that knowledge about the efficacy and safety of ongoing therapies needing further research for obesity among the elderly, motivated a retrospective study in a public outpatient clinic of Endocrinology and Metabology at Hospital das Clínicas, Faculty of Medicine, the University of São Paulo. Medical records of 51 patients, aged ≥ 60 years, were analyzed since the beginning of treatment and followed for an average time of 39.3 ± 26.4 months. Patients who consulted at least once in the 6-month period with a prescription for obesity medication were included. The elderly were assessed for their weight, body mass index, and height both at the beginning of the study and every six months until the total period of 2 years, with good tolerance to long pharmacotherapeutic treatment against obesity.[150]

In endemic areas of tropical diseases, long-term cohort studies are important to investigate whether vector-borne diseases interfere with mortality rates and the establishment of comorbidities in aging adults.[151] As the population over 65 years old increases, infectious diseases in this group become a serious public health problem. The increase in infection rates observed with aging may be due to physiological changes common to "normal" aging or to aging-related chronic diseases, and with medical, surgical, and diagnostic interventions. Epidemiological studies in elderly populations are needed to examine the relationship between specific infectious diseases and risk factors.[152]

The protozoan *T. cruzi* is responsible for causing Chagas disease in Latin America, a serious public health problem that affects approximately 8 million people. A group of elderly adults from a large long-term Brazilian cohort was evaluated on the relationship between Chagas disease and stroke mortality. This was an important cohort study that has generated different publications in medical journals. One of the important results is the causal link between Chagas' disease and the risk of stroke. In addition, type B natriuretic peptide isolated or related to atrial fibrillation has prognostic value for stroke mortality in elderly people chronically infected with *T. cruzi*.[153]

Noteworthy, is the scarcity of such cohort studies, even in high endemic countries, which are key to understanding the burden of the disease with aging. This is an important gap of knowledge that needs to be addressed by the scientific community.

Before 1965, research on cognitive aging was predominantly descriptive, identifying which aspects of intellectual functioning are affected in older adults compared to younger adults. Since the mid-1960s, there has been growing interest in how and why specific components of cognitive domains are affected differently in aging, with an increasing focus on the neuroscience of cognitive aging.[154]

The first investigation focusing on cognitive decline in the elderly in LAC in a low- or middle-income country investigated the association of age, gender, and education with the 10-year cognitive trajectory in a well-defined elderly population in the Bambuí Cohort Study of Aging previously cited in this chapter. Cognition was assessed using the Mini-Mental State Examination (MMSE) in elderly who were followed-up with annually. 12,206 MMSE applications were made to 1,461 people at the baseline. The main conclusions of this analysis demonstrate that people of female gender and of more advanced school level in the initial period of the study obtained significantly higher scores in the MMSE, while the elderly participants demonstrated lower initial levels in this same test. On the other hand, the elderly, women, and people with higher education had a faster cognitive decline.[155]

Another genomic research study was carried out using data from the same Brazilian cohort to observe the influence of American and African ancestry on cognition during aging. A worse cognitive performance was observed in those of African descent, but it did not differ in the cognitive trajectory. The educational level changed the initial association between greater African descent and cognitive performance, as the association was observed only in those with poor education (<4 years). No association was found between the ancestry of American Indians and the underlying cognitive function or their trajectory. Thus, the authors concluded that levels of African and Native American genomic ancestry had no prognostic value for age-related cognitive decline.[156]

In another study, longitudinal data compiled from 14 cohorts involving 12 countries, including Brazil, used a total of 42,170 individuals aged 54−105. It was also found that cognitive performance decreased with age and more rapidly with increasing age in different ethnocultural groups and geographic regions. Other correlations were investigated, such as educational level, sewage, and the individual's genotype compared to the apolipoprotein E ε4 allele. Patients with APOE4 showed a slightly more accelerated loss of cognition than noncarriers, with processing speed being the most affected aspect. It is of great importance to develop international research with multiple scenarios addressing correlations between the genotype of the aging population, risk factors, lifestyle, and cardiovascular health.[157]

Because dementia is a public health problem of special concern among older people, interventions for both dementia patients and caregivers are needed in countries facing rapid population aging. Many older people rely on personal assistance in their homes, often due to disabling health problems. In this sense, it is assumed that the training of people involved in the care of these elderly people results in improvements

in the quality of life. In order to investigate this issue, formal caregivers of elderly people with dementia who have undergone training were evaluated. For six months, two Brazilian health institutions in the state of Belo Horizonte, had their 46-elderly people with dementia and their 25 caregivers accompanied. After analyzing the sociodemographic profile, the individual cognitive prognosis of the elderly, and assessing the workload of observing caregivers, it was found that training aimed at qualifying elderly assistants brings benefits to both the psychological aspects of dementia and to the behavioral symptoms resulting from the disease.[158]

Primary care is a crucial tool for the insertion and promotion of physical activity. However, few studies have evaluated the long-term effectiveness of possible interventions. One study compared the success of three types of elderly health interventions in primary care aimed at increasing physical activity and leisure in elderly Brazilians. This research included 142 elderly residents belonging to an urban cohort in progress in São Paulo called EPIDOSO (Epidemiology of the Elderly) to assess independence in routine activities. Elderly people with a minimum age of 60 years were included in the study and evaluated broadly at the Center for the Study of Aging. Participants were divided into groups regarding the degree of multidisciplinary intervention. The first group consisted of minimal intervention, the second group consisted of medical counseling work, and the third group, in addition to counseling, worked with referrals to accessible programs for stimulating and guiding physical activities. The application of the International Physical Activity Questionnaire clarified aspects about physical activity during leisure and demonstrated that the third group responded to the incentive program significantly higher, including maintaining changes in the habit of performing physical activity among the elderly.[159]

Data from another Brazilian cohort called the Longitudinal Study of Health of the Elderly Brazilian (ELSI-Brazil), were used to verify whether physical activity was a routine in the lives of the elderly participants and sought to investigate what factors were associated with the practice of exercises for the purpose of identifying the determinants of this good habit in old age. 9412 residents of 70 municipalities in different regions of the country were interviewed, and various factors were analyzed according to the literature. Women and individuals with less education showed less physical activity during aging. Due to the importance of this practice for healthy aging, public health programs must be created to reduce the sedentary lifestyle.[160]

In times of the COVID-19 pandemic, comorbidities and life-long illnesses compound to aggravate the risk of mortality due to SARS-CoV-2 virus infections. The utmost need of cohort studies in LAC is even more important to better dissect peculiar environmental and genetic predispositions that influence illness severity and thus the quality of life of the elderly that may tremendously increase public health costs due to disability and rehabilitation.

Table 6.1 brings a summary of the cohort studies enrolling elderly people in Latin America.

Table 6.1 List of selected long-term cohort studies in Latin American countries addressing aging-related disorders and outcomes.

Study authors	Enrolment number and age	Study aims	Methodology	Main conclusions
Costa et al.[161]	$n = 1742 \geq 60$ years	Identify predictors of adverse health events in the elderly.	Annual individual monitoring with interviews, clinical, hematological, biochemical tests, serology for *Trypanosoma cruzi*, anthropometric and blood pressure measurements and electrocardiogram.	Brazilian cohort population of Bambuí for studying the aging process in Brazil.
(Lima-Costa et al., 2010b)[162]	$n = 1606 > 60$ years.	Estimate long-term mortality from Chagas disease in the elderly.	Interviews and verification of death certificates.	*T. cruzi* infection was a strong predictor of mortality among members of the cohort.
(Lima-Costa et al., 2010a)[153]	$n = 1398$ aged 60 in the city of Bambuí, Brazil.	Investigate the relationship between Chagas disease and mortality in elderly people from stroke in a long-term cohort in a large.	Analysis of deaths (1997−2007) due to stroke. Considering the confounding variables: age, sex, conventional stroke risk factors, and high sensitivity to C-reactive protein.	The risk of death from stroke among participants infected with *T. cruzi* was twice that of those not infected.

(Horie et al., 2010)[150]	$n = 51$ patients ≥ 60 years old with at least one visit in 2007, with at least 6 months of treatment for obesity.	Describe the experience of obesity pharmacotherapy in the elderly in a specialized obesity care center in Brazil, with a focus on efficacy and safety.	Verification of age, weight, height, and BMI at study admission, after 6, 12, 18, and 24 months and at the last visit. Analysis of prescription drugs, dose, duration of use, adverse effects, and reasons for discontinuing treatment.	Long-term pharmacotherapy for obesity was effective and well-tolerated by this group of elderly patients.
(Costa et al., 2011)[149]	$n = 79$ elderly people ≥ 60 years	To investigate the quality of anticoagulation control among needy elderly and younger patients.	Analysis of the quality of anticoagulation management. Comparison of elderly patients and younger patients regarding educational level and comorbidities.	Elderly patients may have a similar quality of anticoagulation, despite having higher levels of comorbidities and polypharmacy. There is evidence to suggest the safety of such therapeutic interventions in the elderly.
(Castro-Costa et al., 2011)[155]	$n = 1606$ elderly people ≥ 60 years	To investigate the association of age, gender, and education with trajectories of cognitive decline in the elderly population with low education.	Cognition was assessed annually using the MMSE. This analysis was based on 12,206 MMSE measures of 1461 (90%) baseline participants.	With regard to cognitive decline, female participants, with higher education and older, show faster reductions in MMSE scores.

(*Continued*)

Table 6.1 (Continued)

Study authors	Enrolment number and age	Study aims	Methodology	Main conclusions
(Lima et al., 2012)[163]	$n = 871$ consecutive surviving patients not selected more than 30 days after ischemic stroke (55% men, mean age 66±13 years)	To investigate whether the estimated glomerular filtration rate (eTFG) can be a predictor of death in different age groups after ischemic stroke.	Traditional cardiovascular risk factors and eTFG using The Chronic Kidney Disease Epidemiology collaboration formula were analyzed as predictors of mortality for the entire population of the cohort and stratified by age.	The Chronic Kidney Disease Epidemiology collaboration formula measured on the first day of hospital admission can be a simple and reliable predictor of death after ischemic stroke in people under 65 years of age.
(Sánchez R et al., 2013)[164]	$n = 65$ women aged 70–88.	Describe the clinical characteristics and results of long-term treatment of breast cancer (CM) in elderly women.	Review of medical records of 65 women aged 70–88, with localized CM, treated with surgery, postoperative radiotherapy or systemic therapy at a Hospital de Clínicas in Chile.	Screening mammography in older women detected smaller tumors and was associated with better survival. CM is the final cause of death in approximately half of the cases.
(Pirkle et al., 2014)[143]	$n = 1040$ between 65 and 74 years of age in Canada, Albania, Colombia, and Brazil.	Assess whether there is a link between reproductive history and physical function in old age.	Relationship between early maternal age at first birth (≤ 18 years), multiparity (> 2 births), and low physical performance. Data analysis by logistic regression and general linear models.	Childbirth in adolescence can increase the risk of developing chronic diseases and physical limitations in old age.

(Yiengprugsawan et al., 2016)[165]	$n = 25$ formal caregivers and 46 elderly people with dementia.	To investigate the effects of personnel training for assisted residences administered to formal caregivers of the elderly with behavioral and psychological symptoms of dementia in the institutional setting.	Caregivers and elderly residents were evaluated for demographic data, severity of dementia, global cognition, functional performance, quality of life, behavior, caregiver burden, depression, and anxiety. The total time for data collection was 6 months.	There was a significant improvement in the behavioral and psychological symptoms of dementia, assessed by the neuropsychiatric inventory ($P < .008$), with no changes in the other indexes.
(Lipnicki et al., 2017)[157]	$n = 42,170$ individuals aged 54–105 obtained from 14 cohorts from 12 countries. Period 1989 and 2011.	To investigate how age-related cognitive decline rates vary between international cognitive aging cohort studies. Determine whether gender, education level, and carrier status of the ε4 allele (APOE * 4) were associated with the decline.	The standardized MMSE and memory, processing speed, language, and executive functioning test scores were applied using mixed linear models, adjusted for sex and education, and metaanalytical techniques.	Cognitive performance decreased with age, and more rapidly with increasing age. There were many similarities and associations consistent with education and the APOE genotype.

(*Continued*)

Table 6.1 (Continued)

Study authors	Enrolment number and age	Study aims	Methodology	Main conclusions
(Felício et al., 2017)[166]	$n = 135$ elderly women ≥ 60 years	To examine whether handgrip strength predicts disability in elderly women with acute low back pain.	Handgrip strength was assessed with a Jamar dynamometer and disability was assessed using the Roland Morris questionnaire and gait speed test for 12 months.	Caution is needed regarding the use of HGS as a predictive measure of disability in elderly women with acute low back pain. Changes in gait speed were very small and unlikely to be clinically relevant.
(Franco et al., 2017)[147]	$n = 762$ elderly people ≥ 65 years. 413 started on APD and 349 on CAPD	Describe a cohort of elderly patients on PD and evaluate the influence of the modality on long-term survival.	Patients were followed up until death, transfer to hemodialysis, recovery of renal function, loss of follow-up or transplantation. Demographic and clinical data were assessed at baseline.	Continuous outpatient peritoneal dialysis (CAPD) presented a higher risk of mortality. Beyond 18 months, the APD modality was a protective factor.
(Lima-Costa et al., 2017)[145]	$n = 1495$ elderly people ≥ 60 years	(1) To examine the association between multiple cytokines and chemokines, particularly IL-6, with long-term mortality; (2) examine whether levels of African and Native American genomic ancestry affect the ability of these	Evaluate the associations between baseline serum levels of IL-6 and other cytokines (IL1−2, TNF, IL-10, and IL-1β) and chemokines (CCL2, CCL5, CXCL8, CXCL9, and CXCL10) with	The high level of IL-6 (but not other biomarkers) was associated with an increased risk of death. African and Native American ancestors based on the genome have no impact on the

		biomarkers to predict mortality; and (3) to compare IL-6 ability to predict mortality with that of a comprehensive morbidity score.	15-year mortality in 1191 mixed Brazilians aged 60 or over.	prognostic value of IL-6 for mortality.
(Guerchet et al., 2018)[141]		To study the effect of care dependency among elderly residents on the economic functioning of their families, in research sites in areas of coverage in Peru, Mexico, and China.	Families with elderly people were classified and followed up for 3 years to verify the economic outcomes (family income, consumption, economic pressure, satisfaction with economic circumstances, and health expenses and residents giving up work or education to care).	Income from paid work and government transfers were lower in assisted families than in unassisted families, and health expenditures were higher.
(Torres et al., 2018)[144]	$n = 1014$ elderly people ≥ 60 years	Examine the social support capacity, social network, and basic measures of depressive symptoms to predict the onset of disability in long-term ADL in a western middle-income country.	ADL was measured annually. Psychosocial factors included depressive symptoms, social support, and social network. Potential covariates included sociodemographic characteristics, lifestyle, cognitive function, and physical health scores based on 10 self-reported medical conditions.	Depressive symptoms and the low emotional support of the closest person have a strong predictive value for subsequent disability in ADL.

(*Continued*)

Table 6.1 (Continued)

Study authors	Enrolment number and age	Study aims	Methodology	Main conclusions
(Lima-Costa et al., 2018)[156]	$n = 1215$ elderly people ≥ 60 years	To investigate the association between African and Native American genomic ancestry and long-term cognitive trajectories in mixed-race Brazilians.	Cognitive function was assessed annually using the MMSE, totaling 12,208 measurements. Ancestors were assessed using a broad genome approach.	Levels of African and Native American genomic ancestry had no prognostic value for age-related cognitive decline in this mixed population.
(Peixoto et al., 2018)[160]	8736 participants (92.8%) aged 50 or over from the Longitudinal Study of Brazilian Aging (ELSI-Brazil).	Describe the prevalence of PA among elderly Brazilians and associated factors. In addition, the potential effect modifiers of the association between PA and age were investigated.	Physical activity was measured using the short version of the International Physical Activity Questionnaire. The exploratory variables were age, sex, education, ethnicity, marital status, number of chronic diseases and medical consultations, and knowledge or participation in public programs to encourage PA.	In addition to the association with marital status and health promotion programs, there was significant gender and educational inequality in the decline in PA in old age.
(Meireles et al., 2018)[146]	644 individuals with an average age of 61 years	To assess the mortality associated with CIMT in the participants in the ERICO study.	CIMT was assessed by mode B ultrasound to assess the risk of mortality in 180 days, 1−3 years.	The thickness of the carotid intima was mainly influenced by aging. CIMT was not a good predictor of mortality in this study.

(Novais et al., 2019)[159]	142 elderly people living in an urban cohort—São Paulo (Brazil).	To compare the effectiveness of three primary health care interventions in increasing leisure-time PA among elderly Brazilians.	Randomized participants in three groups: minimum intervention, medical counseling, and counseling group and individual referral to PA programs (CRG). Application of the extended version of the International Physical Activity Questionnaire to assess leisure-time PA at the beginning of the study, 4 years after the start without any intervention, 3 months after the intervention, and 6 months after the intervention.	Interventions with individual referral to PA programs effectively produce sustained changes in PA among older Brazilians.

ADL, Activities of daily living; *APD*, automated peritoneal dialysis; *BMI*, body mass index; *CAPD*, continuous ambulatory peritoneal dialysis; *CIMT*, carotid intima-media thickness; *ERICO*, Strategy of Registry of Acute Coronary Syndrome; *IL*, interleukin; *MMSE*, Mini-Mental State Examination; *PA*, physical activity; *PD*, peritoneal dialysis.

6.6 Conclusion

In summary, this chapter highlights the increasing challenges faced by aging populations in LAC countries due to peculiar tropical environments, endpemic diseases, health systems and accessibility, and cultural and socioeconomic characteristics; calling especial attention to emerging countries coping with socioeconomic, population growth, and nutritional transitions. Such panorama requires continuous preventive and intervention measures and permanent government policies and surveillance to reduce disease burden and to foster healthy aging and the quality of life of the elderly in a short and long term.

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SECTION 2

The biology of aging

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CHAPTER 7

Identification of metrics of molecular and cellular resilience in humans and animal models

Felipe Sierra
Inspire, CHU Toulouse, Toulouse, France

7.1 Biomedicine is focused on disease, not health

The world is aging, very fast, driven by a combination of decreased birth rates and significant gains in longevity. While most of the explosive increase in longevity has been the result of advances that prevent "premature deaths" during childhood and early adulthood (primarily among childbearing females), significant improvements have also occurred in life expectancy after age 60, with large portions of the world population being now in a position to expect a healthy survival into at least their 70s. In fact, it is interesting to note that, at a time when the average chronological age of humankind has increased, the mortality rate has diminished for all age groups, and particularly for the oldest. Thus, a person's chance of dying at any age has diminished, implying that people are aging in better health than ever before, so that now an older adult of 75 years has on average the physiological age of a 65 years old of just a few decades ago.

And yet, most people dread getting there, as old age is still invariably associated with infirmity, disease and frailty. If we have succeeded in significantly postponing death, why have we not succeeded in postponing disease? The answer is that the question is wrong: we have indeed succeeded in delaying both, so that while a diagnosis of cancer, for example, was a death sentence only a few decades ago, people are now living with cancer for years and decades. Similarly, the impact of heart disease has been significantly diminished through the use of statins, stents and other preventive measures, as well as post-event therapies and treatments. However, there is indeed still a catch. While modern biomedicine, nutrition and public health have succeeded in extending both lifespan and health span, there seems to be a disparity between the rate of advancement in each category, where despite both having seen steady increases in the last decades, their rise has not been parallel. Work by Crimmins[1] and others has shown that the increase in longevity lags significantly behind the increase in chronic

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diseases such as diabetes, cancer, stroke, myocardial infarction, and many others, leading to a situation where people are living longer, but in worse health conditions.

Certainly, this dramatic increase in the prevalence and incidence of chronic diseases is at least partially driven by the simple increase in the proportion of older adults within the population, and we need to keep in mind that this is a significant achievement brought about by the advances in biomedicine during the last century. But another culprit lies on biomedicine's use of an antiquated approach, whereby the focus is not on health, but on disease. Traditionally, medicine has been all about curing the sick and the diseased, with only occasional efforts at prevention. That is, the health profession is not focused on health! Of course, tending for the sick is a primary obligation and need, and nobody will argue that such efforts should be abandoned, but the entire approach is not helping us at the scale we require in the 21st century. For most of human history, sickness and death, when not self-inflicted through war and other activities, was caused by external agents that attacked our organism, such as viruses, bacteria and parasites. Focusing on combating those acute attacks by external forces was a reasonable approach that led to the most important advances made in the last century, including a recognition of the paramount role of hygiene, the development of vaccines and the establishment of sensible public health services such as clean water and garbage collection. Unfortunately, the dangers to our health have changed and no amount of garbage collection or clean water will protect us against chronic diseases whose etiology does not lay on external organisms attacking us, but are instead the result of an age-driven decrease in our own body defenses. That is, the current enemy is not a parasite, but it is an internal enemy: our own body.

7.2 Comorbidities are the prevailing characteristic of older age

So, we need to change the paradigm, but why does the current approach lead to higher morbidity, rather than being neutral or positive? The reason is that we are focusing on fighting one disease at a time, and with a strong emphasis on those diseases that produce the highest mortality, such as cancer and cardiovascular diseases. As a result, we have made significant advances in keeping those diseases at bay, thus increasing survival (i.e., lifespan) of those individuals affected by them. But the problem is that virtually all chronic diseases increase with age, not only those that kill us. This is called multimorbidity or comorbidity, and it points to the fact that older adults are rarely affected by only one disease: they have 2, 3, or as many as 10 complications at the same time. Addressing each of them individually, under the control of a different specialist each, leads to polypharmacy and a lower overall quality of life.

Returning to the issue of health span not moving as fast as lifespan as mentioned, we have made big progress against those diseases that kill us, so that a patient affected by a cancer (which would have killed her/him in a short time in the past), is now

allowed a second chance, via chemotherapy, surgery, and other means. The person lives a few extra years, but because none of the other concomitant diseases and conditions were treated, those extra years are spent in the presence of all those non-lethal conditions. That is, the lifespan of that individual was extended, but so was the prevalence of the accompanying diseases. We can conclude that the approach of addressing one disease at a time, with a laser focus on the deadliest ones, appears misguided. Of course, the extra years of life allowed to the patient will likewise accelerate the appearance of new diseases, not the least because some diseases and/or their treatments are known to accelerate the aging process, so that there will also be a negative effect on the "incidence" metric. This is unfortunately a natural downside of increasing the lifespan, irrespective of the approach taken to achieve it, so it will be a negative side effect of both the traditional approach and the new tactics being developed, to be discussed here.

7.3 Chronological versus physiological age

Given that the nature of the "enemy" has changed, then it is obvious that we are not using the right tools to fight, and we need to re-think our approach. In sports, as in war (either in the classical or metaphorical sense), it is necessary to develop two congruent and parallel approaches: an aggressive offense balanced by a solid defense. Since the enemy we are facing in this case is our own body, then it is reasonable to think that a good defense is more appropriate to the task than a vigorous attack. Indeed, much of current medicine is focused on attacking the disease and/or its culprit, and preventive measures aimed at strengthening the host are a relatively new concept that is only recently gaining strength.

Cardiovascular diseases (CVD) represent a prime example where significant gains have been achieved primarily through preventative approaches. The demonstration, using epidemiological approaches, of multiple conditions that predispose an individual to disease (cholesterol, HDL, diabetes, smoking, etc.) lead to the development of statins, drugs that decrease the risk of CVD by lowering cholesterol levels. That early success in turn focused the attention on additional preventive approaches such as diet and exercise. This is undoubtedly a success story. However, all efforts so far have excluded paying attention to the one risk factor which is much stronger than all others combined: aging. Indeed, while a cholesterol level as high as 280 will give a score of 4 in the Framingham scale, being 75 years old gives by itself a score of 15.[2] But this fact is ignored in the medical profession for a very simple reason: the belief that there is nothing we can do about aging, so what's the point of discussing it? That is, however, a fallacy we are only now beginning to realize. There is a big difference between chronological age (that which is in your identification, and which is indeed immutable) and physiological age, the rate at which your body has deteriorated as a function

of the passage of time. A landmark 2015 study by Belsky et al.[3] indicates that, using physiological markers, the biological/physiological age of 38-year olds can vary dramatically, between 28 and 61 years (with a median, as expected, of 38 years). That high level of variability indicates that chronological age is by no means a good substitute for examining the real biological age of an individual. In fact, we intuitively know that while our chronological age cannot be modified, our physiological age is highly malleable, and an unconscious hope that good habits will lead us to a better aging is in large part what prompts us to try to eat properly, exercise, avoid smoking, etc. We hope that by doing so we will be in better health, which is just another way of saying "physiologically younger."

7.4 Linking aging to disease: geroscience principles

The idea that aging is the main risk factor for most diseases and conditions, coupled with advances in the basic biology of aging indicating that physiological age is malleable, led to the development of the concept of geroscience.[4] Geroscience proposes that, since age is the major risk factor for most or all chronic diseases affecting the older adult population, then addressing the so-called pillars/hallmarks of biological aging should lead to a generalized improvement of health and the postponement of diseases and conditions associated with aging.[5] Of course, this concept has existed since times immemorial, but the trigger that made it currently attractive is the definition of major modifiable drivers of the aging process.[6,7] For almost a century now, diet restriction was the only manipulation known to significantly extend lifespan. In the last couple of decades, scientists have used that paradigm and other approaches to identify hundreds of genes which, when individually manipulated, allow a partial recapitulation of life extension afforded by diet restriction. In turn, those individual genes helped identify a network of molecular pathways believed to control the aging process, including proteostasis, mitochondrial metabolism, nutrient sensing, and macromolecular damage/response to stress. In addition to these molecular events, research on cellular biology identified additional drivers of the process, such as cellular senescence, inflammation, stem cell robustness and, at least in some cases, telomere shortening.[6,7] While 2 models were proposed at a close interval, their differences are so minor compared to their similarities that most scientists refer indistinctively to one or the other. Of note, the microbiome and circadian rhythms represent two examples of additional potential drivers that were not identified originally but have come to the forefront in the last few years as potentially important and manipulable players. This underscores the fact that while we do appear to know the major players important in aging biology, we don't yet know all of them, so that more research into the biology of aging is urgently needed.

Inevitably, attempts to validate hypotheses about the relevance of these multiple hallmarks led to the testing of pharmacological interventions to modify each of them.

Drugs that aim at improving one or more hallmarks of aging have been termed "geroprotectors."[8] For example, one of the main pillars of aging identified to date is nutrient sensing, and an important intracellular nutrient sensor is mTOR (which stands for "mammalian/mechanistic Target Of Rapamycin"). Thus, a pharmacological approach was already implied in the name of the sensor, and indeed, an early success was the documentation in 2009 that rapamycin did indeed lead to an increase in lifespan in both male and female mice of a mixed genetic background, the HET3 mice.[9] Subsequent work from many laboratories has indicated that in mice and other species, rapamycin improves multiple health parameters, and provides resilience against many models of age-related chronic diseases. In fact, the data on the positive effects of rapamycin in mice is robust enough that attempts have been made to test the effect of rapamycin or its derivatives in higher animal models, as well as in humans. A small (phase 1) study in pet dogs indicated no side effects, and an improvement in multiple parameters of heart function.[10] Larger placebo-controlled clinical trials on pet dogs are ongoing, as well as smaller trials in other translational relevant animal models like marmosets, a small South American non-human primate. Furthermore, a series of studies in humans has shown that a rapamycin derivative, sirolimus, can improve the immune response of elderly subjects to influenza vaccination.[11,12] Indeed, while it is well known that older adults often respond poorly to vaccination (because of a phenomenon termed "immunosenescence"), a pretreatment with sirolimus and another mTOR inhibitor led to a 30% improvement in anti-influenza titers, 3 months after vaccination.[12] Perhaps more impressive, is that short pretreatment also led to a 40% decrease in overall infections in the treated group. This data is in stark contrast to the clinical literature about rapamycin and its derivatives, which are used as immune suppressors rather than adjuvants. It is possible that the positive effects of the drug on resilience and overall health overcome the negative immune suppressive effects, although it is also possible that rapamycin might not act at all as an immunesuppressor in healthy older adults. It should be noted that a recent phase 3 trial of the drug failed in its primary endpoint, though this endpoint differed from the endpoints tested in phase 1 and 2 trials. Therefore, research is expected, both in relation to anti-influenza and the timelier possible effects on the responsiveness to the newly-developed Covid vaccines. In addition to rapamycin, there is currently significant data accumulated on the positive effects of manipulating other hallmarks of aging, such as mitochondrial metabolism, cellular senescence and others. Of significance, is the strong current interest in senolytics, drugs that "preferentially" induce apoptosis of senescent cells.[13] Multiple senolytics and senomorphs (drugs that blunt the negative effects of senescent cells without actually killing them) have been identified and are being tested now in clinical trials at different levels up to phase 3. In fact, the strong data and interest in senolytics has recently led the US National Institutes of Health (NIH) to launch a major program to map and characterize senescent cells in healthy humans and mice

(RFA RM-21−008), Cellular Senescence Network: Tissue Mapping Centers (U54 Clinical Trial Not Allowed).

Many other geroprotectors are being tested at various levels, either alone or in combination; in models ranging from worms to mice and, in some cases, humans. These include, notably, compounds that boost the levels of NADH,[14] and which have been shown to improve health in small exploratory clinical trials, as well as other chemicals such as a-ketoglutarate, which acts on mitochondrial health.[15] An important but largely untapped approach is the use of combination therapies, either using multiple geroprotectors together, or using geroprotectors as a way to improve effectiveness of disease-specific interventions. For example, Campisi's group has shown that senolysis can improve the response of mice to chemotherapy, by greatly reducing treatment-induced fatigue.[16] Interestingly, the work also showed a significant correlation between chemotherapy-induced fatigue in humans and their levels of cellular senescence in T cells, suggesting that an application in humans might have a similarly positive outcome. Again worth emphasizing: the elimination of senescent cells is not a "directly anti-cancer" approach, but rather, the intervention addresses the issue of aging being the major risk factor for conditions such as chemotherapy-induced fatigue.

In terms of testing multiple geroprotectors at the same time, it is noteworthy that emerging data is beginning to show that many geroprotectors seem to act synergistically upon multiple aging pillars. Thus, for example, improving proteostasis has been shown to lead to positive effects on age-related changes in other areas including mitochondrial metabolism, inflammation, cellular senescence and stem cells.[17] This raises the alluring idea that perhaps it is not necessary to develop interventions against each of the hallmarks of aging, and that maybe combinations of different geroprotectors might allow a reduction of dose for each, thus lowering the possibilities of toxic or negative effects from individual compounds.

7.5 A common thread: improvement of resilience

How is it possible that geroprotectors act on so many different aspects of health and disease? After all, classical medicine has always emphasized the need for therapies to be highly selective, as a way to avoid unwanted secondary effects. But geroprotectors represent a completely different approach, where drugs are not meant to affect individual pathways involved in the physiopathology of specific diseases. Geroprotectors appear to act by altering pathways that increase the resilience of cells against external or internal attacks. In fact, early studies using genetically-modified mice with improved longevity (Dwarf mice) demonstrated that such mice, as well as the fibroblasts derived from them, were resilient to a full panel of chemical and physical stressors, including H_2O_2, heavy metals, UV light and others.[18] The concept of multiplex-stress resistance was used to describe this effect. The demonstration that mice treated with

geroprotectors as described above display resistance to several diseases as well as improved physical and cognitive performance suggest that all (or at least most) geroprotectors work by increasing resilience to both internal and external stresses. Such a characteristic should be a litmus test for whether an intervention can truly be called a geroprotector. But there is a catch: we don't really know yet how to measure resilience, either in mice or in humans.

7.6 Defining resilience at the molecular level

Before we go any further, we need to define resilience. As defined in Webster's Dictionary, resilience is "the capability of a strained body to recover its size and shape after deformation caused especially by compressive stress," or "an ability to recover from or adjust easily to misfortune or change." It is this last definition that has been used mostly in socioeconomic studies of, for example, the effects of early lifetime trauma on late-life health. The literature in this area is extensive, but unfortunately it is relatively lacking in mechanistic explanations of cause/effect. For that reason, for the purpose of the following discussion, I will focus on the concept of "molecular resilience" which has been defined as the ability of every cell in our organism to respond to a challenge and return to homeostasis. Relevant challenges in this context include both physical (UV light, radiation, heat, cold, starvation) and chemical challenges (oxidants, toxins, chemotherapy) that require an immediate response not only from a few "target" specialized cells, but by all cells in our body.[19]

Both physiological and molecular resilience decreases with advancing age. At the macro level, a quantitative example of this is the decrease in records for different sports with advancing age. For example, the speed at which a marathon can be run even by the best trained athlete will decrease as the person ages, because of a loss in the ability to properly respond to the challenge. At the clinical level, survival from surgery decreases with the increase in a patient's chronological age, as does the ability to mount a proper immunological response to vaccination. The molecular basis for this decreased capacity to mount a robust response to a challenge is still poorly understood.

It is important to specify that resilience and frailty are not just two sides of the same coin, and the loss of resilience is not directly equivalent to acquisition of frailty. Indeed, the timeframes of these two processes indicate that they are not equivalent. Frailty, as measured by either the Fried or Rockwood metrics, is something that occurs relatively late in life, usually after significant functional loss and early signs of disease. In contrast, resilience is something that is lost throughout adult life as described above, and it is independent of factors such as disease. It is therefore reasonable to suspect that, as the loss in resilience precedes the appearance of frailty, then the appearance of frailty might be the result of resilience loss passing a certain threshold. Once frailty appears, the organism becomes more susceptible to disease and death. In this

model, recurring challenges throughout life are normally well-handled by the young organism, but each time, the response might lead to a new (and less optimal) setpoint, so that as we age, the setpoint becomes less stable and easy to dislocate, thus leading to the appearance of frailty, where even minor challenges can no longer be dealt with appropriately.

In turn, frailty increases susceptibility to disease and death. Furthermore, these three elements (resilience, frailty and disease) seem to form a vicious circle in which each player can induce the appearance of the other two (Fig. 7.1). Nevertheless, simply based on their time of appearance during the course of life, it seems reasonable to suspect that resilience might be a major driver, since as described above, loss of resilience is already apparent in mid-life, while frailty and disease are more often late events. Of course, this is a generalization based on epidemiological studies, but as described by Ferrucci et al., each individual can have his/her own pathway leading to a loss of health.[20]

Based on these arguments, it seems relevant to develop measurements of resilience that can be applied to a healthy population, to assess risk of developing frailty and disease before these elements appear. Ideally, if we had validated measurements of molecular resilience, it might be possible to quantify the loss of resilience, between 40 and 60 years of age, and with that information, predict the likelihood that the individual will develop frailty and chronic diseases earlier or later than expected for his/her chronological age. To be clear, it would be expected that a decrease in molecular resilience should be observed for all individuals between the two ages chosen. The question therefore is not whether resilience has decreased, but rather, whether that decrease has occurred at the expected rate, or faster, or slower (Fig. 7.2). However, we do not currently have a method for measuring molecular resilience.

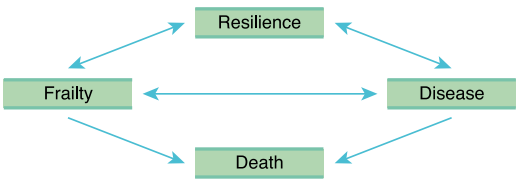

Figure 7.1 ***The relationship between resilience, frailty and disease.*** All three of these elements can and do lead to death, because all three of them interact with each other in a synergistic fashion that leads to overall physical deterioration. Because of these interactions, the initiating problem in a given individual could be any of the three. For example, a serious disease can lead to sudden onset of frailty and loss of resilience. Equally, incident frailty can accelerate the appearance of chronic disease and loss of resilience. On the other hand, as argued in the text, at the population level it appears that loss of resilience predates the appearance of either frailty or disease, and thus it appears reasonable that our efforts should be put in that element.

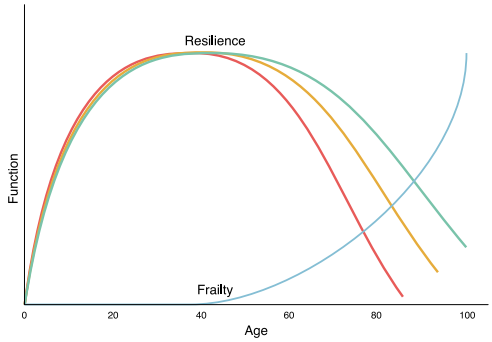

Figure 7.2 ***The value of measuring resilience early in life.*** Frailty is a condition that develops late in life, after the onset of disease and disability. In contrast, loss of resilience occurs throughout adulthood, and even in the absence of disease or significant loss in function. It is proposed that, if measured longitudinally at different times during adulthood (40 and 60 years in the figure) the rate of decay could be measured against the "expected," allowing a physician to assess if an individual is losing her/his resilience at the anticipated speed, faster or slower. If an individual is losing resilience faster than expected, then administration of a geroprotector would be an appropriate prophylactic to reduce the risk of later developing frailty, chronic diseases, or premature death.

7.7 Measuring molecular resilience

Different from measures of frailty, which is a static representation of the current status of the individual, measuring resilience requires the imposition of a challenge, so as to measure the maximal capacity to respond to such a challenge. In a sense, it is akin to the use of Seahorse technology to measure the maximal capacity of mitochondria to respond to successive challenges. However, because the goal is to measure overall resilience, a single stress test will not be sufficiently informative, because what is needed is a multiparametric, or multiplex resilience measurement, and applying multiple such stresses to any individual is obviously problematic, especially if the individual is already suspected of having low resilience capacity. While this issue is particularly difficult in humans, it is also problematic in animal models such as mice. It is in principle possible to calibrate tests to individual challenges such as cold exposure, chemotherapy, surgery, etc., however, application of a battery of such tests to a single animal will lead to negative interactions, where the stress of responding to a given challenge will necessarily impact the ability of the subject to properly react to a second or third stress, even if the stresses are unrelated. Thus, the task ahead is complex: we need to first define which stresses are the most relevant, then assess which recovery parameters are most informative for each of these stresses. Then it will be necessary to calibrate both the stress and the response, so as to obtain measurements that are discriminatory enough among different individuals. Finally, once all those elements have been optimized for the individual challenge/response combinations, then a series of successive tests would need to be devised so that multiple challenges can be applied in close succession. Then each test other than the

very first one would need to be recalibrated for an expected response that takes into account the stress produced by the previous test(s). It seems like a enormous task and it would obviously not be possible to apply it to humans.

Since we are defining molecular resilience as the ability of every cell to respond to challenges, a possible alternative is to develop tests that can be applied, not to the individual, but to cells derived from them. Because millions of cells can be derived from small biopsies (which by themselves could serve as a parallel in vivo test), such cells could be subjected to multiple stresses in parallel. The main limitation of this approach lies in proving whether or not cells in culture retain the resilience capacity shown in vivo by the organism from which they are derived. It has been shown that fibroblasts from long-lived mice indeed display multiplex resistance, as compared to control mice[18]; however, in that case, the difference in lifespan (and supposedly, resilience) was driven by a genetic mutation in the growth hormone pathway, and thus it makes sense that physiological effects of the mutation would be retained in the cells that still contain the mutation. In contrast, for example, it has been shown that while caloric restriction leads to an improvement in immune function when tested in vitro, actual immune function in the live animal is negatively affected by caloric restriction.[21] That is, the protective effect is lost once the cells are no longer under the stress of nutrient scarcity. Current efforts in the stem cell field, where recent work is starting to show that it might indeed be possible to retain epigenetic marks driven by aging after reprogramming, might help alleviate this roadblock.[22] Nevertheless, efforts are currently underway to test whether mouse fibroblasts in culture can recapitulate the resilience observed in live mice, but no results have been published to date.

But where do we start, if we want to standardize measures of resilience but we don't know the role of resilience in future health? It's a chicken-and-egg proposition, unless we accept the premise that decreased resilience early in life will lead to worse late-life health outcomes. The identification of a discriminating stress/response combination could separate young mice with strong and weak response, and then the animals can be left to age and their late-life health/longevity can be measured. This still requires the assumption that the testing itself did not differentially alter future health and longevity. Since the same test was applied to all individuals, this would be a reasonable assumption, except if we consider that, because of the extra effort required to mount a response, an individual with lower resilience might be more negatively affected by the testing itself.

A solution to this conundrum is to begin by testing, not in the individual (which is the ultimate goal), but in a cohort already known to display altered (improved or reduced) late-life health and longevity. Testing could be done in animals that differ in their late life outcomes due to changes in behavioral interventions, rather than genetic ones, such as for example diet-restricted mice, or mice allowed to exercise freely (extended health and longevity), as well as mice under a high-fat diet or exposed to non-lethal stresses such as chronic exposure to toxins (decreased health and longevity).

Such cohorts with pre-known endpoints of interest can therefore be used to standardize resilience measurements, both in the whole organism, and in cells derived from them and maintained in such a way as to preserve the epigenetic marks expected to be introduced by the behavioral manipulations.

To end this topic, ideally a composite measurement of resilience could include a mixture of measurements that can be done in the whole organism and others that need to be done in vitro. For example, even in a human patient, a cardio stress such as exercise to exhaustion could be combined with measuring the rate of closing of a small skin biopsy, which would provide the cells necessary to test more aggressive tests such as exposure to chemotherapy, UV or oxidative challenges.

7.8 We need to develop resilience metrics in animal models

As we have seen, developing resilience tests is not a trivial endeavor, and unless in vitro models can be validated, translating these methodologies to humans is even more challenging. And yet, it is crucial that we undertake this effort, not only as a prelude to deploying such tests in humans, but also because of the intrinsic value of developing the methodology in mice for research purposes.

Since longevity is always a relevant endpoint, research into the basic biology of aging has traditionally taken advantage of short-lived species. But even the roughly 3-year lifetime of mice can be a roadblock to progress in looking for geroprotectors and we need to establish pre-screening methodologies that can accelerate and focus the process. An effort has been made by the creation by the NIH of the *Caenorhabditis* Interventions Testing Program (CITP), designed to test potential geroprotectors in a genetically heterogeneous set of *Caenorhabditis* species (including, but not solely *C. elegans*).[23] The approach allows for the rapid testing of many potential geroprotectors, in multiple related species, a process that in principle allows for the identification of compounds that extend longevity in multiple genetic backgrounds. However, the testing is restricted to a single outcome, longevity, and it is done in species that are evolutionarily quite distant from humans. Development of resilience measurements that can be applied to young mammals and be predictive of future health and longevity would be invaluable in reducing the time, money and effort necessary to provide an early indication of possible efficacy, as well as assessment of potential side effects of new geroprotectors or combinations.

In addition, as with other aspects of biomedical research, developing methodologies in mice allows researchers to control many variables that are not possible to control in humans. Mice also allow for the assessment of the predictability of the technologies. As mentioned above, a major premise of the field is that measurements of resilience at a young or middle-aged point will be predictive of late-life health, morbidity and mortality. While reasonable, that premise has not been proven and, in addition, it is likely that not all possible challenges and response dyads will be equally

predictive. Yet to prove a prediction about health several decades down the line is problematic. For that reason, development of appropriate methodologies in mice is a necessary step before translation can be envisaged.

7.9 Translation of resilience measurements to humans

Measurement of resilience in humans is complicated. First of all, application of physical stresses and challenges is, in many cases, fraught with ethical issues. For that reason, attempts are being made to use "natural" stresses such as elective surgery and events such as bone fractures. In the case of the first category, the limitation is that the population that pursues elective procedures is not representative of the whole population, and in fact it is skewed towards more robust individuals. In the second category, the limitation is the absence of proper pre-event data. Nevertheless, these approaches are likely to provide early indications of possible measurements. On the other hand, events—programmed or not—will not be usable as measurements for the entire population, which has not been subjected to such events (and can't be induced to do so). Other stresses can be used if the response is measured more carefully. For example, exercise to exhaustion is used to measure cardiovascular fitness, and response to vaccines could be used to measure the immune response. While these stresses measure the function of specialized tissues, careful monitoring of the responses, beyond what is done in routine care, might be useful as a substitute measure of resilience.

In addition to measuring responsiveness in the whole patient as above, it would be ideal to develop measurements of resilience in vitro, in cells cultured from a small skin biopsy. However, for this approach to succeed, the same limitations described above apply, in terms of demonstrating that cells (usually fibroblasts) derived from individuals retain their resilience properties in vitro. As in the case of experiments done in Dwarf mice, it is likely that at least the genetic component of resilience might be retained, while the environmental modifiers retained in the epigenetic code might be lost in culture. It is crucial that further work on maintaining the age and environmental marks of cells in culture be accelerated.

7.10 Conclusions

Developing the methodology to measure molecular/cellular resilience is a challenging task. Multiple roadblocks will need to be addressed in order to develop reliable and predictive methodologies. We have discussed some of the issues that need resolution but, because of space limitations, the analysis has been superficial, and a more in-depth discussion within the research community is necessary. The challenges apply to efforts in any species, including humans, but there is a need to develop these methodologies

in mice, before we can begin to think about translating them to the human population.

One exciting possibility discussed is the possible use of cells in culture, rather than the entire individual. This is not only relevant in the case of humans, where ethical considerations would preclude the application of multiple stresses, but it is also an important avenue to explore in mice, since the development of a multiplex-resilience panel would require the application of multiple successive stresses to the same animal, thus severely complicating the interpretation of results. The use of cells in culture would dramatically simplify the task, but many issues remain unresolved, including the development of technologies to allow cells derived from patients (or mice) to retain the epigenetic marks suspected to represent the effect of the environment on the individual's resilience.

In spite of these challenges, development of such measurements is of paramount importance if we are to develop truly preventive health measures and implement geroscience-based approaches to human health.

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CHAPTER 8

A metabolic and mitochondrial angle on aging

Ana I. Duarte[1,2,*], Izabela Sadowska-Bartosz[3,*], Agnieszka Karkucinska-Wieckowska[4,*], Magdalena Lebiedzinska-Arciszewska[5,*], Carlos M. Palmeira[6,7,8], Anabela P. Rolo[1,2,8], Yaschar Kabiri[9], Barbara Zavan[10], Paolo Pinton[11], Fernanda Borges[12], Hans Zischka[9,13,†], John G. Jones[1,†], Grzegorz Bartosz[14,†], Paulo J. Oliveira[1,2,†] and Mariusz R. Wieckowski[5,†]

[1]CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[2]CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[3]Laboratory of Analytical Biochemistry, Institute of Food Technology and Nutrition, College of Natural Sciences, Rzeszow, Poland
[4]Department of Pathology, The Children's Memorial Health Institute, Warsaw, Poland
[5]Laboratory of Mitochondrial Biology and Metabolism, University of Coimbra, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Warsaw, Poland
[6]CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[7]CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[8]Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[9]Institute of Toxicology and Environmental Hygiene, Technical University of Munich, School of Medicine, Munich, Germany
[10]Department of Translational Medicine, University of Ferrara (c/o CUBO), Ferrara, Italy
[11]Department of Medical Sciences and Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy
[12]CIQUP, Department of Chemistry and Biochemistry, School of Sciences, University of Porto, Porto, Portugal
[13]Institute of Molecular Toxicology and Pharmacology, Helmholtz Center Munich, German Research Center for Environmental Health, Neuherberg, Germany
[14]Department of Bioenergetics, Food Analysis and Microbiology, Institute of Food Technology and Nutrition, College of Natural Sciences, Rzeszow, Poland

Abbreviations

TEMPO	2,2,6,6-Tetramethylpiperidinyloxyl
DNP	2,4-dinitrophenol
H_2DCFDA	2′,7′-dichlorodihydrofluorescein diacetate
HNE	4-hydroxynonenal
AICAR	5-aminoimidazole-4-carboxamide ribonucleotide
AMPK	5′AMP-activated protein kinase
8-oxodG	8-oxo-7,8-dihydro-2′ -deoxyguanosine
OGG1	8-oxoguanine glycosylase
AGEs	advanced glycation end-products
AOPP	advanced oxidation protein products
BPDE-I-DNA	benzo[a]-pyrene diolepoxide-I-DNA adducts

* these authors share co-first authorship
† these authors share senior authorship

Aging
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FCCP	carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone
CNS	central nervous system
SOD1	cytosolic superoxide dismutase 1
COX	cytochrome c oxidase
Marf	*D. melanogaster* orthologue of mitofusin
DRP1	dynamin-related protein1
ETC	electron transport chain
GSH	glutathione
GSSG	glutathione disulfide
GPx	glutathione peroxidase
GSSG-R	glutathione reductase
GST	glutathione S-transferase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
AntiOxBEN	hydroxybenzoic acid-derived mitochondriotropic antioxidant
AntiOxCINs	hydroxycinnamic acid-derived mitochondriotropic antioxidants
HO•	hydroxyl radical
TPP	lipophilic triphenyl-phosphonium
MDA	malondialehyde
mTORC	mammalian target of rapamycin complex
MLS	maximum lifespan
MsrA	methionine sulfoxide reductase-A
mtDNA	mitochondrial DNA
polymerase γ POLG	mitochondrial DNA polymerase
MFF	mitochondrial fission factor
FIS1	mitochondrial fission protein 1
mPTP	mitochondrial permeability transition pore
mROS	mitochondrial ROS
SOD2	mitochondrial superoxide dismutase
NAC	N-acetyl-cysteine
NMN	nicotinamide mononucleotide
NAMPT	nicotinamide phosphoribosyltransferase
NAFLD	nonalcoholic fatty liver disease
OXPHOS	oxidative phosphorylation
PCR	polymerase chain reaction
PUFAR	polyunsaturated fatty acid residue
PGI2	prostacyclin synthase
SIRT3	protein deacetylase sirtuin 3
PMF	proton motive force
RNS	reactive nitrogen species
ROS	reactive oxygen species
RISP	Rieske iron-sulfur polypeptide
PUM2	RNA-binding protein Pumilio2
SIRTs	sirtuins
SPF	specific pathogen free
SOD	superoxide dismutase
8-OHdG	the nucleoside 8-hydroxy-2′-deoxyguanosine
Trx2	thioredoxin 2
UCPs	uncoupling proteins
Dnm1p	yeast dynamin-related protein 1

8.1 Aging and longevity: revisiting the evolutionary perspectives and controversies

The increasingly aged human population (mainly in developed countries) represents a significant scientific achievement and privilege associated with medical, social, and economic progress. However, it also poses several challenges to national health and social care systems. Besides, the uncoupling of biological evolution with the fast technical progress achieved by humanity has minimized the role of natural selection and rendered aging almost an undesirable physiological event that most people desire to delay as much as possible. All this has been challenging modern gerontology to focus on potential strategies to extend the lifespan, but primarily to mitigate the negative thoughts often associated with aging and aged individuals (Fig. 8.1).

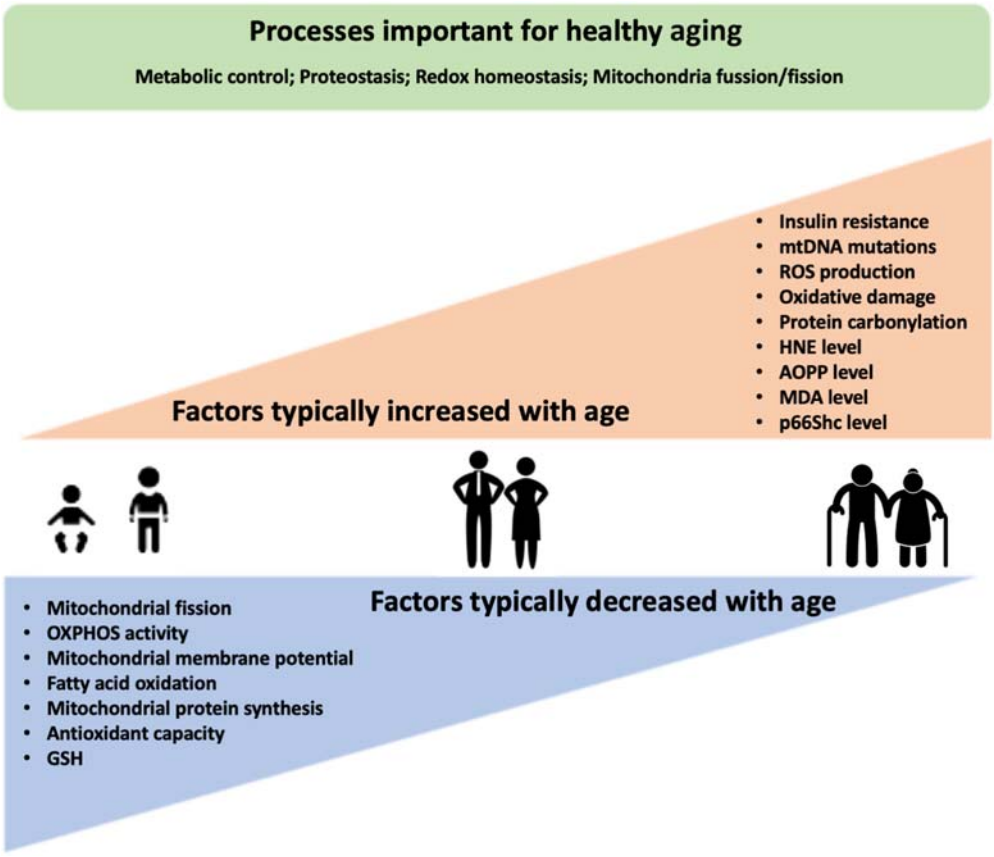

Figure 8.1 Aging-related alterations. Mitochondria- and ROS- related factors gradually increasing or decreasing with age.

Aging is not just a psychosocial matter, it involves molecular, cellular, and tissue changes towards a progressive impairment of metabolic function.[1] Indeed, mortality and morbidity increase exponentially upon aging and, though these factors might be insignificant in early life, the inevitable deterioration of tissues and organs over time leads to a notable increase in mortality in older age.[2] Therefore, we can ask ourselves whether: (1) aging is a gradual, time-dependent programmed event (or sequence of events)—*chronic phenoptosis*, and (2) if it would be advantageous to delay, slow, or even stop such events to further extend the organism's lifespan[1]—*neoteny*, studied in organisms such as the naked mole rat.[3,4] In this respect, we must remember that aging is an essential evolutionary mechanism that promotes diversity within a population (for example, due to the distinct phenotypic changes or development rate across individuals). Aging also constitutes a demographic buffer that accounts for eliminating the more senior and weaker individuals upon adverse conditions to maintain a young, healthy and reproductively competent population, thus increasing the pressure of natural selection and, ultimately, its biological evolution.[1] However, in some species (such as in the naked mole-rat, *Heterocephalus glaber*, or to a lesser extent, in *H. sapiens*) the social organization has minimized the role of natural selection and, therefore, aging became a harmful atavism that is sought to be replaced by an extension of youth (neoteny) to increase longevity.[3]

Humans more frequently aim to counteract the effects of aging, possibly through neoteny mechanisms, namely a rapid growth of the brain from a human embryo or the maintenance of immature brain structures across the lifespan.[5] This is supported by transcriptomic studies demonstrating that the reconstruction of the human prefrontal cortex after birth (particularly for genes associated with neuronal development and synaptic function) is much slower than in other primates,[6–8] thus prolonging the youth of the brain and delaying its senescence (as observed in the naked mole rats, as detailed later). Similar delays occur in lungs, skeletal muscle, eye, hair covering, or sexual maturation, but not in the heart, liver, or kidneys.[1] Taken together, this means that while aging could be considered a programmed sequence of events that are crucial for evolution, and neoteny could effectively extend the longevity of individuals, the lack of clarity on the precise mechanisms limits the development of interventions that could enhance this process.

8.2 Aging and longevity: challenging the traditional views for mitochondrial-derived oxidative stress

Controversies aside, dysfunctional energy metabolism is a well-described and evolutionarily-conserved aging feature.[9–11] In 1972, Harman proposed that aging arises from the progressive accumulation of byproducts of normal respiration within the mitochondria, the so-called mitochondrial free radical theory of aging.[12–14] The theory was based on two premises: (1) free radicals damage biomolecules (as evidenced by radiation chemistry), and (2) free radicals occur in living organisms (which was

demonstrated at this time).[15] Initially, free radicals were postulated to be the culprit of aging; later on, other reactive oxygen species (ROS) were also included. This theory has been developed and modified[16] and gave birth to several daughter theories. Emphasis on oxidative stress as the cause of oxidative damage prompted some authors to reformulate the theory as the oxidative stress theory of aging.[17]

More recent studies extended this notion by focusing on the oxidative injury to mitochondrial DNA (mtDNA) and its eventual effect on gene expression, DNA replication and mutation as putative causes of age-related mitochondrial and tissue damage[18–21] **(**Fig. 8.2**)**. Accordingly, aging can promote mtDNA damage in humans,

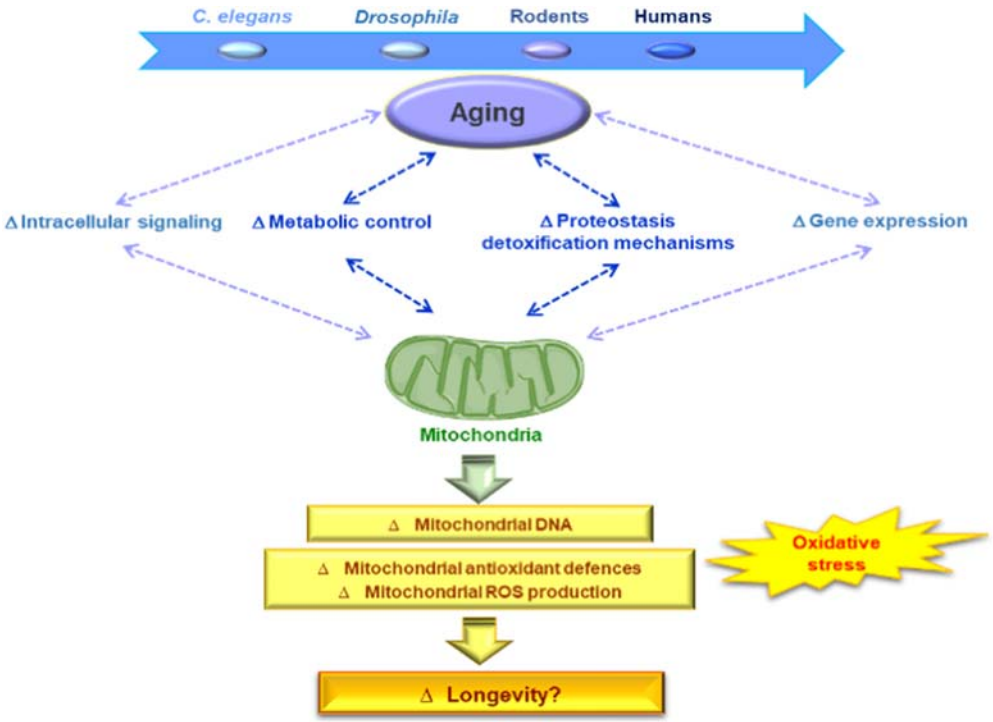

Figure 8.2 Mitochondria-derived oxidative stress in aging and longevity. More than a psychosocial issue, aging comprises many progressive molecular, cellular and tissue changes across time that alter the metabolic and mitochondrial function, ultimately rendering the organism more prone to stress, disease and death. Adding to this, aging is an essential evolutionary mechanism and a demographic strategy to promote diversity within a population and the biological evolution of species. Among the mechanisms that affect and are affected by aging, we emphasize, for example, the changes in intracellular signaling pathways, the regulation of intracellular metabolism and proteostasis/defense mechanisms, or gene expression. These aging-related alterations may negatively impact the mitochondrial function and DNA, leading to excessive (mitochondrial) ROS and a deficit in mitochondrial antioxidant defenses. As a consequence, oxidative stress may arise, ultimately affecting the species' longevity. Abbreviations: Δ- alteration; *ROS*, reactive oxygen species.

rodents, *Drosophila melanogaster* or *Caenorhabditis elegans*,[13,22–25] and such injury is exacerbated in higher organisms lacking proper antioxidant defenses and reduced in those overexpressing mitochondria-targeted catalase (see below). Moreover, in *C. elegans* lacking the *mpst-1* gene, the absence of the mitochondrial enzyme that synthesizes hydrogen sulfide downregulated this antioxidant/modulatory molecule, thus increasing ROS levels and mtDNA damage, and reducing life and healthspan.[26–28] Analogous results were obtained in *C. elegans* lacking the *mev-1* gene that encodes for a key component of the mitochondrial complex II - the succinate dehydrogenase cytochrome b: complex II activity was downregulated, increasing mitochondrial ROS production and oxidative damage, and ultimately reducing their lifespan.[29–31] Nevertheless, and despite the more extensive nuclear and mitochondrial DNA oxidative damage, the longevity of mice heterozygous for the mitochondrial superoxide dismutase (SOD2) did not change significantly.[32] Similar results occurred in *C. elegans* depleted of SOD2.[21] Others also failed to correlate the mtDNA content in guanine-cytosine with the resting metabolic state, further determining mammalian longevity.[33–35] One possible explanation for such apparent discrepancies could be the methodological limitations in the measurement and availability of validated markers of the oxidative damage to mtDNA, in contrast with nuclear DNA assessments.[21] Moreover, we cannot exclude that such inconsistencies may reveal that mtDNA changes may not control aging and longevity or that this relation may not be as simple as initially expected.[21] It is also possible that a compensatory activation of, for example, intracellular signaling, ROS production/detoxification, or DNA repair/turnover mechanisms might contribute to such contrasting observations.[21] Moreover, it remains debatable whether such increased mtDNA injury is a cause or a consequence/symptom of aging.

Better metabolic regulation and improved proteostasis/intracellular quality control mechanisms may exert beneficial roles in delaying aging and extending lifespan; all mechanisms in which mitochondria play a crucial role.[21] Accordingly, recent studies revealed the importance of mitochondrial dynamics, including fusion and fission events, in maintaining cellular homeostasis and, ultimately, in healthy aging and longevity.[36] The disruption of mitochondrial fusion and fission mechanisms affects the mitochondrial morphology in different organisms.[37–41] Although Byrne et al. observed that locomotor activity was impaired in *C. elegans* depleted from mitochondrial fission or fusion genes in an age-dependent manner, such changes were more visible in those lacking the fusion machinery.[36] These fusion-depleted nematodes also presented more evident alterations in mitochondrial morphology, especially at the earlier stages of muscle development.[36]

Conversely, fission proteins' loss was more detrimental in the *C. elegans* neurons, especially at the later stages of adulthood.[36] In a sophisticated approach, Chen et al. observed that blunting fusion mechanisms in disrupted fission models improved tissue function and lifespan.[42] In contrast, fission disruption rescued the deficits in

mitochondrial morphology, locomotion, and neuronal function upon losing fusion genes.[36] Others found that regardless of the mitochondrial dynamic mechanism affected, the health and behavior of *C. elegans* was reduced, thus reinforcing the need for a tight balance between mitochondrial fusion and fission mechanisms across aging, with fusion playing a homeostatic role in mitochondrial health and longevity.[36,43] On the other hand, fission may intervene mainly in later life to counteract age-related stress (for example, by promoting the clearance of abnormal mitochondria through mitophagy).[36,44]

An alternative key role of mitochondria in the aging process is activating the innate immune system, which stimulates ATP and ROS production to initiate antiviral and antibacterial mechanisms.[45] Studies demonstrated that the impairment of the mitochondrial respiratory chain elicited immune mechanisms in several organisms (as further detailed in Mao et al.).[46] A recent paper described that mutation of the mitochondrial chaperone *hsp-6/mtHSP70* gene activated transcriptional signaling mediated by MDT-15 and the nuclear hormone receptor, NHR-45 to initiate a xenobiotic or immune response.[46] Notably, this process was blunted by removing *mdt-15* or *nhr-45* and improved the health and lifespan of *C. elegans*.[46]

8.3 Changes of mitochondrial function and structure associated with aging

8.3.1 Oxidative phosphorylation and aging

Mitochondria, the center of oxidative metabolism, are crucial in both health and in many human diseases' pathogenesis. Thus, it is not surprising that the mitochondrial bioenergetics' alterations have been repeatedly associated with aging and cellular senescence.[47–50] In the early 1990s, a progressive loss of complex IV activity in the human extraocular muscles had already been demonstrated as a hallmark of the aging process.[51] A similar observation was reported in human limb muscles and in the diaphragm.[52] The authors observed first sporadic alterations in the 3rd and 4th decade, and then more obvious alterations in the 6th to 9th decade of life.[52] The studies conducted in Chinese populations of various ages (from 31 to 76 years old) showed that respiratory control and ADP/O ratios measured in liver mitochondria decrease with age.[53] Pharmacologic inhibition or genetic manipulation of oxidative phosphorylation (OXPHOS) complexes has been shown to promote the aging process. Inhibition of complex I by rotenone[54,55] or knockdown of the complex I assembly factor NDUFAF1[47] have been shown to induce cellular senescence. Likewise, decreasing complex II activity by desferroxamine mesylate can also induce premature senescence.[48] Compromised complex III activity by either knockdown of the mitochondrial Rieske iron-sulfur polypeptide (RISP)[56] or complex III inhibition with antimycin A can also induce a senescence arrest in cells.[57]

The aged mammalian brain shows a decreased capacity to produce ATP by oxidative phosphorylation, and it is assumed that this decreased capacity for energy production becomes a rate-limiting factor under physiological conditions in aged individuals. Detailed analysis of bioenergetic parameters in the brain and heart mitochondria from young (2 months) and old (28 months) rats performed by Cocco et al. showed an age-related decrease in brain respiratory fluxes but not in heart mitochondria. The age-related decrease in the respiratory rate in the presence of NAD-dependent substrates was associated with a consistent decline in complex I activity in brain mitochondria. On the other hand, heart mitochondria showed an age-related decline of complex II activity.[58] Mitochondria from senescent cells show decreased mitochondrial membrane potential, increased proton leak, and increased ROS generation.[59–61] It was recently reported that mitochondria from senescent cells had decreased fatty acid oxidation, resulting in increased accumulation of lipids.[62]

Pandya et al. investigated alterations of mitochondrial bioenergetics parameters in liver, lung and heart of young, adult, middle-aged, and old-aged male Brown Norway rats, and found a decreased ATP-generating respiration (state III respiration) in heart and lung mitochondria of the aged animals. Interestingly, an opposite result was observed in liver mitochondria. Moreover, heart and liver mitochondrial bioenergetic rates and enzyme activities remain higher than those in the lung across all stages of aging.[63] The studies performed by Navarro et al. showed that mitochondria isolated from the brain of aged animals have decreased membrane potential and increased size and fragility.[64,65] In comparison to young (4 months) rats, hippocampal mitochondria isolated from aged (12 months) and senescent (20 months) animals had decreased state III respiration with NAD-dependent substrates as well as decreased in the activities of complexes I and IV of OXPHOS.[65] Age-dependent rat brain alterations in OXPHOS complexes and further mitochondrial proteins, such as HSP60, mitochondrial aconitase and ATP synthase have been demonstrated by Dencher et al. Interestingly, the authors found age-related alterations in the oligomerization state of the ATP synthase and age-related changes in the supramolecular architecture of OXPHOS complexes, all of which are consistent with a decreased rate of ATP production with age.[66]

Another tissue particularly affected by age-related degenerative symptoms is the muscle. Muscle wasting is a hallmark of aging and the primary reason for declining physical abilities.[67] Rooyackers and colleagues studied a decrease in muscle endurance capacity due to mitochondrial decay that follows the aging process intensively. They described a decline in muscle mitochondrial protein synthesis rate in middle-aged human individuals (54 ± 1 year) compared to young individuals (24 ± 1 year). Interestingly, no further decline in the rate of mitochondrial protein synthesis occurred at an advanced age (73 ± 2 years). Observed muscle proteome changes were accompanied by decreasing activities of mitochondrial enzymes accelerating with increasing age.[68] Quantitative proteomic analysis of skeletal muscle obtained from healthy

individuals aged 20 to 87 years showed that in muscles from older persons, proteins related to energetic metabolism were downregulated. On the other hand, proteins involved in innate and adaptive immunity, proteostasis, and alternative splicing were upregulated.[69]

Comparison of bioenergetic parameters and protein expression levels in synaptic mitochondria isolated from mature (5 month old), old (12 month old) and aged (24 month old) mice performed by Stauch et al. revealed that proteomic alterations found in synaptic mitochondria correlate with preservation of mitochondrial function.[70] Further studies by Stauch et al. showed that despite changes in the expression profiles of several metabolic pathways, including glycolysis, the tricarboxylic acid cycle, and oxidative phosphorylation, mitochondrial bioenergetic parameters were unaffected. The authors postulated that the observed proteomic changes during aging may compensate for functional defects aiding in mitochondrial function preservation.[71]

Genomic studies performed using *C. elegans* and *D. melanogaster* also confirmed that the mitochondrial electron transport chain components are affected by aging. In both cases, an approximately twofold decrease in the expression level of several genes encoding proteins involved in ATP synthesis and mitochondrial respiration has been found.[72] Comparison of the transcription profiles of genes encoding mitochondrial proteins in atrial tissue from aged ($\geq$65 years old) and comorbidities-matched adult ($<$65 years old) patients showed that the expression of 87 (out of 1008) genes encoding mitochondrial proteins were downregulated (87/101) what was accompanied by a significant decrease in the functional activity of complex I.[73] An interesting study showing alterations in gene transcripts and functional activity of mitochondrial oxidative phosphorylation complexes in aging hearts found that almost 90 out of 614 genes encoding mitochondrial proteins in ventricles of aged rats were downregulated. Among them, 39 affected genes were encoding proteins involved in oxidative phosphorylation. Alterations in the heart of aged animals' gene expression profile resulted in reduced mitochondrial functional capacity, with decreased complex I and complex V activities.[74]

A decline in mitochondrial function can be associated with normal aging and the development of a wide range of age-related diseases such as intestinal disorders (e.g., mucosal barrier dysfunction, intestinal dysmotility, and chronic constipation), that are highly prevalent in the elderly population. Schneider et al. showed that intestinal disorders are associated with an age-dependent decline of mitochondrial function.[75] It has also been demonstrated that age-related accumulation of mutations in mtDNA affecting OXPHOS complexes may harm the intestinal epithelium function.[75,76] Multiple reports suggest that cytochrome c oxidase-deficient crypts increase in size with age, possibly due to the accumulation of mtDNA mutations during aging.[76–79] Greaves et al. found that in colonic epithelium from human subjects aged 18–84 y, approximately 50% of crypts present some form of respiratory chain deficiency or decreased expression of subunits of multiple complexes.[75,76]

8.3.2 Mitochondrial morphology and dynamics in aging

Mitochondrial morphology in living cells is heterogeneous and can range from small spheres to interconnected tubules. Changes in mitochondrial shape have been related to many different cellular processes such as development, neurodegeneration, calcium signaling, ROS production, cell division, and apoptotic cell death.[80] An interesting study compared the impact of aging on the ultrastructure and mitochondrial function in mouse tissues and in *D. melanogaster*. They found that cardiac mitochondrial function and ultrastructure were maintained during aging. About 50% of mitochondria in aged flies showed altered inner membrane organization and compromised respiratory activity.[81] Sastre et al. showed that in both intact hepatocytes and isolated liver mitochondria, decreased mitochondrial membrane potential and increased mitochondrial size can be correlated with aging, whereas the number of mitochondria per cell and mitochondrial mass was unchanged.[82]

Several proteins involved in mitochondrial fission are dysregulated with age, which likely contributes to the changes in mitochondrial network architecture seen in old organisms.[83] Aged mice demonstrate reduced DRP1 activity and altered mitochondrial morphology in several tissues, including neurons, skeletal muscle, and oocytes.[39,84] Alterations in mitochondrial dynamics (fusion and fission) have been shown to trigger cellular senescence.[85–87] The fission 1 (FIS1) protein is downregulated during senescence, leading to mitochondrial elongation.[88] On the other hand, processes that stimulate mitochondrial fission reduce senescence-associated phenotypic changes.[86,88] Changes in mitochondrial dynamics associated with the aging process were observed in different models including *D. melanogaster*, and fungal models (*Podospora anserina* and *Saccharomyces cerevisiae*). In fungal models, dynamin-related protein 1 (Dnm1p) deletion, mediates fission, retards aging, and extends lifespan.[89] On the other hand, *D. melanogaster* models show an opposite trend. Upregulating Drp1 expression in midlife extends the lifespan of *D. melanogaster*.[90] Similarly, overexpression of Parkin in *D. melanogaster* extended lifespan, and was accompanied by a reduced level of the *D. melanogaster* orthologue of mitofusin (Marf), which typically increases in amount with age in flies.[91] Interestingly, in *D. melanogaster* ovarian germline stem cells, mitochondrial dynamics shifted toward fission during aging.[92]

Aged skeletal muscle in mice shows an increased mitofusin 2/DRP1 ratio and longer intermyofibrillar mitochondria.[93] Furthermore, aged human endothelial cells (HUVECs) show a similar downregulation of both DRP1 and FIS1 expression, as well as elongated mitochondrial networks.[85] Recently, it was found that the RNA-binding protein Pumilio2 (PUM2) binds Mitochondrial Fission Factor (MFF) mRNA and prevents its translation. Coupled with the finding that PUM2 levels increase with age in humans, mice, and nematodes, this study demonstrates a post-transcriptional mechanism by which mitochondrial fission and mitophagy are impaired.[94] Moreover,

both anterograde and retrograde mitochondrial trafficking decrease with age.[83,95] Mitochondrial dynamics link to existing pathways that regulate lifespan in *C. elegans*. Mitochondrial trafficking in distal neuronal processes declines progressively with age of *C. elegans* but in the case of long-lived *daf-2* mutants, the authors observed delayed age-associated changes in mitochondrial morphology, constant mitochondrial density and maintained trafficking rates.[96] Thus, at present, there is no clear association between either fusion or fission and lifespan; and promoting either process may have diverse effects, depending on the signaling and metabolic context. Several lines of evidence support the notion that a decline in mitochondria functions contributing to aging is generally accompanied by alterations in the activity and/or the level of mitochondrial enzymes. Changes in the mitochondrial morphology and dynamics have been summarized by Sun et al.[97] as well as by Giorgi et al.[98]

8.4 A metabolic angle on aging

From the large number of studies on aging that have been made on numerous organisms representing all parts of the tree of life, a common theme is that aging is associated with fundamental alterations in the central metabolic fluxes that sustain energy generation and biosynthesis of cell components. In mammals, the essence of these alterations can be thought of as a systemic breakdown in energy and carbon homeostasis[11] that is likely visible in all tissues, although some are much more affected than others. Given the importance of intracellular substructures and organelles in metabolism, the ultimate factors of metabolic dysfunction related to aging, such as the onset of oxidative stress above and beyond the tonic level of normal physiological function and the accumulation of damaged proteins, are intimately intertwined with a damage of these structures. Here, we will highlight aging effects by focusing on dietary carbohydrate, lipid, and protein metabolism. Nutrient metabolism provides interesting case studies that illustrate both the multifactorial and feed-forward mechanisms that contribute to cellular dysfunction and death. It also underpins the most effective intervention currently known for delaying the effects of caloric restriction aging.

8.4.1 Carbohydrate metabolism and aging

Carbohydrate metabolism has many aspects that make it susceptible to the factors of aging. The main product of nutrient carbohydrate metabolism is glucose. It is the primary fuel for the brain and central nervous system (CNS); hence a highly complex system that coordinates glucose metabolism in many peripheral tissues such as the liver, kidney, and skeletal muscle has evolved to ensure the constant supply of glucose to the CNS independently of its dietary availability. This is enabled by maintaining a remarkably constant concentration of circulating glucose through the selective control of glucose uptake by tissues that are not obliged to oxidize glucose for energy, such as

the skeletal muscle. Glucose uptake is coordinated with tight regulation of endogenous glucose production by the liver and kidney. While there are various biological control mechanisms involved in maintaining glucose homeostasis, the most important is the insulin-glucagon axis. When there is a surfeit of glucose, such as during meal absorption, the principal actions of insulin are to attenuate any rises in blood glucose levels by stimulating glucose uptake in tissues such as adipose tissue and muscle while at the same time suppressing endogenous glucose production and promoting glycogen synthesis in the liver. When glucose becomes scarce, and blood glucose levels fall, glucagon restores normal glucose levels by stimulating endogenous glucose production through glycogen mobilization and promoting de novo synthesis of glucose from other precursors, a process known as gluconeogenesis. One of the most well-known processes associated with aging is the development of insulin resistance, where the efficacy of insulin-mediated glucose uptake by adipose tissue and skeletal muscle and its suppression of hepatic glucose output are impaired. This results in a chronic elevation of blood glucose levels, a condition known as hyperglycemia.

Among other consequences, an overabundance of glucose results in the deleterious modification of the structure and function of many proteins by glycation,[99] a spontaneous process mediated by the reactive aldehyde functionality of the open-chain form of glucose. Thus, these glycated proteins contribute to the overall accumulation of damaged proteins. Another consequence of excess glucose is that it begins to be metabolized by non-canonical pathways that generate even more reactive glycating agents, notably methylglyoxal, that further add to the burden of damaged proteins.[100] Alongside this disruption of glucose homeostasis, oxidative stress products directly impair glucose's glycolytic metabolism in affected tissues by inactivating glyceraldehyde-3-phosphate dehydrogenase (GAPDH). They also cause aggregation of inactivated GAPDH to form a highly cytotoxic entity that causes mitochondrial dysfunction.[101] Thus, in summary, both the systemic regulation of glucose levels and its metabolic fate are compromised by aging. The consequences include the erosion of glycolytic capacity and an increased burden of damaged intracellular proteins. As mentioned, insulin resistance is a crucial driver of these derangements, which begs the question of how this condition originates in the first place. It seems that the answer to this, at least in part, lies in aging-induced alterations of nutrient lipid metabolism.

8.4.2 Lipid metabolism and aging

In most mammals, lipid oxidation accounts for most whole-body energy generation, accounting for ~80% of ATP synthesis in cardiac muscle and more than 50% for aerobically exercising skeletal muscle. Lipids, specifically triglycerides, are also the most compact and inert form of energy substrate storage, for which adipose tissues have evolved to sequester and efficiently mobilize when required. Importantly, when there is an

excess of other macronutrients, notably carbohydrates, lipids are converted to triglycerides by both the liver and adipose tissue. After an extended period of overfeeding, which is defined as an excess of caloric intake over that expended, triglycerides accumulate. Adipose tissues accommodate this by expanding the size of individual adipocytes and generating additional adipocytes through adipogenesis. When there is a demand for lipid oxidation by other tissues such as muscle, triglycerides are hydrolyzed to fatty acids and glycerol, and these are released into the bloodstream. Any fatty acids that are not oxidized are re-esterified by the liver and transported back to adipose tissue. While triglycerides themselves are metabolically inert, free-fatty acids,[102] as well as some precursors of triglyceride synthesis such as fatty acyl CoA and 1,2-diacylglycerols,[103] can have highly disruptive effects on metabolic control, in part by interfering with insulin signaling. Aging promotes the increase of the above and other so-called lipotoxic species in many different tissues, but in particular, liver, heart, kidney and skeletal muscle.

Lipotoxicity is mediated in two main ways. The first is through promoting the loss of mitochondrial function and their metabolic capacity to oxidize free fatty acids, thereby attenuating the main route for fatty acid clearance via oxidation.[104] Thus, there is an accumulation of fatty acids and long-chain acyl-CoA species in the cytosol, where they have access to insulin-signaling components. The second is by promoting a shift in lipid distribution from adipose tissue to other tissues to form so-called ectopic lipid pools.[105] Aside from some special exceptions, such as high-endurance athletes, the accumulation of ectopic lipid in a given tissue is invariably associated with metabolic perturbations that in part are mediated by lipotoxicity. In the liver, this manifests as nonalcoholic fatty liver disease (NAFLD), while in the skeletal muscle, this is associated with impaired insulin-mediated glucose uptake. Thus, age-induced dysfunctions in lipid metabolism also promote loss of glucose homeostasis through the interference of insulin actions.

8.4.3 Protein metabolism and aging

Dietary protein is digested into amino acids, and a significant fraction of these are utilized to synthesize muscle protein. Muscle protein is continually turning over, and a stable lean body mass represents a balance between anabolic synthesis from amino acid precursors and catabolic degradation back into amino acids. A positive balance between synthesis and breakdown provides the basis for muscle remodeling and increased muscle mass, while a negative balance results in atrophy and loss of strength. This balance between anabolic and catabolic fluxes is regulated by the mammalian target of rapamycin complex, (mTORC)[106] which is responsive to both nutrient and endocrine states. Anabolic fluxes are highly upregulated with meal ingestion and elevated amino acid levels, increased insulin concentrations, and high intracellular ATP levels, contributing to the activation of protein synthesis via mTORC.[107]

In comparison, the rate of muscle protein breakdown to amino acids is relatively constant. Aging is associated with muscle mass loss and, consequently, body strength in a process known as sarcopenia.[108] This is in part due to an attenuated stimulation of muscle protein synthesis in the postprandial state and, may reflect decreased tissue ATP levels secondary to mitochondrial dysfunction[109] and impaired insulin actions.[110]

8.4.4 Nutrient metabolism and caloric restriction

In many different organisms, caloric restriction prolongs lifespan and delays or attenuates much of the aging comorbidities.[111] Concerning macronutrient metabolism, many of the adverse factors associated with aging are promoted or exacerbated by excessive food intake, particularly lipids and carbohydrates. While the most significant effects of caloric restriction on both longevity and quality of life are obtained through an entire lifetime of adherence,[112] it appears that engaging in caloric restriction at any stage of life, including advanced age, can bring benefits.[113] In this regard, it is notable that for type 2 diabetes and NAFLD, diseases that have factors common to aging, such as loss of insulin sensitivity and dyslipidemia, caloric restriction is also a highly effective intervention.[114,115]

8.5 Oxidative stress and aging

8.5.1 Reactive oxygen species and their reactions

Energy production in aerobic cells is mainly based on reactions of biological oxidation. However, apart from the reactions that fuel cellular energetics, many uncontrolled oxidation reactions lead to ROS formation **(**Fig. 8.3**)**. ROS are formed in endogenous reactions, including autoxidation of reduced compounds such as metal ions and thiols, as by-products of some enzymatic reactions, and induced by exogenous factors such as ionizing and UV radiation, ultrasound and many xenobiotics. The main cellular sources of superoxide are mitochondria, in which a fraction of oxygen is reduced in a one-electron pathway instead of being fully reduced to water[116] and NADPH oxidases, generating superoxide as the main product.[117] In mammalian mitochondria, seven major sites of superoxide production have been identified: the ubiquinone-binding sites in complex I (site IQ) and complex III (site IIIQo), of the electron transport chain (ETC) glycerol 3-phosphate dehydrogenase, the flavin in complex I (site IF), the electron transferring flavoprotein:Q oxidoreductase of fatty acid beta-oxidation and pyruvate and 2-oxoglutarate dehydrogenases.[118] Estimates of the fraction of oxygen which is subject to one-electron reduction in the mitochondrial respiratory chain by various researchers differ in a broad range (0.1%–5%), although likely to be in the lowest part of this range. The factors that control ETC generation of ROS in vivo are not fully understood. Much of our current understanding stems from

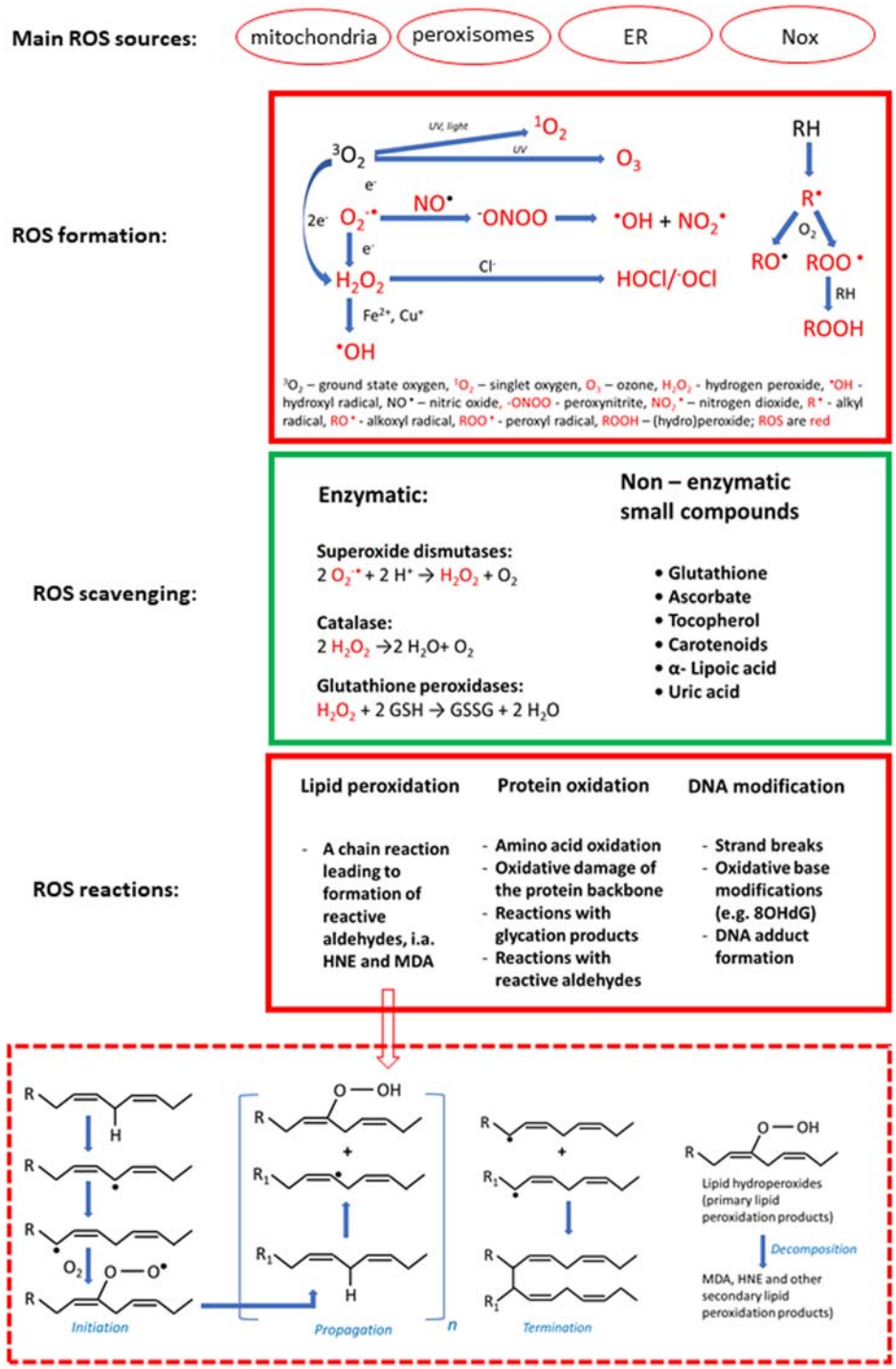

Figure 8.3 Formation of main reactive oxygen species (ROS), lipid peroxidation and ROS scavenging. Excitation of ground-state (triplet) oxygen molecule $^3O_2$ generates a singlet oxygen. One-electron reduction of molecular oxygen forms superoxide radical anion, two-electron reduction leads to the formation of hydrogen peroxide. The reaction of H_2O_2 with transition metal ions is the primary source of hydroxyl radical, the most reactive ROS. Oxidation of chloride ions by H_2O_2 forms hypochlorite. The reaction of superoxide with nitric oxide generates peroxynitrite (the two latter species are also called reactive nitrogen species, RNS).

studies with isolated mitochondria and cells.[119] It has been found that the primary determinant of mitochondrial ROS (mROS) production is the redox state of the ETC. For example, inhibition of ETC electron carriers typically causes them to be more reduced, which increases their propensity to generate superoxide. Another major determinant of mROS production is the proton motive force (PMF) across the inner mitochondrial membrane. The PMF is generated as protons are extruded out of the matrix into the intermembrane space by complexes I, III, and IV of the ETC. An increase in mROS production is observed when PMF increases.[120]

ROS are generated under physiological conditions and play a role in cellular signaling.[121,122] Their lifetime and local concentrations, and thus reactions are controlled and kept within physiological limits by antioxidant enzymes and antioxidants. However, situations may occur in which there is an excessive generation of ROS, or the levels of antioxidants/antioxidant enzymes are too low to keep control of ROS; such situations are called oxidative stress (OS). Classically, oxidative stress was defined as "a disturbance in the pro-oxidant-antioxidant balance in favor of the former."[123] A more recent definition refers to oxidative stress as "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage."[124]

ROS differ in their reactivity, the most reactive being the hydroxyl radical $HO^{\bullet}$, which has the highest standard redox potential of 2.31 V (thus oxidizing all biomolecules) and reacts with most molecules at diffusion-controlled rates (second-order rate constants of an order of 10^9 M/s). Thus, reactions of $HO^{\bullet}$ are rapid and non-selective. Hydroxyl radicals may abstract the α-hydrogen from polyunsaturated fatty acid residues (PUFARs), initiating the chain reaction of lipid peroxidation, damage proteins, carbohydrates, and nucleic acid bases.[125]

Superoxide anion radical and hydrogen peroxide are considerably less reactive and can react with a smaller group of substrates. In the cell, their main targets are FeS clusters and thiol groups. Reversible oxidation of the most reactive thiol groups of proteins is the main mechanism of "redox signaling."[126] Protein phosphatases appear to be especially susceptible to controlling their activity by ROS in this manner, as they possess a reactive cysteine that can be reversibly oxidized in their catalytic domain, and such oxidation inhibits their dephosphorylation activity.[127] Specific examples of protein phosphatases known to be regulated in this manner are protein tyrosine phosphatase 1B (PTP1b), phosphatase and tensin homolog, and mitogen-activated protein kinase (MAPK) phosphatases.[128]

Abstraction of the α-hydrogen from a polyunsaturated fatty acid residue (PUFAR) of a phospholipid, initiating lipid peroxidation, is relatively easy from a chemical perspective. Moreover, lipid peroxidation is a chain process, which may proceed with a high yield, so its products are relatively abundant. For these reasons, lipid peroxidation was the first and most extensively studied consequence of OS. Abstraction of the

α-hydrogen leads to the formation of an alkyl radical and rearrangement of double bonds. The alkyl radical reacts with oxygen forming a peroxyl radical, reactive enough to abstract a α-hydrogen from another PUFAR, which propagates the chain reaction and forms a lipid hydroperoxide (a primary product of lipid peroxidation).

Further transformations of lipid hydroperoxides (mainly β-scission) generate secondary products of lipid peroxidation, among them malondialehyde (MDA), 4-hydroxynonenal (HNE) and 4,5-dihydroxydecenal **(**Fig. 8.3**)**. Consequently, a popular, although unspecific, assay for measuring oxidative stress via lipid peroxidation is the colorimetric assay based on the reaction of MDA with thiobarbituric acid.[129,130] Lipid peroxidation also generates isoprostanes; F2-isoprostanes are considered better biomarkers of lipid peroxidation and in vivo indicators of oxidative stress than the thiobarbituric-reactive substances on which the MDA assay is based.[131]

Aldehyde groups of reducing sugars react with thiol and amino groups of other entities (mainly proteins, as these are, in terms of mass, the most abundant biomolecules in the organism) forming Schiff bases. This reaction is the first step of the nonenzymatic glycosylation (glycation) of these compounds. Rearrangement of Schiff bases initiates reactions leading to the formation of advanced glycation end-products. As ROS are formed and participate in these reactions, and oxidation reactions accompany glycation, the whole process is often referred to as glycoxidation.[132,133] Apart from glycoxidation, carbohydrates can also be targets for ROS action, which can cause strand scission and modification of saccharide residues. Since carbohydrates often play a role in protein and cell recognition and in antigens, oxidative modifications may lead to alterations in their antigenic properties and abilities to serve as substrates for recognition.[134]

Aggressive ROS can also induce formation of protein peroxides (protein peroxidation)[135] which, like lipid peroxides, are relatively unstable and enter into further reactions forming more stable products. ROS's most commonly assayed protein oxidation products are carbonyls, resulting from oxidation of mainly amine groups to carbonyl derivatives. The main carbonyl protein oxidation products are: glutamic semialdehyde, a product of oxidation of arginine; aminoadipic semialdehyde, a product of lysine oxidation; 2-pyrrolidine, a product of histidine oxidation; and 2-amino-3-ketobutyric acid, a product of threonine oxidation.[136] The two significant compounds which contribute to protein carbonyl groups are γ-glutamyl semialdehyde and aminoadipic semialdehyde. After metal-catalyzed oxidation, they are the main oxidation products of proteins and can account for 55%–100% of the total carbonyl content.[137] Proteins can also react with sugar glycation/oxidation products and lipid peroxidation (such as MDA and HNE). Such modifications may also introduce carbonyl groups on proteins.

Other oxidative protein modifications include methionine and thiol oxidation; the first oxidation steps of these reactions are reversible. Reactions with reactive nitrogen species (RNS) lead to nitrosylation of protein thiol groups and nitration of mainly

tyrosine and (with much lower yield), tryptophan and histidine residues. Reactions with hypochlorite form chlorotyrosine. There is a set of specific products of oxidative modifications of individual amino acids for example, 2-oxohistidine, dityrosine, or N-formylkynurenine.[133,138] A particular class of protein modification products, consisting of oxidized, dityrosine-containing, crosslinked proteins formed mainly by reactions of chlorinated oxidants with plasma proteins, predominantly albumin, are called advanced oxidation protein products (AOPP). In vivo, the chlorinated oxidants are generated by activated phagocytic cells containing myeloperoxidase.[139,140]

Oxidative damage to nucleic acids includes strand breaks and oxidative modifications of nucleic acid bases, which may cause mutations and malfunction of nucleic acids.[141] The nucleoside 8-hydroxy-2′-deoxyguanosine (8-OHdG) or 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), present in DNA or released from DNA in the process of DNA repair, is the most frequently assayed marker of oxidative DNA damage, although other oxidative modifications are also assayed.[142]

8.5.2 Reactive oxygen species as the cause of aging

The garbage catastrophe theory of aging postulates that the process of aging may derive from poor clearance of oxidatively damaged molecules, which further hinder cellular catabolic and anabolic functions and increase the probability of death. In young organisms, this undigested material is diluted due to intensive growth; when development is complete, age-related damage accumulates and mainly affects postmitotic, non-proliferating cells and extracellular matrix.[143] These damaged molecules may be generated by ROS and other nonenzymatic reactions of compounds constituting living organisms (e.g., glycation or spontaneous protein denaturation).[144] The term "parametabolism" has been proposed for this set of unavoidable spontaneous reactions due to the inherent chemical reactivities of selected metabolites and macromolecules.[145,146] Nonenzymatic ROS formation is, indeed, a key component of parametabolism.

Several lines of evidence support the free radical theory of aging. Generally, accumulation of oxidative damage to macromolecules is observed with aging. Caloric restriction, the first and most studied experimental manipulation demonstrated to increase lifespan and retard aging, has been shown to reduce oxidative damage to lipids, DNA, and proteins.[147] Mutations in the insulin/IGF-1 signaling pathways (*age-1*, *daf-2*, and *daf-16* mutants) increased the lifespan of *C. elegans*, which was correlated with reduced oxidative damage and increased resistance to oxidative stress.[148] Overexpression of human cytosolic superoxide dismutase 1 (SOD1) in motor neurons of *D. melanogaster* increased the lifespan of the flies by up to 40%.[149]

However, many experiments have cast doubts on the general validity of the free radical theory of aging. If aging is due to ROS's damaging reactions, supplementation with exogenous antioxidants should extend the lifespan. Experiments brought about

conflicting results. For example, in *C. elegans* vitamin E (α-tocopherol) increased lifespan in two studies, but not in a third. Likewise, trolox, an α-tocopherol derivative, increased lifespan in one study, but not in another. Lifespan was also slightly increased by a mixture of tocotrienols, antioxidants similar to α-tocopherol. α-Lipoic acid increased lifespan (two studies), but N-acetylcysteine and vitamin C did not. Superoxide dismutase mimetics, EUK-8 and EUK-134 compounds, increased the lifespan of *C. elegans* in one study, but four other groups did not see this effect.

Even when antioxidant administration increases the lifespan, the question remains: is the effect indeed due to reduced oxidative damage? Doses of trolox and α-lipoic acid that increased lifespan also increased resistance to heat shock, suggesting the possibility that these compounds might increase lifespan by inducing a stress response, that is by a hormetic effect, and not by their antioxidant action.[150] A large-scale analysis of 68 randomized trials including 232,606 participants from the general population, including patients with heterogeneous diseases has generally reported no effect of antioxidant supplements on overall mortality or even a significant increase in mortality in subjects receiving β-carotene, vitamin A, and vitamin E.[151] A metaanalysis including 50 randomized controlled trials with 294,478 participants showed no evidence to support the use of antioxidant supplements to prevent cardiovascular diseases.[152]

8.5.3 Antioxidant defense in aging

The most effective defense system against oxidative stress is the glutathione system. Under oxidative conditions, reduced glutathione (GSH) is reversibly oxidized to glutathione disulfide (GSSG). From the concentrations of GSH and GSSG, the glutathione system's redox potential can be calculated according to the Nernst equation.[153] During the aging process, the plasma cysteine concentration in humans is constantly declining while the amount of oxidized cysteine (cystine) is likewise increasing, leading to a constant age-related increase in the cystine/cysteine redox potential by 0.2 mV/year. Other studies suggest this change is not linear throughout life: the redox potential of GSSG/GSH was found to be constant up to the age of 45 y, but then increases at a rate of 0.7 mV/year.[154] Thiol groups of human serum albumin, which provide most of the blood plasma's thiol groups, also become oxidized with age.[155]

Several groups have observed an age-related decline in intracellular glutathione concentration and a decrease of the antioxidative capacity.[156–160] However, there are other reports, which did not find such a decrease, and some studies even show an age-related increase in the glutathione content of some tissues.[161]

Uric acid is a hydrophilic radical scavenger and is present in high concentration both in intracellular and extracellular compartments. Its effects are more important in the extracellular compartment, which is poorer in other antioxidants compared to the intracellular milieu. Some authors reported augmentation of uric acid levels with age

in human plasma,[162,163] while others found no significant difference[156] or even lower uric acid plasma levels.[164–166]

Another group of compounds protecting cells against oxidative damage is the antioxidant vitamins A, C, and E (retinol, ascorbate and α-tocopherol). The concentrations of vitamins A, C or E are strongly dependent on individual nutrition. This could be the reason why some groups reported higher values of retinol in older adults[167] while others showed no effect[168] of age or even decreasing amounts of retinol in the plasma of older adults.[169] Similar divergent data have been published for α-tocopherol.[167,169]

Regarding the main antioxidant enzymes, some investigators found a decrease in the total superoxide dismutase activity with age.[158,170–172] Other researchers could not detect any age-related difference[173] or even increased SOD activity with age.[174,175] These discrepancies may be related to the fact that total cellular SOD activity is contributed by both cytoplasmic SOD1 and mitochondrial SOD2. Previously, no age-related differences in total activity or SOD1 activity in human skeletal muscle were reported, while the activity of SOD2 was found to increase.[176]

Glutathione peroxidase (GPx) oxidizes glutathione to remove hydrogen peroxide. It was reported that an increased GPx activity,[157,170,172] or a decreased activity[158,160,164,171] accompanied aging or that age had no impact on the GPx activity.[173,177] For glutathione reductase (GSSG-R) and glutathione S-transferase (GST), negative correlations[157,160,172] or no changes with age[177] were reported. Reduction,[160] no significant change[158,171,173] or an increase of catalase activity with increasing age[170,176] were also reported.

As a note of caution, many of these results cannot be generalized as they are influenced by sex, nutrition status, and the respective tissue examined. For example, catalase and SOD2 activities decreased in old males' hearts but increased in the hearts of old females.[178] In the rat, MDA concentrations increased in old males and decreased in old females.[179]

Manipulation of the endogenous defense (i.e., the main antioxidant enzymes) provided results inconsistent with the free radical theory of aging. SOD1, predominantly a cytosolic enzyme, but also found in the mitochondrial intermembrane space (IMS), is one of the major antioxidant enzymes in the cell. Knockout and overexpression studies yielded unexpected results. Whole-body knockout *Sod1* mice, for example, presented an increase in age-related loss of muscle mass, whereas its muscle-specific knockout did not show this phenotype.[180] The authors concluded that this phenotype is caused by redox-mediated signaling effects rather than ROS-induced damage. Interestingly, *Sod1* expression in *C. elegans* increased the median lifespan of affected animals by 33% while increasing peroxide levels and oxidative damage to proteins.[181] According to the authors, this increase in lifespan may result from increased daf-16-mediated stress response instead of a ROS decrease. While early studies in *D. melanogaster* did not show any effect in animals overexpressing SOD1, similar studies nine years later reported an up to 48% increase in median lifespan.[182,183] In contrast, SOD1

overexpression in mice did not affect animals' lifespans, while its targeted inactivation resulted in a 30% reduction of the lifespan.[184,185] In summary, genetic studies on SOD1 in different animal species resulted in vastly different outcomes, shedding confusion on its antioxidative effects in the aging process.

Overexpression of SOD2 in transgenic mice reduced oxidative damage (protein carbonyls and F2-isoprostanes) but did not affect their lifespan.[186] However, the overexpression affected the incidence of age-related pathologies.[187] Methionine sulfoxide reductase-A (MsrA) repairs oxidized methionine residues within proteins and may function as a general antioxidant. $MsrA^{-/-}$ mice have been reported to increase hyperoxia sensitivity, but their mean and maximal lifespan was not affected.[186,188] Thioredoxin 2 (Trx2), the mitochondrial form of thioredoxin, is the electron donor for several antioxidant enzymes (peroxiredoxins, MsrA, etc.), but also plays a major role in repairing the oxidized cysteine residues in proteins. Trx2 null mice are embryonically lethal.[189] $Trx2^{+/-}$ mice were viable and showed reduced levels of Trx2 in all tissues studied, increased levels of oxidative damage to DNA, lipids, and protein, and diminished mitochondrial functions (decreased ATP synthesis and increased ROS production) in several tissues.[190] However, the $Trx2^{+/-}$ mice showed only a slight decrease (7%) in mean lifespan and a 16% decrease in the maximum lifespan.[186] Partial deletion or even, in some cases, total deletion of relevant antioxidant enzymes did not affect the animals' lifespan. From among single and double deletions, SOD1/SOD2 ($Sod1^{-/-}/Sod2^{+/-}$); SOD1 and glutathione peroxidase (GPx)1 ($Sod1^{-/-}/Gpx1^{-/-}$), SOD1 and GPx 4 ($Sod1^{-/-}/Gpx4^{+/-}$), SOD2 and Gpx1 ($Sod2^{+/-}$ and $Gpx1^{-/-}$); SOD2 and Gpx4 ($Sod2^{+/-}/Gpx4^{+/-}$) and Gpx1 and Gpx4 ($Gpx1^{-/-}/Gpx4^{+/-}$), only the double mutants that were null for *Sod1* showed significant differences in the survival curves compared to WT mice (defined as ~20% decrease in mean lifespan and a more than 30% decrease in maximum lifespan).[185] Contrarily, transgenic mice carrying additional genes for SOD1, SOD2, catalase, GPX4, SOD1 + SOD2, or SOD1 + catalase did not have a prolonged lifespan, although they showed decreased markers of oxidative damage.[186] Interestingly, in *C. elegans*, knockouts of peroxisomal catalase, peroxiredoxin, thioredoxin or glutathione S-transferase was reported to decrease the lifespan.[150]

In addition, even evidence has been gathered that increasing the generation of ROS may increase longevity in some cases. The apparent explanation may involve the dual role of ROS: they cause molecular damage and serve as signals and act as modulators of physiological processes. To consider this aspect, the cell signaling disruption theory of aging has been proposed.[191]

It was pointed out that interventions preventing oxidative damage mostly do not affect longevity but have an apparent effect on preventing frailty and its transition to disability. Frailty is a geriatric concept by which a more senior person shows a lack of well-being, unintentional weight loss, a relatively low grip strength, lowering the speed of

walking, difficulty standing, etc. The free radical theory of frailty postulates that free radicals (and other ROS) are the leading cause of frailty rather than healthy aging.[192]

8.5.4 Mitochondrial free radical theory of aging

One of the features of the mitochondrial free radical theory is the central role that mitochondria play in the generation of ROS from the electron transport chain, ATP production, and the numerous potential feedback loops in the regulation of mitochondrial and cellular function, in which redox state and ROS might create "vicious cycles."[193] This theory's basic idea is that ROS formed as a byproduct of oxidative phosphorylation, is responsible for accumulating somatic mutations in mtDNA. Some mammalian mtDNA characteristics were thought to make it highly susceptible to oxidative damage, including its proximity to ROS production sites from the respiratory chain, lack of protection by histones, and limited capacity to repair DNA damage. Steady-state levels of oxidative modifications observed in mtDNA, especially 8-OHdG, are several-fold higher than those in the nuclear DNA.[194] According to the assumption of the mitochondrial theory of aging, mtDNA mutations result in the production of faulty components of the respiratory chain complexes, leading to still more ROS production. This vicious cycle of mitochondrial ROS production and damage leads to global oxidative stress, disruption of cell and tissue functions, and organism aging. Almost all of the 1200 or so proteins of the mammalian mitochondrial proteome are encoded by the nuclear DNA, translated in the cytosol, and imported into mitochondria. mtDNA only encodes about 1% of the mitochondrial proteome corresponding to 13 proteins that are essential for mitochondrial function (they are all critical components of the OXPHOS complexes). Moreover, the human mitochondrial genome codes for two rRNAs (12S and 16S) and 22 tRNAs essential for protein synthesis in the mitochondria. Because of the crucial role of the mitochondrial genome in the respiratory chain function, accumulation of mtDNA mutations can result in an energy crisis, oxidative stress, or cell death, contributing to the aging phenotype. Mutations of mtDNA are known to lead to severe impairment of cellular energy conversion and tissue dysfunction, as documented by identifying many mitochondrial diseases with pleiotropic symptoms.[195]

Observational and experimental evidence for the validity of the mitochondrial free radical theory of aging is equivocal. Birds live much longer than mammals of the same size. For example, pigeons live 35 years, whereas rats only live four years. When free radical production is studied in pigeons' and rats' isolated mitochondria, pigeon mitochondria produces less ROS.[196] Available data supports a negative correlation between free radical production in isolated mitochondria and lifespan in homeothermic vertebrates and flies.[196–199] However, there is an interesting exception to this rule: the naked mole rat, *H. glaber*. The naked mole-rat is the longest-living rodent with a

maximum lifespan of 32 years. Despite the extraordinary longevity of this species, naked mole-rat mitochondria do not produce fewer free radicals than mouse (maximum lifespan: 4 years) mitochondria. Due to elevated mitochondrial ROS production and low antioxidant activity,[200] naked mole-rats have even higher levels of oxidative damage to lipids, proteins, and DNA than mice.[201]

Overexpression of human catalase in mitochondria of transgenic mice increased their median and maximum lifespans (by 10%), delayed cardiac pathology and cataract development, decreased H_2O_2 production, reduced oxidative damage including aconitase inactivation, and diminished mitochondrial DNA deletions and point mutation of mtDNA in heart and muscle tissues. These results suggest that mitochondrial H_2O_2 plays a vital role in aging and determining the lifespan of the animals and supports the mitochondrial free radical theory of aging.[202–204] Interestingly, similar overexpression of catalase targeted to peroxisome, its standard location within the cell, or to the nucleus, had modest and insignificant effects on murine lifespan. This indicates that the catalase's mitochondrial localization is key to lifespan extension in this model.[202] In line with this finding, the mitochondria-targeted antioxidant SkQ1, prolonged the lifespan of inbred male mice in specific pathogen-free conditions and outbred mice, and dwarf hamsters in conventional or outdoor cages.[205]

Decreasing the rate of mitochondrial ROS generation can prolong the lifespan. Additional evidence for mitochondrial ROS involvement in aging comes from observations of mice with a mutation of the *p66Shc* gene, which encodes three adaptor proteins (p66Shc, p52Shc and p46Shc). These mice show reduced ROS generation and increased resistance to ROS-mediated apoptosis, thereby having a prolonged lifespan.[206] Further studies have shown that p66Shc is a mitochondrial redox enzyme located in the mitochondrial intermembrane space, producing H_2O_2 from electron leakage during oxidative phosphorylation.[207] Phosphorylated p66Shc accumulates within mitochondria, activates mitochondrial Ca^{2+} response and induces apoptosis.[208] While apoptosis is beneficial in developing organisms, excessive apoptosis in adult or aging ones may deplete organs of cells and limit their functionality. Interestingly, primary fibroblasts obtained from centenarians and liver, heart, lungs, skin, and diaphragm from adult mice express higher levels of p66Shc than the younger counterparts.[209,210]

Caloric restriction decreases mitochondrial ROS generation (though short-time calorie restriction was reported to lack effects) while increasing various organisms' lifespan.[211] Caloric restriction is a complex intervention affecting the organism at various levels. Further experiments, which shed light on the relevance of the mitochondrial free radical theory of aging, were based on modifying the specificity of the mitochondrial DNA polymerase (polymerase γ POLG). Homozygous knock-in mice that express only a proof-reading-deficient version of *PolgA*, the nucleus-encoded catalytic subunit of POLG, develop a mtDNA phenotype with a three- to fivefold increase in

the levels of point mutations, as well as increased amounts of deleted mtDNA. This increase in somatic mtDNA mutations is associated with reduced lifespan and premature onset of aging-related phenotypes such as weight loss, reduced subcutaneous fat, alopecia (hair loss), kyphosis (curvature of the spine), osteoporosis, anemia, reduced fertility, and heart enlargement, proving, according to authors, a causative link between mtDNA mutations and aging phenotypes in mammals.[212] However, despite the increased mutational load in mtDNA, mitochondria from these mice did not show increased oxidative stress. Levels of H_2O_2, markers of oxidative damage to DNA (8-OHdG), proteins (protein carbonyls), and lipids (F2-isoprostanes) were not significantly different in the somatic tissues between mutant and wild-type mice.[213] The in vivo levels of H_2O_2 were not increased in young *PolgA* mutant mice, although they carried an excessive amount of mtDNA mutations at this stage. In a subset of tissues, studies of end-stage *PolgA* mutant mice showed a slight increase of intramitochondrial H_2O_2 compared to wild-type controls.[214] Another criticism of this experiment was that the levels of mutations in the mutator mice were more than an order of magnitude higher than typical levels in aged humans. In particular, point mutations in aged human tissues are much less abundant than those causing premature aging in mutator mice.[215]

Another group reported data on heterozygous knock-in ($PolG^{-/+}$) mice. These mice accumulated 500 times more point mutations than control animals, but they did not show any significant reduction in the lifespan. These data do not support the role of mtROS in the regulation of longevity.[204] However, only point mutations were analyzed, so it is impossible to discard a relevant role for deletions in aging.

Experiments on modifications of mitochondrial superoxide dismutase level do not support the mitochondrial theory of aging either. While mice homozygous $Sod2^{-/-}$ knockouts result in prenatal or neonatal lethality associated with dilated cardiomyopathy and lipid accumulation in the liver,[216] the heterozygotes are viable. The $Sod2^{+/-}$ mice have reduced (by 50%) SOD2 activity in all tissues throughout life. They show an augmented oxidative damage, including increased 8-oxodG level in mtDNA in liver and brain compared with wild type (WT) mice and a 100% increase in tumor incidence in old $Sod2^{+/-}$ mice compared with the old WT mice. The $Sod2^{+/-}$ mice showed partially reduced (30%–80%) scavenging activity for superoxide anions in all tissues throughout life and increased oxidative damage to mitochondria compared with the $Sod2^{+/+}$ mice.[32,216–218] However, their mean and maximum lifespan were identical to those of the WT mice. Also, biomarkers of aging, such as cataract formation, immune response, and formation of glycoxidation products carboxymethyl lysine and pentosidine in skin collagen, changed with age to the same extent in both $Sod2^{+/-}$ and WT mice.[32] Overexpression of SOD2 did not alter either lifespan or age-related pathologies.[187]

It has also been hypothesized that oxidative stress might promote longevity and metabolic health according to the concept of mitochondrial hormesis (mitohormesis). The mitohormesis theory postulates that low levels of oxidative stress induced by either calorie restriction, exercise,[219] or other stimuli may trigger adaptive responses that improve overall stress resistance, probably via increasing endogenous antioxidant defense, which may eventually reduce chronic oxidative damage[220] and subsequently achieve lifespan extension. This concept is supported by a study in *C. elegans* demonstrating that respiration inhibition increases mitochondrial ROS production and significantly increases lifespan via mitochondrial ROS mediated activation of hypoxia-inducible factor-1 (HIF-1).[221] Low levels of oxidative stress induced by dietary restriction, especially glucose restriction, have been shown to enhance mitochondrial metabolism and extend lifespan in various model organisms, including *D. melanogaster*[222] and *C. elegans*.[223] For instance, an increase in the *C. elegans* lifespan by glucose restriction has been ascribed to mitochondrial respiration and oxidative stress induction. This effect was demonstrated to be dependent on the 5′-AMP-activated protein kinase (AMPK), and the lifespan extension is abolished by pretreatment with antioxidant N-acetyl cysteine, which suggests that ROS are required for lifespan extension by dietary restriction.[223] Although definitive evidence of hormesis in lifespan regulation in mammalian models is still lacking, care should be taken when developing antioxidant therapy. The theory of mitohormesis could have important translational implications as an ideal antioxidant therapy that prevents oxidative damage induced under pathological conditions without interfering with ROS needed for hormesis and cellular signaling. Mitochondrially-targeted catalase may play a role in such a mechanism since the K_m of the catalytic activity of catalase is >10 mM, so that this enzyme is less likely to be effective at the lower intracellular H_2O_2 concentrations that may be involved in signaling or hormesis but may protect against damaging concentrations of hydrogen peroxide.[193]

8.5.5 Accumulation of reactive oxygen species-induced damage with aging

Generally, numerous studies are demonstrating an accumulation of oxidative damage during aging.[147] However, it should be kept in mind that the level of products of ROS-induced damage in cells or extracellular fluids is a resultant of three factors: (1) the rate of ROS generation, (2) the activity of antioxidants/antioxidant enzymes, and (3) the rate of repair or removal of products of ROS reactions; all can change with age and be affected by such factors as nutrition and age, which contributes to the variability of results.

During the aging process, the concentration of F2-isoprostanes in the urine was found to increase,[224,225] although similar increases in serum F2-isoprostane concentration of young and older men after eccentric exercise have also been reported.[226]

Numerous studies have revealed a significant age-related increase in the plasma concentration of MDA.[156,164,168,170,171,227–229] The level of protein-bound MDA residues, detectable with antibodies, increased with age.[168] A positive correlation between the HNE concentration in the blood plasma and the individual's age has been found. The HNE values correlated positively with the MDA values and negatively with the glutathione system's antioxidative status.[156]

It has been estimated that in senescent cells, almost 30% of proteins involved in enzymatic or structural functions are dysfunctional due to oxidative damage.[230] The age-related increase of protein-bound carbonyl content has been found in tissues such as the heart, muscle or brain[176,231–233] and in the blood plasma of healthy people.[156,171,228,234] Carbonylation may alter the polypeptide chain's conformation, which leads to partial or total inactivation of the protein function. The consequent loss of function or structural integrity of carbonylated proteins can have many downstream functional consequences and may underlie the subsequent cellular dysfunctions and tissue damage.[235] Protein carbonylation was demonstrated to modify enzymes and functions of other proteins like DNA binding of transcription factors.[236] Carbonylation can lead to functional impairment of proteins involved in insulin signaling.[237] The level of AOPP has also been reported to increase with advancing age.[133]

Not all proteins are uniformly susceptible to oxidative damage. Using an immunochemical probe for oxidative damage, mitochondrial aconitase was shown to be particularly vulnerable to oxidative damage accompanying aging in *D. melanogaster*. Similarly, the mitochondrial adenine nucleotide translocase, glutamine synthetase, and creatine kinase were shown to show high vulnerability. These proteins can also be inactivated by nitration.[238]

An increase in the nitrated proteins has been observed during aging in various human and animal tissues,[239] however only a limited number of proteins undergo nitration. A proteomic approach identified about 40 nitrated proteins out of 1000 during an inflammatory challenge. These included a large number of mitochondrial proteins that regulate cellular energy metabolism. One of these proteins was SOD2, which is selectively nitrated at Tyr-34 and inactivated by nitration. Prostacyclin synthase (PGI2) is another protein that is nitrated and inactivated by very low peroxynitrite levels. Nitration of tyrosine residues in glutamine synthetase by peroxynitrite leads to inactivation of the enzyme and compromises regulation of glutamine synthetase by adenylation. Peroxynitrite-mediated nitration of lymphocyte-specific tyrosine kinase inhibits its ability to phosphorylate tyrosine residues, which is the basis of a signal transduction mechanism involved in a myriad of pathways, including those mediated by neurotrophins. Creatine kinase is another key intracellular enzyme regulating energy metabolism that is nitrated and inactivated by peroxynitrite.[238] Covalent modification of various proteins by tyrosine nitration is associated with the modification of

biological functions of various organs/tissues such as skeletal muscle, heart, brain, and liver in aging.[240]

Irreversibly oxidized proteins usually are repaired or degraded by the proteasome and/or lysosomes and replaced by de novo synthesized proteins. If the oxidative damage is faster than the proteolysis rate, oxidized proteins accumulate within the cells. Depending on their location and composition, these aggregates are called inclusion bodies, plaques, lipofuscin, ceroid, aggregosomes or Lewy bodies. The waste material which is stored in lysosomes is called lipofuscin or age pigment. Apart from proteins, lipofuscin contains triglycerides, free fatty acids, cholesterol and phospholipids, some amounts of carbohydrates and metals (Fe, Cu, Al, Zn, Ca, and Mn). Lipofuscin composition significantly varies between different types of cells, but all lipofuscin pigments are resistant to degradation, most likely due to the presence of peptides crosslinked by aldehydes into plastic-like structures.[241] As the proteasomal and lysosomal systems cannot degrade these aggregates, the accumulation is a progressively and continuously ongoing process over the cell lifetime. These high molecular weight aggregates are inhibitors of the proteasomal proteases and enhancement of their formation compromises the removal of damaged proteins.[242] Because of their ability to bind transition metals such as iron and copper, coupled with the fact that lipofuscin is a fluorochrome, these aggregates seem to sensitize lysosomes and cells to blue light, a process which might be important for the pathogenesis of age-related macular degeneration.[241,243] In mitotically active cells, lipofuscin is diluted with every cell division, but in postmitotic cells, a steady increase of lipofuscin accumulation is observed, and it is an established hallmark of aging. In crustaceans, lipofuscin has been used for the determination of age. Mitochondria may contribute to lipofuscin's cellular accumulation, especially under conditions of inhibition of mitochondrial fission or Lon protease downregulation.[244]

There is consensus on the decline of proteasomal activity in mammals during aging, although the exact reasons for such alteration are still under discussion. It may be explained by changes in the proteasomal system's composition and/or structure, at least in part induced by modifications of proteolytic enzymes, both irreversible oxidative damage and reversible (also redox-dependent) posttranslational modifications.[244]

It has been estimated that up to 200 oxidative modifications of guanine bases per cell occur each day.[245] Oxidative damage to nuclear DNA and mtDNA of tissue cells increases with mammalian age.[194] Oxidized form of guanosine, 8-oxodG, which is present at a frequency of about 10^{-5}–10^{-6} per dG in extracted DNA.[246] A higher level of 8-oxodG was detected in mtDNA compared to nuclear DNA, suggesting that mtDNA is more susceptible to oxidative damage.[22,247] The 8-oxodG level in mtDNA increases with age.[22] Since 8-OHdG lesions can be efficiently repaired, imbalances between oxidative modification rates of guanosine and repair mechanisms may explain the conflicting results on the role of 8-OHdG accumulation in aging. Whereas some groups found an age-related increase in damaged bases,[224,248] others detected no

change or even a decrease in the 8-OHdG level of elderly subjects.[226,245,249] Diet and the metabolic rate of the individuals are the other factors influencing the amount of detectable DNA damages.[245,250]

Furthermore, research was carried out in different organs and it is possible that 8-OHdG damage is accumulating in some organs, while in others, the repair process is more efficient and no DNA modification is observed. There are many other oxidative DNA modifications, and it is not clear whether efficient repair systems exist for all of them. Such unrepairable damages may accumulate in ever-increasing amounts with aging.

Reactive organic molecules can react irreversibly with nucleic acids and form covalently bound DNA adducts. The adduct spectrum is broad, ranging from simple methylation of guanine to bulky adducts such as benzo[*a*]-pyrene diolepoxide-I-DNA adducts (BPDE-I-DNA), also called I-compounds. Several researchers described an age-related increase of I-compounds.[251–253] In contrast, other authors found no difference in the concentration of 7-methyl-, 7-(2-hydroxyethyl)-guanine and other adducts in peripheral lymphocytes from subjects at different ages.[254,255]

The length of telomeres can be a valuable biomarker of oxidative stress during the aging process. In dividing cells, telomeres are shortened by each cell division as DNA polymerases cannot fully duplicate the ends of the chromosomes. Several studies have linked telomere attrition with the aging process.[256] In addition to cell division, telomeres can also be shortened by oxidative damage and/or mutations.[257]

8.5.6 Age-related oxidative modifications of mitochondria

With increasing age, mitochondria show changes in morphology and oxidative phosphorylation activity.[258] Moreover, proteasomal activity decreases, autophagy is impaired, and mitochondrial dysfunction emerges with an activity decrease of OXPHOS, β-oxidation, and the Krebs cycle and an increase of ROS production that, in turn, may further damage mitochondria.[259] In a longitudinal study, mitochondrial respiratory activity estimated in ex vivo muscle samples showed an age-related decrease; this decrease correlated with the in vivo decline of oxidative capacity, cardiorespiratory fitness, and muscle strength.[260]

A decline in mitochondrial function accompanies aging in model organisms and this decline might, in turn, contribute to the observed age-dependent decline in organ function. A decline in mitochondrial function in humans has been observed, and this decrement may predispose to specific age-related diseases.[261] Mitochondrial mutations increase in frequency with age in both animal models and humans,[262,263] although the levels and kinds of mutations appear to differ between tissues and even within tissues.[264] While some have speculated that the increased levels of mitochondrial mutations contribute to aging and age-related diseases,[265] others have questioned whether

these mutations ever reach a significant enough level to contribute to the aging process.[266] Indeed, since mitochondrial DNA exists in hundreds to thousands of copies per cell, the detection of mutated mitochondrial DNA does not in itself imply dysfunction, as it is generally believed that mutational load must exceed a threshold value (perhaps exceeding 60% of all mitochondria within a given tissue) to become a significant phenotype.[267]

Several studies have reported that changes in mtDNA copy number occur in aging organs, whereas other studies found no such changes.[268] One possible confounding factor when assessing mitochondrial function and mtDNA copy number is that the relative proportion of different cell types and tissue composition often change in aging organs due to regeneration, fibrosis, and inflammation. In addition to mtDNA-driven effects, which are comparatively easy to diagnose, the mitochondrial function can also be influenced by exogenous signals such as hormones[269] and physical activity.[270] This makes it difficult to distinguish between direct and indirect causes of mitochondrial dysfunction in aging.

The rates of mitochondrial superoxide and hydrogen peroxide generation increase with age and correlate inversely with the lifespan of animals[271]; and they are higher in the ad libitum fed than in calorie-restricted mice in the brain, heart, and kidney at each age. In contrast, there was no clear-cut overall pattern of age-related or dietary-related changes in antioxidant defenses provided by SODs, catalase, and glutathione peroxidase.[272]

The 8-oxodG lesion is commonly considered a mutagenic lesion, because it can base pair with adenosine, leading to a G to T transversion.[273] However, these transversion mutations might be rare in mitochondria because in vitro assays have shown that POLG has a decreased efficiency in incorporating nucleotides opposite to 8-oxodG during replication of mtDNA.[274] Also, when POLG was able to add a nucleotide opposite to 8-oxodG, the correct cytosine was much more likely to be inserted than adenosine,[275] which argues that G to T transversion mutations in mtDNA are rare and that this type of oxidative stress-induced lesion is a less prominent source of point mutations in mtDNA.

Furthermore, 8-oxo-dGTP is actively removed from the nucleotide pool by MTH1[276] and from mtDNA by the base excision repair machinery, shared among mitochondria and the nucleus.[277] The base excision repair (BER) enzyme 8-oxoguanine glycosylase (OGG1) recognizes and removes the 8-oxodG base,[278] followed by gap-tailoring, gap-filling, and DNA ligation performed by APE-1, POLG and DNA ligase 3, respectively.[279] If adenosine is erroneously inserted opposite of 8-oxodG, the adenine DNA glycosylase (MUTYH) removes the adenosine before 8-oxodG removal by OGG1.[280] There are reports on the increase in the 8-OHdG in mitochondrial DNA.[281,282] Surprisingly, *Ogg1*$^{-/-}$ and *Mutyh*$^{-/-}$ double-knockout mice do not accumulate mutations of mtDNA.[283] Descriptive data from humans

comparing the mtDNA mutation load in the brain from young and aged individuals showed an apparent increase in point mutation load with age, but the mutation pattern was not consistent with G:C to T:A transversion mutations caused by oxidative damage.[284] Instead, the reported mutations were mainly transition mutations consistent with replication errors and/or spontaneous deaminations.[285] Similar mutation patterns have also been reported in tumors and aging mice.[286,287] Experimental studies in Ogg1-deficient *Drosophila melanogaster*, which also have reduced Sod2 activity, surprisingly showed no increase in G:C to T:A transversion mutations.[288] Thus, several lines of evidence make it reasonable to conclude that oxidative damage may not be a primary contributor to mtDNA mutations.

Mitochondrial DNA damage has been shown to increase with age, and its rate is higher compared to nuclear DNA. However, it is necessary to distinguish point mutations from deletions, for example, it has been argued that the point mutations do not[204] whereas deletions do correlate with the lifespan of mice.[203] This may be related to mitochondria having multiple DNA copies, protecting from heteroplasmic mutations.

Multiple studies have robustly documented that somatic mutations in mtDNA progressively accumulate with age in a variety of tissues of humans,[289–292] rhesus monkeys,[293] and rodents,[294,295] which leads to a mosaic dysfunction of oxidative phosphorylation only affecting a subset of the cells in a tissue. Currently, a matter of debate is whether these mutations play a causal role or just correlate with aging. However, there are strong indications that they can contribute to aging phenotypes. Many lines of studies have revealed that multiple deletions of mtDNA occur in the skeletal muscle, heart, and brain of aged humans and mice and suggest that a broad spectrum of mtDNA deletions accumulate with age. Many types of tandem duplications in the D-loop region of mtDNA were identified in various tissues of elderly subjects.[296,297] Some pathogenic point mutations in mtDNA's tRNA genes[298,299] and high levels of point mutations in the D-loop region of mtDNA[300,301] have also been found to accumulate with age in human tissues and cultured human skin fibroblasts.

Terminally-differentiated tissues with active oxidative metabolism, such as skeletal muscle, heart, and brain, accumulate relatively higher levels of mutated mtDNA during the aging process. Many of these mtDNA mutations start to occur after the mid-30s and accumulate with age in postmitotic tissues of humans.[291,302] The occurrence of mtDNA deletions was correlated with reduced mitochondrial respiratory chain enzyme activities in aging human skeletal muscles.[303,304] Here, most studies showed that the overall percentage of a specific mutated mtDNA is lower than about 0.1% of total mtDNA molecules in any somatic tissue examined.[305] These levels are far lower than a threshold of mtDNA mutation required for a defective electron transport system in patients with mitochondrial myopathies.[306] However, most investigations are

screened for mtDNA mutations in the whole tissue rather than individual cells. The mutated mtDNA molecules may be unevenly distributed and can accumulate clonally in specific cells, causing a mosaic pattern of respiratory chain deficiency in somatic tissues during aging. Moreover, the unevenly distributed mutated mtDNA molecules may have been primarily diluted by wild-type mtDNA when the whole tissue was used to screen for mtDNA mutations. The idea of a mosaic pattern of mtDNA damage was supported by some evidence showing that the proportion of mtDNA with a large-scale deletion correlated well with that of cytochrome c oxidase (COX)-negative fibers in the same subjects.[307] Using a single-fiber PCR method or the laser-capture microdissection technique,[308,309] it was further demonstrated that COX-negative fibers in the skeletal muscle of normal elderly subjects and aged rats contain reduced levels of full-length mtDNA and high levels of mtDNA deletions. Most mtDNA deletions, regardless of whether they are causing mitochondrial disease or are found in aging, affect the major arc of mtDNA[310,311] and are classified into two categories. The majority of the large deletions are type I deletions flanked by homologous or near-homologous repeats. Deletions not flanked by repeats are known as type II deletions and have been reported to colocalize with secondary DNA structures such as hairpins, cruciform, and G-quadruplex structures.[310,312]

Using both histological and polymerase chain reaction (PCR) analyses, it was shown that mtDNA deletions clonally accumulate to high levels within COX^{-}/SDH^{+} regions ($>90\%$ of total mtDNA) in vastus lateralis muscle of human subjects aged 49–93 years.[313] Moreover, the amplitude of decline in the deletion-containing mtDNA levels in the transition regions and mitochondrial electron transport chain-normal regions immediately adjacent to the transition regions, suggests that the accumulation of mtDNA deletions precedes the electron transport chain deficiency.[313] The high levels of accumulated mtDNA deletion were also observed to link to enzymatic and morphological abnormalities of mitochondria in muscle fibers from aged rats.[314] These observations strongly support the notion that mtDNA deletions play a causal role in mitochondrial dysfunction of skeletal muscle fibers with age, a process that may ultimately lead to fiber loss.

Parkinson's disease patients carry high levels of mtDNA deletions in some dopamine neurons in substantia nigra.[315–317] High levels of deleted mtDNA are also found in a subset of dopamine neurons in normal aging human brains, but the levels are generally higher in Parkinson's disease patients.[315] Notably, the proportion of mtDNA with deletions increased significantly with age, and neurons containing over 60% of deleted mtDNA molecules were associated with a striking loss of COX activity. These findings suggest that mtDNA mosaic seems to parallel the occurrence of the bioenergetic mosaic, and mtDNA mutations can reach relatively high levels in some cells of elderly subjects. Consequently, the accumulation of mtDNA mutations with age in human and rodent tissues can cause adverse effects and play a causal role in aging.

The presence of mtDNA deletions was also found to correlate with aging and aging-related diseases in humans. In these cases, the overall amount of mtDNA deletions is low in the whole tissue, but the levels can be very high in individual cells. The levels of mtDNA deletions increase with age in brain,[318] retina,[319] skeletal muscle,[314] sperm,[320] ovaries,[321] hepatocytes,[302] and heart.[262,322] Interestingly, cells with high levels of mtDNA deletions are not a feature of every age-associated neurodegenerative disease and is, for example, not present in patients with Alzheimer's disease.[323] There has been a debate about whether these changes represent clonally-expanded mtDNA deletions generated early, late in life, or both.[318,324] Although the correlation between the occurrence of clonally-expanded mtDNA deletions and aging is evident, the amount of mtDNA deletions varies between various tissues and even between individual cells of a given tissue due to the random drift of the deleted mtDNA molecules.[295,313,314,325] Because of this mosaicism, PCR-based methods typically identify low levels of mtDNA deletions in tissue homogenates ($<10\%$), whereas single-cell-based detection approaches can result in very high levels of mtDNA deletions in individual cells.[326]

Muscle fiber regions with the highest number of deletions colocalize within regions of COX deficiency and muscle atrophy in aging individuals.[308,309] A mouse model with an inducible expression of a mitochondrially-targeted restriction enzyme has been generated.[327] These mice accumulate mtDNA deletions, leading to decreased OXPHOS capacity, thus demonstrating that mtDNA deletions can have detrimental in vivo effects when present at high enough levels. These results strongly suggest that mtDNA deletions are one driving force behind the occurrence of at least some cells with age-related OXPHOS dysfunction.

The content of lipid peroxides in mitochondria is increased with age.[328] The extent of lipid peroxidation was also correlated with alterations in mitochondrial respiration and OXPHOS activity, inner membrane barrier properties, maintenance of mitochondrial membrane potential, and mitochondrial Ca^{2+} buffering capacity.[329] Cardiolipin resides primarily in the inner membrane of mitochondria, and the highly unsaturated nature of the fatty acyl chains in cardiolipin is required for the optimal function of many of the proteins involved in the mitochondrial respiratory chain. It has been shown that increased ROS production from mitochondria may result in oxidation and depletion of cardiolipin and inhibition of cytochrome c oxidase activity.[330] Peroxidation of cardiolipin can impair the inner membrane's barrier function and facilitate the detachment of cytochrome c from the mitochondrial respiratory chain, thereby facilitating the initiation of apoptosis.[331]

The amounts of proteins with oxidative modifications in mitochondria, such as oxidation of the sulfhydryl groups of proteins or protein carbonyl formation, are increased with age.[132,332] The age-related increase in the level of oxidized proteins was also observed in human skin fibroblasts and the mitochondrial fraction

accumulating higher protein carbonyl levels than the whole-cell lysate of these skin fibroblasts. This increase in the level of proteins with oxidative modifications correlated with a decline in the intracellular ATP level.[333] Aconitase and adenine nucleotide translocase are preferred targets of oxidative damage to mitochondrial proteins during the animals' aging.[334,335] In addition, DNA polymerase γ was shown as one of the major targets of oxidative damage in the mitochondrial matrix, which may reduce mtDNA replication and DNA repair activities in mitochondria.[275] Oxidative modification of proteins can alter protein structure and/or result in a loss of their normal function, which might lead to further ROS production from mitochondria.

Various authors have suggested that abnormal mitochondrial ROS production and detoxification contributes to mitochondrial dysfunction and cardiomyopathy in old age.[336–338] An age-dependent reduction in cardiac mitochondrial OXPHOS function is related to the decline in mitochondrial state 3 respiration due to diminished activity of electron transport complexes I and IV (both have subunits encoded by mtDNA). In contrast, complexes II, III, and ATP synthase are relatively unaffected.[64] Impaired electron transport chain function is directly related to elevated electron leakage and generation of mROS. As the heart has a high metabolic demand and is rich in mitochondria, it produces considerable amounts of ROS within mitochondria as a byproduct of OXPHOS and is especially susceptible to oxidative damage. Mitochondrial production of ROS significantly increases in hearts with advanced age.[339] The age-related cardiac phenotype was significantly attenuated in mice overexpressing catalase localized in mitochondria (mCAT). They also have lower age-dependent accumulation of mitochondrial protein carbonyls, suggesting prevention of mitochondrial oxidative damage as a mechanism for cardiac aging protection. Peroxisomal overexpressed catalase or exogenously-introduced N-acetyl-cysteine (NAC) were ineffective, underscoring mitochondrial specificity's importance in the antioxidant intervention.[340]

Sarcopenia is the loss of skeletal muscle mass and function with age. Sarcopenia is a significant public health concern due to exercise intolerance, increased morbidity, and loss of independence in the elderly. Skeletal muscle, like heart, relies on mitochondria to meet the majority of the ATP demands for sustained muscle contraction. Mitochondrial function in skeletal muscle is very dynamic in which the metabolic rate can vary by at least an order of magnitude during rest to work transitions and varying with the nutritional state. One consequence of this variation in mitochondrial function is that increased mitochondrial ROS production periods are a normal part of skeletal muscle physiology. These transient increases in ROS modify muscle function and play an essential role in the beneficial adaptations to exercise training.[341] Mitochondria in aged skeletal muscle have an increased capacity to produce H_2O_2 when measured under ex vivo conditions.[342] In aged skeletal muscle of both mice[343] and humans[344] reduced mitochondrial coupling (P/O) and depression of skeletal muscle metabolism

was demonstrated. Mitochondrially-overexpressed catalase preserved mitochondrial function and insulin sensitivity in the skeletal muscle of aged mice.[345]

The current evidence strongly supports an essential role of mitochondrial oxidative stress in declining skeletal muscle function with age, while its role in skeletal muscle atrophy with age is still controversial. The most robust evidence supporting a role for oxidative stress in age-related muscle atrophy comes from mice lacking SOD1; the absence of SOD1 leads to mitochondrial dysfunction associated with a premature loss of skeletal muscle mass in aging mice.[346] Muscle fibers from SOD1 mice accumulated mitochondria around the neuromuscular junction, and showed a loss of motor units and disruption of neuromuscular junctions. Muscle-specific knockout of SOD1 did not result in muscle atrophy, increased oxidative stress or mitochondrial dysfunction,[347] but did cause a loss of specific force throughout life.

Age-related sensorineural hearing loss or presbycusis is the gradual loss of hearing with aging. The sensorineural hearing loss is usually more severe for high-pitched sound, which eventually leads to difficulty in understanding speech. The pathology is characterized by age-dependent loss of sensory hair cells, spiral ganglion neurons, and stria vascularis cells in the inner ear cochlea. Mice with the deletion of the mitochondrial pro-apoptotic gene *Bak*, attenuated age-related apoptotic cell deaths and prevented presbycusis. Oxidative stress induced BAK expression in primary cochlear cells, while mCAT suppressed BAK expression, reduced cell death, and prevented presbycusis. These findings suggest a central role of mitochondrial ROS-induced apoptotic pathway in presbycusis. Caloric restriction prevents presbycusis via reduction of oxidative damage by NAD-dependent protein deacetylase sirtuin-3 (SIRT3). In response to caloric restriction, SIRT3 directly deacetylates and activates mitochondrial isocitrate dehydrogenase 2, leading to increased NADPH levels and an increased ratio of reduced-to-oxidized glutathione in mitochondria, thereby enhancing the mitochondrial glutathione antioxidant defense system.[348]

Enhanced activation of mitochondrial permeability transition pore (mPTP) by aging in different tissues and organisms was observed. While short, infrequent, opening of the mPTP also triggers protective pathways, increasing the frequency and duration of the mPTP is associated with more persistent oxidative damage that may contribute to aging. Since mPTP opening contributes to the ROS production which in turn, further potentiates mPTP activation, mPTP opening may constitute another "vicious circle" of enhanced mROS production.[349]

8.6 Potential strategies against aging to increase longevity

8.6.1 Metabolic control-related approaches

In the face of the above evidence on the promising benefits of targeting the metabolic pathways and, in particular, the numerous roles of mitochondria within the cell that

are affected by aging, we will discuss below some of the most recently metabolic and/or mitochondrial approaches that aim to prevent, maintain or recover metabolic/mitochondrial functions across aging, and to ultimately extend an individual's lifespan. Among the most promising antiaging metabolism-targeted strategies, are caloric/dietary restriction, the recovery of melatonin levels/function, sirtuins activation, mitochondrial uncoupling, and physical activity.

8.6.1.1 Caloric/dietary restriction

During the last decade, dietary restriction (the reduction of calorie intake without causing malnutrition)[350] arose as one of the most promising 'eternal youth elixirs', with proven beneficial results in a plethora of species, from fungi to humans.[1,351–353] Such benefits were achieved either by reducing caloric intake by 30%–40% (rather than complete starvation) or by reducing the consumption of specific amino acids (such as methionine, instead of the simultaneous reduction of all the essential ones).[202,354–357] When blood methionine levels are depleted, the organism delays the aging process to focus its energies in overcoming what it perceives as a starvation period.[1] Moreover, caloric restriction blunted the age-related mitochondrial dysfunction that affects multiple tissues.[358] Strikingly, recent studies demonstrated that intermittent fasting also ameliorates blood cholesterol and glucose levels[359,360] and could therefore represent a promising antiaging approach.

8.6.1.2 Melatonin

Another promising antiaging player appears to be the hormone melatonin, mainly produced within the brain pineal gland. Melatonin most likely acts through its major role in the circadian rhythm-related tight control of cellular metabolism.[361] This hypothesis was supported by the dietary restriction-mediated rescue of melatonin's daily peak (usually at midnight) upon aging,[361–363] alongside a dietary restriction-induced rejuvenation of circadian rhythm-related genes (especially those associated with stress resistance) in the aged liver, muscle, and epidermis.[364,365] Following the putative antiaging role of melatonin, the "eternally young" naked mole rats showed an atrophied epiphysis, together with a mutations in the genes coding for the two main melatonin receptors and downregulation of enzymes involved in the brain metabolism of serotonin to melatonin.[366,367]

8.6.1.3 Sirtuins activators

Sirtuins (SIRTs) are one of the first families of longevity proteins to be revealed, which induce changes that promote survival during times of adversity in response to signals like the amount of energy, daylight timing, and other types of environmental stress. Sirtuins are a family of NAD-dependent enzymes first identified as deacetylases that catalyze the posttranslational modification of histone and non-histone proteins.[368]

Among the seven SIRTs found in mammals, SIRT 3, 4, and 5 are present in mitochondria.[369]

Although SIRTs are considered critical modulators of aging and aging-related diseases, their role in lifespan extension remains an intense matter of debate.[350] Nevertheless, the increasingly described involvement of SIRTs 1−7 in the benefits of calorie restriction points towards a promising therapeutic role of SIRT activation against age-related disorders (such as diabetes, metabolic syndrome, cardiomyopathies, nonalcoholic hepatic steatosis, chronic inflammation, neurodegenerative diseases, and cancer).[370−372] Regarding the role of SIRTs in blunting cellular senescence and promoting organismal longevity, some authors suggest the involvement of insulin/IGF-1 signaling and AMPK.[369] Moreover, SIRTs may be involved in the caloric restriction-associated lifespan extension and in mitohormesis processes.[373,374]

Exogenous SIRTs activators or molecules capable of replenishing cellular NAD^+ pools[371,375] were involved in various biological models of aging-related diseases. Nicotinamide mononucleotide (NMN), a key NAD^+ intermediate, could restore several critical aspects of aging in mice, such as muscle-type switching, insulin sensitivity, oxidative phosphorylation, and gene expression,[376] as well as it was able to restore NAD^+ levels and prevent diet- and age-induced type 2 diabetes.[377] Among the exogenous SIRT activators, the most well-known is resveratrol.[375] The discovery of resveratrol (3,5,4-trihydroxystilbene), a stilbenoid present in many fresh fruits (including grapes, blueberries, and raspberries), as an activator of sirtuin 1,[378,379] lead many to propose an explanation for the unusually low rates of mortality from chronic heart diseases in French people, from regions with high consumption of their local wine.[380] Associated to sirtuin 1 activation, resveratrol improves mitochondrial function by stimulating mitochondrial biogenesis, an effect mediated by a decrease in the acetylation of peroxisome proliferator-activated receptor-gamma coactivator (PGC-1α), as the master regulator of mitochondrial biogenesis, binding to and promoting the activity of various transcription factors that ultimately lead to increased mitochondrial numbers.[381] Nevertheless, over the years, resveratrol's insolubility and low bioavailability blunted a search for new possible synthetic activators that could be more potent and efficacious.

8.6.1.3.1 Resveratrol showed cytotoxicity against several cancers (namely leukemia, skin and prostate cancer)[382,383]

More than 14,000 SIRT activators have been identified after resveratrol, including plant-derived metabolites that activate *sirt1* (e.g., flavones, stilbenes, chalcones and anthocyanidins),[375] and synthetic molecules. Among the latter compounds that activate *sirt1* allosterically,[384,385] examples include imidazothiazoles (namely SRT1720), thiazolopyridines (namely STAC-2), benzimidazoles (namely STAC-5), bridged ureas (namely STAC-9), cilostazol, paeonol, statins, hydrogen sulfide and persimmon.[386−392] Other natural molecules with potential antiaging properties include quercetin, butein,

fisetin, kaempferol, catechins and proanthocyanidins.[393] The strategies to replenish intracellular NAD^+ pools may involve the stimulation of enzymes from NAD^+ biosynthesis or the inhibition of CD38 hydrolase.[394–396] Indeed, mounting evidence suggest that such NAD^+-boosting molecules (including NMN and nicotinamide riboside)[376,397–399] may recover the intracellular NAD^+ pools and activate all SIRT 1–7 isoforms in elderly individuals.[350] Additionally, recycling NAD^+ from nicotinamide mimics calorie restriction and increases yeast longevity.[400,401] Among the enzymes that regulate NAD^+ levels, which inhibition could present a therapeutic potential, examples include CD38, CD157, NAMPT (Nicotinamide phosphoribosyltransferase), and malate dehydrogenase-1.[350] Of note, besides its role in the Krebs cycle, during which NAD^+ is reduced to NADH, malate dehydrogenase 1 appears to also be involved in cellular senescence.[350] To our knowledge, the currently available CD38 inhibitors include apigenin, quercetin, and GSK897–78c.[395,402]

8.6.1.3.2 Food nutrients

Increasing attention has been paid to functional food nutrients, that is natural compounds included in the diet, as potential antiaging, pro-longevity, preventive and/or therapeutic strategies by interacting with SIRTs. Among them, dietary polyphenols appear to be beneficial against neurodegenerative, cardiovascular, metabolic, and inflammatory diseases, and cancer, most likely by stimulating SIRT1 deacetylase activity.[350,403]

8.6.1.3.3 *Curcumin* (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione), a diarylheptanoid derived from the rhizome of *Curcuma longa*, is also a promising antiaging compound, despite its effects as a mitochondrial uncoupler in isolated mitochondria[404]

Its antiaging actions may be related to its anti-inflammatory and antioxidant properties.[405] Low doses of curcumin may act as a protective agent, whereas in high doses, it could act as a cytostatic, cytotoxic, and genotoxic agent,[405] observations that are consistent with hormetic actions. However, the action of curcumin on aging is controversial. At low concentrations, curcumin could neither postpone replicative senescence nor protect cells from doxorubicin-induced senescence but did increase sirtuins and AMPK in vascular smooth muscle cells undergoing replicative senescence.[406] Curcumin also increased the positive effect of exercise and prevented fatigue in animals, an effect associated with an increased level or activity of AMPK and sirtuin 1.[407,408] Although some natural compounds offer the advantage of being nontoxic, readily available, and with the potential to be included in supplements, their clinical preventive/therapeutic potential in humans remains unestablished.[350]

8.6.1.4 Sirtuin inhibitors

While sirtuins' stimulation might be a promising therapeutic strategy against aging and its associated disorders, their selective inhibition may, on the contrary, constitute potential anti-cancer approaches. However, in this case, a potent sirtuins inhibitor must necessarily be specific to a single sirtuins isoform, being processed by the enzyme into a non-catalytic intermediate that competes with the standard substrate and binds with higher affinity to the active site of that sirtuin isoform.[409] The currently known sirtuins inhibitors include nicotinamide (NAM), thioacyllysine-containing compounds, sirtinol, and its analogs, splitomicin and indole derivatives and tenovin and its analogs; the latter four classes inhibit sirtuins via non-covalently binding to the active site.[350] More specifically, Compound 6 potently inhibits the deacetylation-catalyzed by *sirt1* and, to a lower extent, by *sirts2* and *3*; SirReal 2, Compounds 7 and 8, and AK-7 potently inhibit *sirt2*; Tenovin-6, salermide, and benzodeazaoxaflavin are small-molecule inhibitors of *sirt1* and *sirt2*; Suramin binds into the B- and C-pockets of the NAD^+ binding site and to the substrate-binding site of *sirt5*.[409–414] Interestingly, the *sirt2* inhibitor AK-7 can cross the blood-brain barrier, decreasing neuronal polyglutamine inclusions and cholesterol levels, thereby exerting neuroprotective effects.[415]

8.6.1.5 Mitochondrial uncoupling

Although the chemiosmotic proton circuit across the inner mitochondrial membrane links ATP production to substrate oxidation,[416,417] the basal leak of protons into the mitochondrial matrix (or basal mitochondrial proton conductance) is responsible for a mild mitochondrial uncoupling.[369] Under physiological conditions, this evolutionarily-conserved proton leak contributes to 20%–25% of the metabolic rate, constituting an important energy drain.[418] Interestingly, the basal mitochondrial proton leak has been hypothesized to protect against the overproduction of reactive oxygen species and, ultimately, against oxidative stress—the "uncoupling to survive hypothesis".[416] Though the mechanisms underlying such basal mitochondrial proton leak remain debatable, some authors suggested a role for the SLC25 superfamily of mitochondrial solute carriers, while others hypothesized that it may result from the activation of the mitochondrial ADP/ATP carrier.[419]

Besides the basal mitochondrial proton leak, the activation of uncoupling proteins (UCPs) may result in an inducible proton conductance,[420,421] which appears to play a role in cancer cell metabolism and tumor progression.[369] Despite some controversy, such mitochondrial uncoupling may promote tumor cells' metabolic reprogramming, thereby decreasing oxidative stress and cell death mechanisms and ultimately allowing cancer cells' proliferation.[422] Conversely, others reported that female mice with higher metabolic rates and higher mitochondrial uncoupling also showed an increased lifespan.[423] This study further supported the "uncoupling to survive hypothesis."

8.6.1.5.1 *UCP1* was first identified in the 1970s and is the most well-known member of UCPs[424,425]

UCP1 is predominantly expressed in brown adipose tissue, being stimulated by the sympathetic nervous system's activation by cold temperatures and the subsequent release of free fatty acids. Then, UCP1 dissipates the proton gradient across the inner mitochondrial membrane and uncouples electron transfer from ATP synthesis. The electron transfer chain activity increases, releasing the energy as heat.[426,427] Indeed, UCP1 was traditionally associated with the thermogenesis mechanisms in mammals.[424,425] More recently, others proposed that UCP1 may constitute a symporter for protons and fatty acid anions.[419]

8.6.1.5.2 UCP2, UCP3 and UCP4

UCP2 is ubiquitously expressed throughout the body, including the brain and pancreatic β-cells.[369] Though Dietrich and Horvath hypothesized that UCP2 could be a pivotal player in regulating lifespan and senescence,[428] studies performed in mice with upregulation or downregulation of UCP2/UCP3 provided contradictory data.[369] For example, McDonald et al. did not observe significant alterations in mice's lifespan overexpressing or lacking UCP2 and UCP3.[429] Conversely, others reported that mice lacking UCP2 had a shorter lifespan, while UCP2 overexpression failed to extend their survival.[430] *UCP4* stabilizes calcium homeostasis and maintains human neural cells' mitochondrial function while protecting against oxidative stress and apoptosis.[431]

Given these controversial results and the lack of knowledge on the role of UCP2 and 3 in basal mitochondrial uncoupling,[419,420,432] one can hypothesize that other mechanisms may crosslink mitochondrial uncoupling to longevity. One such mechanism could be the caloric restriction-like features of mitochondrial uncoupling (including the decrease in mitochondrial membrane potential and in ATP synthesis), which has been demonstrated to extend longevity[358] (as previously discussed). Other putative mechanisms could be the lower ATP/AMP-induced activation of AMPK, the inhibition of mTOR and induction of autophagy/mitophagy, and the activation of mitochondrial biogenesis.[369,433] Of note, the increased respiration and NADH oxidation rates that arise from the mitochondrial uncoupling may in turn increase the NAD^+/NADH ratio and activate SIRTs and their antiaging effects[369] (as previously detailed).

8.6.1.5.3 2,4-dinitrophenol (DNP) and carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone (FCCP) are well-studied pharmacological uncoupling compounds[369]

DNP and FCCP are lipid-soluble, weak acids that function as protonophores, increasing the metabolic rate and, therefore, appearing to constitute promising anti-obesogenic treatments.[369] DNP was even considered a potential metabesity therapy, which includes obesity-related complications, neurodegenerative disorders and

accelerated aging.[434] Indeed, some authors observed that chronic administration of low doses of DNP to mice reduced diet-induced obesity, insulin resistance and also prolonged their life.[435,436] Increased tissue respiratory rates accompanied these observations, as well as normalization of blood glucose, triglycerides, and insulin levels and a reduction in hydrogen peroxide levels in DNA and protein oxidation.[435] In line with this, Goldgof et al. observed that chronic DNP treatment reduced body and fat mass, and hepatic steatosis in females fed a high-fat diet, which also displayed a higher energy expenditure without changes in food intake, and by the recovery in their glucose tolerance.[436] These beneficial effects of DNP may be mediated by a decrease in lipid storage and the stimulation of insulin sensitivity in adipose tissue, skeletal muscle and liver.[433] Moreover, we cannot exclude an effect of DNP in neurons from appetite-regulating brain areas and/or in neuroinflammatory mechanisms.[433] Thus, chronic administration of low, nontoxic, doses of DNP could be a potential therapeutic approach against obesity and type 2 diabetes.[433] More recently, studies demonstrated that low doses of DNP also protected mouse neurons against Parkinson's disease and could thus constitute a promising treatment herein.[437,438]

However, we must bear in mind that the low selectivity/efficiency, the severe side effects (including hyperthermia), and the toxicity (or even death) associated with high doses render the therapeutic potential of DNP and FCCP very limited.[434,439] In this respect, Kamour et al. reported an increase in the number of humans presenting toxicity symptoms or even dying after exposure to DNP.[440] In a recent study, Quirós et al. observed that FCCP decreased mitochondrial membrane potential and increased superoxide levels in HeLa cells, without significantly changing ROS measured by H_2DCFDA dye.[441] Furthermore, another protonophore, CCCP, was found to dissipate mitochondrial membrane potential in HeLa cells and to inactivate the fusion protein, OPA1 mitochondrial dynamin like GTPase (OPA1).[442] Similar observations were made in HeLa cells and mouse embryonic fibroblasts with other uncouplers, which appeared to induce mitochondrial shrinkage into spheroid structures.[443] According to the authors, these mitochondrial morphology and function changes may allow their clearance through mitophagy,[443] as discussed elsewhere.

To overcome the limitations associated with the "traditional" mitochondrial uncouplers, several safer and therapeutically promising mitochondrial- or tissue-targeted uncouplers (e.g., the targeted expression of UCP1 in skeletal muscle[444,445] are currently under development).[417] For example, niclosamide-induced mitochondrial uncoupling targets p53-deficient tumor cells for apoptotic death.[446] Furthermore, a mild mitochondrial uncoupling activity of UCP1 targeted towards mice's skeletal muscle improved their energy expenditure and metabolism, reduced their muscle mass, and extended their longevity,[447–450] also decreasing the incidence of lymphoma.[444]

Additionally, oxidative phosphorylation capacity was attenuated in the skeletal muscle from the HSA-UCP1 mice,[451] whereas their glucose uptake, fatty acid oxidation, and insulin sensitivity were recovered.[452–454] Of note, this improvement in peripheral glucose homeostasis of HSA-UCP1 mice was independent from the amelioration of obesity.[454] Surprisingly, isolated mitochondria from skeletal muscle of HSA-UCP1 mice had lower superoxide levels,[451] but both the production of reactive oxygen species and the activity, for example, of catalase, were increased.[452] This suggests that the mitochondrial uncoupling-induced activation of antioxidant defenses in skeletal muscle may correspond to a mitohormesis process.[455] A mild mitochondrial uncoupling may elicit the integrated stress response pathways, including antioxidant defenses, mitochondrial biogenesis, and the unfolded protein response, to ultimately resist cellular stress and increase longevity.[455–457] However, prolonged or intense activation of the integrated stress response may culminate in cell death.[458]

Another striking observation in muscle from HSA-UCP1 mice was the downregulation of the Itpr1 gene that encodes the IP3 receptor type 1, the main calcium-release channel at the endoplasmic reticulum and, thus, a key molecule in the intracellular calcium homeostasis and signaling.[459] This appears to correlate with the increased endoplasmic reticulum stress markers reported in these mice.[459] Additionally, Ost et al. described that ULK1, a pivotal regulator of mitophagy, was activated in an AMPKα2-dependent manner in muscle from HSA-UCP1 mice under mild mitochondrial uncoupling.[453] According to these authors, this could constitute a compensatory mechanism for maintaining cell viability and mitochondrial homeostasis upon aging.[453,460,461] Therefore, AMPK activation upon mitochondrial uncoupling might be more effective than dietary restriction in delaying aging.[439]

8.6.1.6 Physical activity

Physical activity is another increasingly studied antiaging approach. Although physical exercise appears to increase health span rather than lifespan,[462] Holloszy demonstrated that the physical activity associated with running in a wheel extended the lifespan of rats by 10%.[463] Beyond this, we must emphasize the importance of physical activity in maintaining and recovering muscle function,[464] or even preventing its degeneration along with aging.[465] Accordingly, long-term moderate physical exercise delayed the loss of grip strength and improved the physical performance in aged rats.[466,467] Hence, regular physical exercise could be more efficient in preventing/recovering skeletal muscle from age-dependent degeneration (and ultimately prolonging lifespan) than dietary restriction.[439]

Among the molecular mechanisms underlying such positive effects of physical activity/exercise on skeletal muscle upon aging, examples include stimulating antioxidant defenses, general muscle function, and mechanisms that protect against its atrophy.[439] Strikingly, there are already some exercise mimetics, that is pharmacological

compounds that stimulate the above mechanisms and appear to benefit muscle function and other tissues.[439,468,469] These exercise mimetics include, for example, metformin and resveratrol, and were found to extend the lifespan of several organisms.[439,468,469] However, we must bear in mind that none of the currently available exercise mimetics alone can activate many molecular mechanisms affected by physical activity. Therefore, they may not fully reproduce the effects of physical activity on skeletal muscle.[439] An example of this intricate crosstalk is the alternate stimulation of the opposite signaling pathways mediated by AMPK and mTOR induced by physical activity.[439]

Although pharmacological compounds activate these cascades (the so-called performance-stimulating drugs in sports), mTOR's overactivation may antagonize AMPK, promoting cellular glycolysis and ultimately inflammatory processes.[439] On the other hand, using AMPK activators (most of them anti-diabetic drugs) upon high nutrient levels may exacerbate ATP production, leading to mitochondrial hyperpolarization, the consequent formation of reactive oxygen species, and oxidative stress injury. The abnormally high levels of ATP may be also accompanied by a decrease in ADP content that may, in turn, affects muscle function.[439] Although the well-known AMPK stimulant 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) demonstrated a beneficial effect on muscle tissue, its chronic administration also induced muscle atrophy.[470]

These controversial results point to the need for some caution when considering exercise mimetics to delay age-related (muscle) degeneration and prolong longevity. Although further studies are needed, Knorre and Severin hypothesized that the use of mitochondrial uncouplers could be interesting in this context, by allowing a moderate, controlled decrease in mitochondrial membrane potential and ATP synthesis (as detailed previously).[460]

8.6.2 Mitochondria-related antioxidant approaches

8.6.2.1 What can we learn from the "eternal youth" of the naked mole rat?

Besides dietary restriction, recent evidence also points towards the antiaging potential of mitochondria-targeted antioxidants.[1] This is mostly based on studies showing the involvement of mitochondria-derived ROS in aging,[471–473] and the higher vulnerability of aged animals to oxidative stress-mediated damage (namely to the brain).[474,475] In contrast, newborn animals and naked mole rats were more resistant to oxidative stress, and the short-lived nematode *C. elegans* (lifespan $\sim$2 weeks) presented low ROS levels that inhibited apoptosis.[476,477] In this respect, we must emphasize several embryonic-like features that naked mole rats present during adulthood and aging that may provide valuable insights: the lack of a mitochondrial reticulum in skeletal muscle formed by small fused mitochondria, low levels of adenine nucleotides in heart mitochondria, partial downregulation of the ADP-induced mitochondrial

respiration/membrane potential upon the decrement in the content of this adenine nucleotide in heart and liver, lower lipid peroxidase index, unchanged (or even higher) levels of ROS and unsaturated fatty acids, unchanged antioxidant parameters (especially the activities of catalase and superoxide dismutase, and the levels of reduced glutathione), entire proteasomal content, the inclusion of serine residues in their β-actin structure, as opposed to the easily oxidizable cysteine residues.

These hallmarks may account in part for these rodents' high resistance to age-related oxidative injury,[3,366,478,479] thus reinforcing the concept of ROS involvement in aging and age-related disorders.[1] Accordingly, aging involved different observations, including, apart from changes in the activities of antioxidant enzymes, discussed before, the activation of the cytochrome c—p66shc complex within the mitochondrial intermembrane space, the activation of the lipoate dehydrogenase at the mitochondrial matrix and the stimulation of monoamine oxidase at the outer mitochondrial membrane, ultimately blunting the detoxification and allowing an overaccumulation of (mitochondrial) ROS.[1] Of note, Ku et al. correlated the lower levels of the protein deacetylase sirtuin 3 (SIRT3) upon aging with the downregulation of mitochondrial antioxidant enzymes.[196]

The naked mole rats are also protected against glucose tolerance changes, glycated hemoglobin, or blood vessel elasticity upon aging, and thus may be less prone to alterations in glucose homeostasis, diabetes, or blood vessel disorders.[1] Strikingly, these animals preferentially metabolize fructose (rather than glucose) through the fructose pathway,[1] thus rendering their metabolism more versatile in terms of substrate choice. However, their high intracellular fructose levels could also exacerbate protein (including hemoglobin) glycation,[480] whereas the formation of glyceraldehyde, lactate, and protons could eventually increase their ROS levels.[1] Since neither oxidative stress nor glycated hemoglobin increased in naked mole rats, this implies strong and effective antioxidant defense mechanisms against aging.[481–483] Moreover, the expression of the GluN2D subunit of the NMDA receptor for glutamate in their adult hippocampal neurons, in contrast with its decrement upon aging in other rodents, may further constitute an adaptive mechanism against oxidative stress- and hypoxia-mediated damage.[1] This may likely occur by efficiently regulating the Na^+ and K^+ (and indirectly also Ca^{2+}) gradients across the plasma membrane under hypoxia (and its consequent downregulation of ATP synthesis in mitochondrial oxidative phosphorylation). Consequently, their neuronal depolarization might be shortened, as well as the triggering of apoptosis.[1] Despite normal neurogenic and migrational events, hippocampal and olfactory neurons from adult naked mole rats also present features of a prolonged maturation, including the expression of structural plasticity markers, or morphogenic and spatial synaptic rearrangements[484] that may ultimately render their brains more plastic and less prone to age-related neurodegenerative diseases.[1]

8.6.2.2 Mitochondria-targeted antioxidants in delayed aging

Endogenous small molecule antioxidants play an important role in any organism to alleviate oxidative damage. Many antioxidative small molecules are either synthesized by the human body (glutathione, bilirubin) or absorbed from food (vitamin E, polyphenols). Naturally, supplementation with small molecule antioxidants has been regarded as a promising therapy against ROS-induced conditions as well as aging. A problem all these substances have in common is that their delivery is untargeted, meaning they are distributed widely in the body and might not even enter the cellular compartments where oxidative damage occurs, for example, in mitochondria. Multiple clinical trials with antioxidant supplementation were conducted, but an increase in various diseases and even increased mortality warranted their quick abortion (for a comprehensive review, see[151]).

It is plausible that mitochondria-targeted antioxidants may play a beneficial role against aging and ultimately prolong the lifespan. Using the classical antioxidants *N*-acetylcysteine and ascorbate or catalase stimulation, Griffith et al. observed protection against thymus alterations upon aging.[485] Other authors showed that the administration of the novel triphenylamylphosphonium cation before reoxygenation rapidly downregulated the activity of mitochondrial complex I and the subsequent formation of hydrogen peroxide in an animal model of hypoxia/reoxygenation. The observed effect was a reduction in mitochondria-related oxidative stress and in the area of myocardial infarction, most likely by stabilizing protein sulfhydryl groups.[486] One strategy that has been tested during recent years is the use of mitochondria-targeted antioxidants, not only to specifically inhibit mitochondria-generated ROS in the context of different pathologies (reviewed in[487–490]), but also attempting to increase lifespan.

Mitochondria drug discovery programs are lengthy and complex processes that primarily focus on identifying hit/lead compound(s) that can target the dysfunctional organelle, or any specific intrinsic target, without changing healthy parameters.[491] After this process, lead optimization is needed to refine the structure(s) and produce more potent and selective compounds.[492] As well, in this course, the pharmacokinetic properties of the lead structure are also fundamental. The tailored structural modifications are regularly based on analog design, including the use of (bio)isosterism, to provide libraries enriched in chemical diversity that can establish robust structure-property-activity/toxicity relationships. The lead optimization process must be systematically carried out and supported by structure-based drug design techniques using molecular modeling, among others.[493] In parallel, attention is also focused on the detailed profiling of physicochemical (e.g., solubility, lipophilicity, ionization, stability), permeability and in vitro ADME-Tox (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties to maximize the therapeutic index and minimize side effects.[494] If one or more of these properties is less than ideal, it might be necessary to screen many more compounds specifically for those properties. If successful, the candidate(s) will be tested during in vivo model(s),

and a drug candidate can be elected to undergo clinical development and ultimately become a marketed medicine.[495]

As the drug discovery process is complex and risky, some shortcuts can accelerate the discovery of new chemical entities, such as drug repurposing/repositioning[496] or bioinspired chemistry that is based on the principles used by nature. Dedicated drug delivery systems for targeted delivery and/or controlled release can also be a valuable tool to facilitate the lead optimization process, including the modulation of ADMET-Tox properties.[497] Based on these concepts, we highlight the discovery of the mitochondria-targeted antioxidants described below, namely MitoQ, SkQ1 and AntiOxBEN$_2$ and AntiOxCIN$_4$. Generally, the core is a natural endogenous or exogenous scaffold and the carrier the TPP cation. The modulation of the ADMET properties has been performed in the linker by a molecular modification strategy called homologation. The list below represents some examples of mitochondria-directed antioxidants in which the potential to decrease mitochondrial oxidative stress was studied. Only a few were tested in the context of lifespan extension.

8.6.2.2.1 MitoQ

Mitoquinone (also known as MitoQ) is one of the most well-studied mitochondria-targeted antioxidants. MitoQ comprises the lipophilic triphenyl-phosphonium (TPP) cation covalently bound to the endogenous antioxidant ubiquinone (which is also a pivotal component of the mitochondrial electron transport chain) via a ten-carbon alkane.[498–501] Of note, the three phenyl groups in the MitoQ structure allow the TPP cation's stability and protect the molecule against nucleophilic attack.[500] Although MitoQ readily crosses the neuronal cell membrane due to its negative membrane potential and to the lipophilicity and hydrophobicity of MitoQ, the positive charge of TPP drives the molecule immediately into the matrix face of the inner mitochondrial membrane, where it accumulates at a 100-fold higher concentration.[500–502] Then, MitoQ can be reduced by the mitochondrial complex II to the ubiquinol form (Mitoquinol), which can, in turn, be oxidized to ubiquinone (Mitoquinone) and then rapidly reduced again to ubiquinol. These cycles of reduction-oxidation allow MitoQ to maintain an efficient chain-breaking antioxidant capacity, targeting mainly lipid hydroxyl radicals[499,500,503–505] and the formation of a superoxide[506] to protect neurons against mitochondria-derived oxidative stress.[500] Besides preventing lipid oxidation,[503] MitoQ attenuates age-related impairment in the rodent brain mitochondrial respiratory chain, ATP synthesis, and membrane potential.[507] Furthermore, MitoQ decreases neuronal inflammation and demyelination, improves remyelination mechanisms, and mitigates neuronal axonal loss.[500,501] As a result, MitoQ recovered cognitive and memory function.[500,501]

MitoQ also reduced oxidative damage and cell death in cells cultured from patients with Friedreich ataxia (a rare neuronal disorder).[500] Similar beneficial effects of MitoQ were described in mouse models of spinocerebellar ataxia type 1,[508]

amyotrophic lateral sclerosis,[509] Parkinson's disease,[510] together with the inhibition of cytochrome c release from mitochondria, inhibition of caspase 3 and DNA fragmentation in bovine aortic endothelial cells.[500] Administration of MitoQ in drinking water for four weeks was associated with preservation of vascular endothelial function in older mice, including decreased superoxide generation by arterial mitochondria; also, MitoQ reversed an age-related increase in endothelial susceptibility to acute mitochondrial damage.[511]

MitoQ decreased heart dysfunction and mitochondrial injury in a rat model of ischemia-reperfusion injury.[500] Furthermore, MitoQ stimulates the mitochondrial uptake of CoQ_{10}, ultimately protecting cells from animal models and human patients of Alzheimer's disease against oxidative damage.[500,501,512] These findings were confirmed in a *C. elegans* model overexpressing human Aβ, in which MitoQ extended lifespan and mitochondrial electron transport chain efficiency.[513] Additionally, MitoQ promoted neurite growth and cell viability, downregulating the expression of mitochondrial fission markers in N2a cells expressing the mutant APP protein.[514] It also stimulated PGC1α expression, prevented membrane depolarization, and free radical formation, while stimulating cytochrome oxidase activity, thereby protecting against Aβ-mediated toxicity.[514]

Hence, we can hypothesize that MitoQ may constitute a promising therapeutical approach against still incurable neurodegenerative diseases (namely Alzheimer's, Parkinson's, and Huntington's disease). Accordingly, Yin et al. (2016) observed that MitoQ alone protected STHdh^{Q111} cells against mutant huntingtin-induced oxidative injury, mitochondrial dysfunction and synaptic impairment.[515] Interestingly, these authors also reported a synergistic effect of MitoQ plus the SS-31 peptide (further detailed later) in diminishing mitochondrial fission and upregulating mitochondrial fusion in such a cellular model of Huntington's disease. In a recent study, Pinho et al. described that the MitoQ-induced recovery of the motor coordination in the R6/2 mouse model of Huntington's disease was associated with protection against their muscle markers for oxidative injury (including carbonyl groups and mitochondrial superoxide dismutase 2).[516] This was accompanied by reduced muscle reactive oxygen species-related autophagy upon MitoQ administration, despite no significant changes in their molecular chaperones' levels.[516] These observations contrast with the MitoQ-related reduction in ROS's mitochondrial formation and the resulting decrease in mTOR and ULK1 phosphorylation in Atg proteins and LC3-II conversion into LC3-II, that culminated in the protection of C2C12 myoblasts against autophagy.[517] According to Pinho et al., the selective protective effects of MitoQ in muscle from R6/2 mice may be explained by its higher bioavailability within this tissue, in contrast with the lower brain levels of the antioxidant.[516] Hence, MitoQ may improve peripheral rather than central nervous system defects (e.g., at the muscle level) associated with Huntington's disease.

In light of this, it is not surprising that MitoQ is used in currently undergoing (as well was used in already completed) several clinical trials on, multiple sclerosis (NCT04267926), nonalcoholic fatty liver disease (NCT01167088), asthma (NCT04026711), sickle cell anemia (NCT04109820), Parkinson's disease (NCT00329056) or Hepatitis C (NCT00433108), (http://clinicaltrials.gov). However, MitoQ dosage could be a limitation in clinical trials, since concentrations above 0.3 μM were toxic for neuronal cells.[518] Furthermore, Sakellariou et al. did not observe a protective effect of MitoQ against the loss of muscle mass and function or oxidative markers in aged mice.[519] Effects of MitoQ on aging-related phenotypes has been recently reviewed.[520]

8.6.2.2.2 SkQ1

One of the most promising mitochondria-targeted antioxidants is SkQ1, a molecule that consists of a TPP cation covalently linked to a plastoquinone core.[521] This molecule not only efficiently slowed down age-related mechanisms and disorders (e.g., in human eyes and animal models), but also prolonged the mean lifespan of plants, fungi, crustaceans, insects, fish, and mammals (including old, immobilized animals).[205,522–524] Strikingly, SkQ1 or its rhodamine analog, SkQR1, also protected against renal disorders,[490,524–530] myocardial infarction, stroke[522,523,531] and neurodegenerative diseases (such as Alzheimer's disease and multiple sclerosis).[490,532] Moreover, SkQ1 is currently undergoing clinical trials on dry eye syndrome (NCT02121301; NCT03764735; NCT04206020; http://www.clinicaltrials.gov).

Although the antioxidant effect of SkQ1 remains incompletely understood, it appears to be dose-dependent. In small concentrations, SkQ1 may scavenge the free radicals resulting from cardiolipin oxidation, whereas, in higher doses, SkQ1 may mildly uncouple the transmembrane uptake of fatty acids by acting as a protonophore, similarly to the well-known uncoupler dinitrophenol.[527,533] However, one cannot exclude a possible interference of SkQ1 with the complex cytochrome c—p66shc to blunt the formation of superoxide anion from oxygen. As already mentioned, the knockout of the *p66shc* gene was shown to extend mice's longevity.[206,534,535] Together with evidence that low concentrations of dinitrophenol may extend the lifespan of *D. melanogaster* and mice,[435,536] further suggests a promising role for SkQ1 and its uncoupling-like effect. In line with these findings, mitochondria-targeted antioxidant SkQ1 has been shown to prolong the lifespan of inbred male mice in specific-pathogen-free (SPF) condition, outbred mice, and dwarf hamsters in conventional or outdoor cages.[205]

Regarding the potential therapeutic effect of SkQ1 or SkQR1 against age-related neurodegenerative diseases, Kapay et al. observed that, similarly to another mitochondria-targeted antioxidant (MitoQ), pretreatment of hippocampal slices with SkQ1 or SkQR1 completely abolished the damage induced by the amyloid-β

peptide,[532] which accumulates within the brain upon aging and plays a role in Alzheimer's disease pathogenesis.[532,537] Although the precise mechanisms underlying such protective effects of SkQ1 and SkQR1 remain elusive, mitochondrial-derived ROS and their consequent induction of the mitochondrial permeability transition pore upon amyloid-β accumulation may trigger a damaging cascade towards neuronal death that may ultimately lead to Alzheimer's disease.[538,539]

Despite the above-described promising results from preclinical studies involving MitoQ and SkQ1, their approval by the Food and Drug Administration faced several challenges.[490] As such, new mitochondria-targeted compounds are urgently needed to overcome these limitations and provide efficient and safe therapies.

8.6.2.2.3 AntiOxBEN$_2$ and AntiOxCIN$_4$

In line with the limitations described above, novel hydroxybenzoic and hydroxycinnamic acid-derived mitochondriotropic antioxidants were developed (AntiOxBENs and AntiOxCINs, respectively).[490] To date, AntiOxBEN$_2$ and AntiOxCIN$_4$, which resulted from the conjugation of gallic acid with the lipophilic TPP cation[540] and the conjugation of caffeic acid with the TPP cation *via* an alkyl spacer, respectively, revealed important antioxidant and iron-chelating properties.[490,540–542] Mitochondria accumulate both compounds in a membrane potential-dependent manner.[540–542] Significantly, the accumulation of AntiOxCIN$_4$ within the mitochondria does not interfere with its morphology or polarization.[541,542] Regarding its antioxidant effect, AntiOxBEN$_2$ was shown to protect against lipid oxidation and oxidative stress in different cell types, including rat cardiomyoblasts, human skin fibroblasts, and hepatocyte cells.[540] Similarly, AntiOxCIN$_4$ rescued oxidative stress-related injury and maintained the intracellular pool of reduced glutathione in isolated liver mitochondria and hepatic cells.[541] In a very recent study, our group demonstrated that the chronic treatment with both compounds for 72 h upregulated reduced glutathione, mitochondrial NAD(P)H and SOD2 levels in cultured primary human skin fibroblasts without significantly affecting the mitochondrial membrane potential or SOD1 expression.[543] These alterations were accompanied by a common upregulation of the regulator of cellular antioxidative stress response, NRF2, by the AntiOxBEN$_2$ and AntiOxCIN$_4$, whereas the expression of the antioxidants HMOX1 and NQO1 was affected differently by these compounds.[543] Although neither AntiOxBEN$_2$ nor AntiOxCIN$_4$ inhibited skin fibroblast death resulting from loss of glutathione, AntiOxBEN$_2$ successfully counteracted hydrogen peroxide-induced cell death.[543] These results, together with the increment in reactive oxygen species promoted by the chronic administration of AntiOxBEN$_2$ and AntiOxCIN$_4$, suggest that they may elicit endogenous protective mechanisms to mitigate oxidative stress under such conditions.[543] However, further studies are warranted to clarify these issues and the therapeutic potential of AntiOxBEN$_2$ and AntiOxCIN$_4$ against age-related disorders.

8.6.2.2.4 Other mitochondrially-targeted antioxidants

8.6.2.2.4.1 TEMPO and Mito-TEMPO Other mitochondrially-targeted antioxidants showed similarly promising results. Mito-TEMPO on the one hand, consists of 2,2,6,6-Tetramethylpiperidinyloxyl (TEMPO) coupled to a TPP cation. TEMPO is a stable radical with strong antioxidative effects and has alleviated renal failure in mice with partial nephrectomy.[544] Another promising candidate is tiron (4,5-dihydroxy-1,3-benzene-disulfonic acid), a chemical entity that serves as an antioxidant and an iron chelator. Tiron treatment has proven superior to MitoQ against both hydrogen peroxide- and UV-radiation-induced ROS, possibly due to the unique combination of its properties.[545]

8.6.2.2.4.2 Szeto-Schiller (SS) peptides It has been demonstrated that the synthetic SS peptides' interaction with mitochondrial cardiolipin is what affects mitochondrial membrane curvature, however, they also can act as a mitochondrially-targeted antioxidant. The structure of this family of small molecules comprises four amino acids alternating with aromatic groups (one of which is a 2′,6'-dimethyltyrosine residue) and a cation renders them potent antioxidants[518,546,547] that are also able to accumulate at the inner mitochondrial membrane in a mitochondrial membrane potential-independent manner.[499] This later feature of SS-peptides is due to their positive charge and lipophilic properties of aliphatic chains and hydrophobic aromatic rings[518,547] and may ultimately allow these antioxidants to target damaged mitochondria, thus rendering them promising against mitochondria-associated disorders.[548] To date, four SS-peptides were developed: *SS-02* (Dmt-D-Arg-Phe-Lys-NH_2), *SS-20* (Phe-D-Arg-Phe-Lys-NH_2), *SS-31* (D-Arg-Dmt-Lys-Phe-NH_2), and *SS-19* (Dmt-d-Arg-Phe-atnDAP-NH_2).[518,547]

Regarding *SS-02* and *SS-19*, studies showed their high potential for internalization within the cell and their immediate selective uptake by mitochondria which, in the case of *SS-19*, may be partially dependent on mitochondrial membrane potential.[518,547] *SS-02* also reduces hydrogen peroxide to oxygen and water and counteracts the oxidation of linoleic acid and low-density lipoproteins.[518,547] Similarly, *SS-20* and *SS-31* bind to cardiolipin, an essential lipid for the inner mitochondrial membrane's integrity.[518,547] *SS-20 binding to cardiolipin* maintain mitochondrial cristae's stability, prevents release of cytochrome c and restores mitochondrial electron transport chain's proper functioning upon ischemia-reperfusion .[500,518,547,549] Given the widely described involvement of amyloid-β (Aβ, a pivotal player in Alzheimer's disease neuropathology) on cardiolipin loss and the subsequent compromise in mitochondrial cristae and electron transport chain integrity, *SS-20* and *SS-31* peptides may also exert a beneficial effect herein.[550]

To our knowledge, *SS-31* appears to be the most studied SS-peptide. It contains the same amino acids as *SS-02*, but in a different arrangement that raises the antioxidant potential of *SS-31*.[518,547,551,552] Its linear uptake kinetics, independent from receptor- or transporter-mediated mechanisms, suggests that SS-31 is readily taken up by endothelial,

renal, and intestinal epithelial cells, myotubes, cardiomyocytes, macrophages or neurons, whereby it accumulates at the inner mitochondrial membrane (rather than at the mitochondrial matrix).[551] Importantly, SS-31 appears not to affect normal mitochondria structure or function,[551] while it effectively mitigated lipid oxidation, the production of reactive oxygen species, and scavenging of hydrogen peroxide, for example, in rodent models of amyotrophic lateral sclerosis and myocardial infarction.[551,553] *SS-31* also counteracted mitochondrial reactive oxygen production and mitochondrial dysfunction upon ischemic conditions, increasing ATP production, most likely by blunting the peroxidase activity of cytochrome c.[551] Besides, binding of *SS-31* to cardiolipin at the inner mitochondrial membrane stabilizes cytochrome c function within the electron transport chain in mitochondrial damaging conditions.[551] Manczak et al. further demonstrated that SS-31 stimulated neurite growth, protected against the dysfunctional cytochrome c oxidase, maintained mitochondrial membrane potential, and improved ATP synthesis, thereby preventing $A\beta_{25-35}$-induced mitochondrial dysfunction and cell death in mouse neuroblastoma N2a cells and in primary neuronal cells expressing the mutant amyloid precursor protein (APP).[514,554] This neuroprotective role of SS-31 might also involve the downregulation of mitochondrial fission and the upregulation of PGC1α, FOXO1, NMDA receptor, and Prxs genes,[514] as well as the improvement in synaptic activity in neurons expressing mutant APP.[514,554] Similar therapeutic effects of SS-31 were reported by Calkins et al. in mitochondria and synapses of the Tg2576 mouse model of Alzheimer's disease.[552] More specifically, SS-31-treated Tg2576 mice showed a recovery in their axonal mitochondria transport, number, and size, as well as with their protection against oxidative damage.[552] Adding to this, SS-31 exerted a synergistic effect when co-administered with the small molecule antioxidant Mdivi1 to N2A cells transfected with mutant APP, by improving mitochondrial function, mtDNA copy number, and cell viability, and lowering $A\beta_{1-40}$ and $A\beta_{1-42}$ levels.[553] These pieces of evidence further reinforce the therapeutic potential of SS-31, alone or in combination with other antioxidants, against Alzheimer's disease.

In another age-related disease, sarcopenia, Sakellariou et al. reported that, despite no effect on muscle mass and function loss, chronic subcutaneous treatment with SS-31 mitigated oxidative muscle stress in 28 month-old mice.[555,556] However, others demonstrated that both acute and chronic administration of SS-31 recover muscle mitochondrial structure, function, and homeostasis in aged mice, increasing their tolerance to physical exercise.[342,557] More recently, SS-31 was shown to mitigate the mitochondrial production of reactive oxygen species and proteolytic activity in muscle from mice treated with doxorubicin (a highly potent chemotherapeutic drug that also damages muscle tissue).[558] This reinforced the therapeutic/antioxidant potential of SS-31 against sarcopenia. Notably, SS-31 increased SIRT1, and downregulated TNFα and NFκB in leukocytes from type 2 diabetes patients, ultimately reducing their inflammatory markers.[559] Moreover, SS-31 could reenergize mitochondria and improve cardiac function in a rat model of ischemia.[560]

8.7 Conclusions

Current data from animal studies, ranging from flies to mice, cast doubts on the generality of the mitochondrial free radical theory of aging. Thus, efforts are underway towards a more general theory of aging, to account for conflicting results from many studies. Although mitochondria seem to play a vital role in aging, mitochondrial ROS generation is likely only one (and potentially secondary) factor out of many others. It has been repeatedly shown that cells can easily manage ROS's physiological levels and, more importantly, a certain level of ROS is even crucial for proper cellular signaling and development.[561] While it seems intuitive that alleviating high levels of ROS is beneficial for cellular function, conclusive evidence that ROS-reducing treatments positively affect the human lifespan is difficult to produce. A further difficulty regarding human aging studies is that any pharmacological or dietary intervention likely has to happen early in life and probably for many decades, while an unsurpassable number of confounding factors may shroud any potential effects.

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CHAPTER 9

Intercellular communication and aging

Teresa M. Ribeiro-Rodrigues[1,2,3], George Kelly[4], Viktor I. Korolchuk[4] and Henrique Girao[1,2,3]

[1]Coimbra Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[2]CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[3]Clinical Academic Centre of Coimbra (CACC), Coimbra, Portugal
[4]Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Campus for Ageing and Vitality, Newcastle Upon Tyne, United Kingdom

9.1 Importance of intercellular communication

Fine-tuned intercellular communication, between cells, tissues and organs, is crucial for the homeostasis of a healthy organism and its deregulation has been associated with aging and the development of several age-related disorders. Indeed, derailment of intercellular communication has been considered a hallmark of the aging process, together with cellular senescence, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, stem cell exhaustion, telomere attrition, genomic instability and epigenetic alterations.[1] These alterations in intercellular communication are not isolated processes making their study and the development of possible interventions intricate and complex. Indeed, it is difficult to discern whether deregulation of intercellular communication is a cause or consequence of the aging process. In fact, the way by which "aging pillars" mutually influence each other suggests that a holistic vision of the aging process is required. In the same way, cells need to ensure a well-balanced communication to mount a synchronized response of the organism. One of the examples of the deregulation of intercellular communication during aging is the inflammaging phenomena. Inflammaging is a low-grade, sterile and chronic inflammatory state observed in aged individuals, caused by exacerbated or continuous stimulation of the innate immune system by endogenous misplaced or altered molecules, persistent infections, components of our body as a *meta*-organism (microbiome) and the presence/accumulation of senescent cells.

In this review, we aim to provide a comprehensive and critical perspective of the impact and role of intercellular communication in aging (Fig. 9.1). Although intercellular communication during aging can assume different forms, we will focus on: (1) the canonical senescence associated secretory phenotype (SASP), (2) direct cell-to-cell communication through gap junctions or tubule-like structures and, (3) long distance communication, involving extracellular vesicles and small metabolites released through Connexin-containing hemichannels that underpin paracrine communication.

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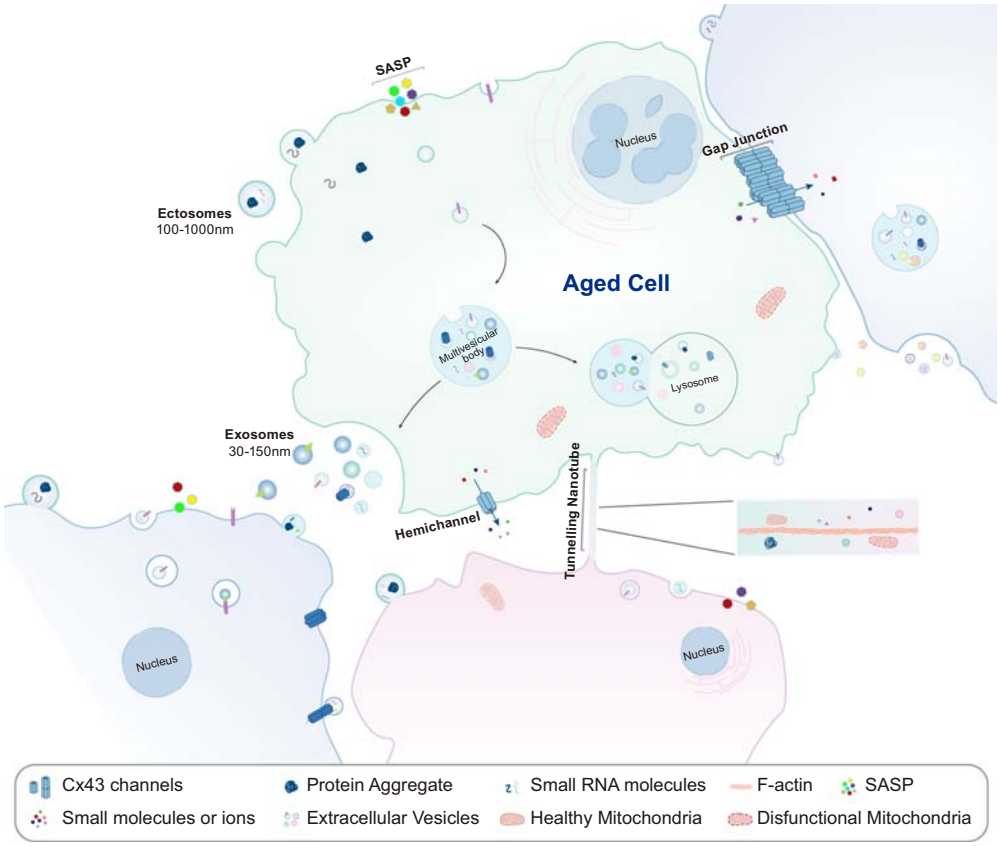

Fig. 9.1 Schematic representation of the different types of intercellular communication. Connexins composed hemichannels allow the exchange of small molecules between the cytoplasm and the extracellular milieu, or when in the form Gap Junctions facilitate communication between neighbor cells. Extracellular vesicles result either from the fusion of multivesicular bodies (MVBs) with the plasma membrane (PM) releasing small vesicles called exosomes, or from PM outward budding originating ectosomes. When in the extracellular space, these vesicles can fuse, dock or be endocytosed by the recipient cell triggering a response. According to its properties, tunnelling nanotubes (TNTs) ensure communication between connected cells by transference of organelles, protein aggregates, genetic material and small molecules. Aged cells can be characterized by a particular secretory phenotype, senescence associated secretory phenotype (SASP), which components can propagate to other cells and throughout the organism potentially modulating younger cell response.

9.2 The defining features of senescence

Aging can be defined as a time-dependent decline in function that affects most living organisms compromising their homeostasis and survival. Several hallmarks have been described that manifest during the aging process whilst accelerating its development. These include genomic instability, loss of proteostasis, mitochondrial dysfunction and cellular senescence.[1]

Derived from the Latin word senex, meaning old man or old age, senescence is used to describe the gradual deterioration of organisms and lack of capabilities to respond to external changes following their maturation.[2,3] Over fifty years ago, the term cellular senescence was used to describe cells that had terminated dividing in culture.[3,4] Leonard Hayflick and his colleagues showed that human fibroblasts had a limited ability to proliferate. Gradually over many population doublings, proliferation decreased until cells lost the ability to divide despite the abundance of nutrients and growth factors in the medium.[4] Penned by Hayflick, replicative senescence is still used to this day to describe this phenomenon and the maximum proliferative lifespan is known as the Hayflick limit.[5]

Senescent cells display several phenotypes which are considered to define when a cell is in a senescent state.[2] These include growth arrest, apoptosis resistance and altered expression of cell cycle mediating genes. Senescent cells and their nuclei increase in size whilst also appearing flattened and vacuolated.[6,7] First described in 1995 by Dimri et al.,[8] senescence associated beta-galactosidase (SA β-gal) activity has been one of the most utilized biomarkers of cellular senescence mainly because of its specificity to senescent cells and simple protocol. Senescent cells harbor an increase in lysosomal activity allowing them to be identified by this method at pH 6.0. Using a chromogenic substrate such as X-gal in situ, cells that are senescent show a dark blue precipitate at the site of β-galactosidase and X-gal interaction.[8,9]

Mitotic cells often spend long periods of time in a quiescent state where growth is temporally arrested. Certain signals can then cause a response by the quiescent cells where they re-enter the cell cycle in times of need and continue to proliferate. Senescence differs in that the mitotic cells enter a state of irreversible growth arrest whilst remaining metabolically active.[10] This growth arrest occurs due to an upregulation in cell cycle inhibitors, the most common being p16 and p21.[11] Simultaneously there is a down-regulation in cell cycle promoters such as cyclin A/B, c-fos and proliferating cell nuclear antigens. Furthermore, markers of cell proliferation such as Ki67 are lacking in senescent cells.[2,12–14] There is no physiological way of rescuing cells from a senescent state without external intervention.[15]

9.3 The mechanisms responsible for the induction of cellular senescence

Different mechanisms have been implicated in cellular senescence. One involves telomere shortening which is the main cause behind replicative senescence, initially described by Leonard Hayflick.[3,4] Telomeres are complexes found at the end of chromosomes that are made from stretches of TTAGGG DNA repeats and several proteins. Their purpose is to protect the ends of linear chromosomes from degradation or intervention from the DNA repair process. Known as the end replication problem, cells lose approximately 50–100 base pairs from their telomeric DNA through every S phase. Eventually after a certain number of replications, the telomere will become

dysfunctional through critical loss of DNA.[16,17] Persistent DNA damage response signals will be released causing cell cycle arrest and resulting in senescence. For that reason, telomeres are often referred to as the biological clocks of the cell.[18]

External sources such as ionizing radiation and chemotherapy can also cause DNA injury and trigger the DNA damage response. Senescence is then induced if the DNA damage is too extensive such that it cannot be repaired.[18] Telomere regions are common targets of a persistent DNA damage response because of the inhibition of nonhomologous end joining by shelterin components, such as TRF2. This, therefore, results in a constant DNA damage response, promoting a stable cell cycle arrest. DNA damage response factors such as phosphorylated histone-2A.X (γ-H2A.X), ataxia telangiectasia mutated and p53-binding protein 1 (p53BP1) are recruited to these double strand breaks and stimulate signaling of the p21 pathway leading to senescence, a mechanism referred to as "stress-induced senescence."[19,20] Oxidative stress can be another cause of senescence due to increased levels of intracellular ROS that lead to p53 mediated upregulation of p21.[21] Accordingly, it has been demonstrated that antioxidant treatment can prevent oxidative stress-mediated senescence.[22]

Another mechanism that acts through the DNA damage response pathway is oncogene-induced senescence (OIS). Research has shown that senescence can occur in different tumors in vivo, arresting tumor development and progression. Senescence has therefore been described as a potent protective anti-tumor mechanism.[23] OIS involves the expression of the CDKN2A locus, which includes INK4A and ARF. This encodes key tumor suppressor genes such as p16INK4A, at low levels that can increase with age. Therefore, CDKN1A expression inhibits the cell cycle through p16 or p21, via activation of p53. CDKN2A expression can also be observed in replicative senescence. It can be activated independently from the DNA damage response, however damage associated with ROS or extensive telomere shortening will promote a robust DNA damage response resulting in senescence mediated by p53/p21.[24]

9.4 Senescence associated secretory phenotype

Senescent cells produce a range of cytokines, a phenomenon known as the SASP. The SASP is described as a complex pro-inflammatory response including the release of pro-inflammatory cytokines such as interleukin 8 and 6 (IL-8 and 6), growth factors such as transforming growth factor-β (TGF-β), reactive oxygen species (ROS) and proteases responsible for degrading the extracellular matrix.[25] The SASP reinforces the senescent phenotype in an autocrine manner by creating an inflammatory environment.[26] However, these secretory factors are also capable of inducing senescence in neighboring cells in vivo, commonly termed the "bystander effect."[27] This paracrine induction of senescence is mostly mediated by TGF-β and ROS, by inducing the DNA damage response and promoting induction of p15, p21 and p27 in neighboring

cells.[27,28] It is believed that the purpose of the SASP is to act as a signaling mechanism for the recruitment of immune cells to clear senescent cells. This can therefore dampen the damage that can occur as a result of their accumulation.[29] These immune cells include T cells, neutrophils, natural killer cells and macrophages, and have all been implicated in the clearance of senescent cells.[30] The exponential production of ROS that is observed as part of the SASP occurs as a consequence of metabolic changes associated with mitochondria dysfunction.[31] In accordance, within senescent cells, the mitochondria have an increased mass and reduced membrane potential alongside increased ROS production.[32]

Both the bystander effect and SASP attributed to senescent cells indicate that they actively interact with their environment. Several mechanisms that mediate intercellular communication and provide coordinated physiological responses can also be involved in the spread of senescence and contribute to the process of aging as a whole. Growing evidence indicates the role of short- (gap junctions, tunneling nanotubes) and long-range (extracellular vesicles) cell-cell communication in both the suppression and facilitation of the age-related functional decline.

9.5 Intercellular communication mediated by gap junctions and connexin channels

Gap junctions are intercellular channels that allow the conveyance of second messengers, ions, small metabolites, linear peptides or small regulatory RNAs between adjacent cells. This type of communication has been shown to be crucial to ensure homeostasis in various organs and tissues, participating not only in short term processes like the rapid and coordinated conduction of the action potential and spreading of signaling molecules, but also in slower physiological processes such as cell growth and development.[33,34] Gap junction channels are formed by the apposition/docking of two hexameric channels, called hemichannels or connexons, present at the plasma membrane of each of the two adjacent cells. When unposed, hemichannels allow the exchange of molecules between a cell's cytosol and the extracellular milieu constituting a form of paracrine communication, typically associated with pathological triggers such as a decrease in extracellular Ca^{2+} concentration, oxidative stress and membrane depolarization.[35–40] Each hemichannel is composed of six identical (homomeric channels) or different (heteromeric channels) subunits of members of the connexin (Cx) protein family. The human genome encodes twenty-one different connexin genes, of which the most widely expressed and studied is Connexin43 (Cx43). All the connexins share a common topology, that is a transmembrane protein with four transmembrane domains, one cytoplasmic and two extracellular loops, and cytoplasmic amino-terminal and carboxy-terminal domains. The communication mediated by Cx channels, either hemichannels or gap junctions (Gap Junction Intercellular

Communication—GJIC), can be regulated at different levels such as transcription and channel oligomerization, intracellular trafficking, gating and degradation.[41] It has been described that the general aging process is associated with a decrease in Cx43 protein levels coincident with gap junction (GJ) loss and decrease in intercellular communication, observable in different cells, systems and organs.

GJIC is important in several organs and tissues to guarantee both electrical and metabolic coupling. For example, in the heart, where Cx is mainly localized at the intercalated disks of cardiomyocytes, ensuring a rapid and efficient anisotropic propagation of the electrical impulse generated at the sinoatrial and atrioventricular nodes, GJIC allows the heart to work as a functional syncytium. Not surprisingly, impairment of GJIC, due to a Cx remodeling that encompasses alterations on channel levels, activity and distribution, have been associated with electric conduction defects underlying cardiac disorders, namely age-related cardiovascular diseases.[42] Indeed, in the heart, despite Cx43 RNA levels being similar between young and old animals, a decrease in Cx43 was observed in aged hearts, concomitant with a remodeling of the intercalated disc, slowed impulse propagation through GJ and consequent decline of cardiac function.[43–46] This phenomenon can be explained by the shortening of Cx43 mRNA UTRs, in consequence of the activation of stress-response pathways, which limits GJA1−20k (Gap Junction Protein Alpha 1−20 kDa) translation, required for full-length Cx43 trafficking and intercalated disk localization.[44] Nevertheless, a previous study showed that in Hutchinson-Gilford progeria syndrome, a model of premature aging, the total levels are not significantly altered but it was observed in the lateralization of Cx43 in cardiomyocytes, which can contribute to the electrical conduction defects.[47] A decrease in Cx43 protein levels was described in hepatocytes and astrocytes of aged animals which correlated with impaired intercellular communication and cellular function.[48]

Additionally, Cx43 expression is also decreased in the bones of aged animals possibly due to the increased oxidative stress observed in older animals.[37,49,50] In fact, Cx43 knockdown in osteocytes increases susceptibility to oxidative stress-induced cell death, thus ascribing to Cx43 as a protective role.[51] Moreover, depletion of Cx43 in osteocytes of young animals is sufficient to induce osteocyte apoptosis and decrease bone strength, which resembles the phenotype observable at the old age.[50] In accordance, Cx43 overexpression prevents the decrease in bone formation and susceptibility to the damage observed during aging, suggesting that Cx43-mediated communication contributes to the amelioration of age-induced bone changes.[49]

The importance of Cx43-mediated communication in aging and aging-related processes was also demonstrated in hematopoietic stem cells (HSC). It was described that Cx43 deficiency in HSC and progenitors (HSC/P) cells leads to a decrease in survival and cell cycle quiescence, associating the absence of Cx43 with propensity to senescence. In fact, the absence of Cx43 culminates in an inability of HSC/P to drain reactive oxygen species to the hematopoietic microenvironment, leading to an

accumulation of ROS within these cells and consequently resulting in senescence.[52] In accordance, re-expression of Cx43 results in rescued communication of HSC/P with bone marrow stromal cells showing that Cx43-mediated communication exerts a protective role against senescence and stem cell exhaustion, as an example of the interconnectivity of the aging hallmarks.

The function of GJ composed of Cx36 is also known to decline during aging in β-cells of mouse and human islets. This correlates with a marked impairment of the coordination between [Ca^{2+}] dynamics and insulin secretion, thereby contributing to glucose intolerance. This age-dependent decline in islet function that contributes to reduced insulin sensitivity and secretion, and possible development of type 2 diabetes, was restored by pharmacological GJ activation, connecting age-dependent alterations of Cx with functional outcomes and a possible development of age-related diseases.[53]

On the other hand, the maintenance of cell-cell communication can also be detrimental during stressful conditions and aging, due to the propagation of harmful signals and damage through bystander effect. In fact, GJ channels have a dual role, as on one hand they allow the "dilution" of harmful signals like ROS thus promoting cell survival, whilst on the other hand this spread of small metabolites may also contribute to spreading of cell damage and cell death. Example of that is the response during myocardial ischemia/reperfusion, in which GJIC appears to mediate cell injury to the myocardium at a distance from the infarct zone contributing to cardiac dysfunction.[42,54] Additionally, Cx43-mediated IC has also been associated with a detrimental response to chronic stresses like endoplasmic reticulum stress in hepatocytes, where cell-cell coupling facilitates the transmission of signals that propagate stress and consequently promote cell dysfunction in a "bystander response," preventable by Cx43 depletion.[55] ER stress can be triggered by a variety of stimuli including some observed to be present in aging, like increased oxidative stress, perturbance of calcium homeostasis, impaired protein folding, accumulation of protein aggregates and decreased protein degradation resulting from proteasomal and autophagy dysfunction.[56] Additionally, it is also described that the unfolded protein response, activated upon ER stress, declines and becomes less efficient with age,[57] suggesting that an increased and continuous ER stress occurs with aging, and can be potentially propagated by Cx channels from cell to cell, similarly to what is described for ER stress activated by metabolic stress.

In the case of hemichannels, their opening induced by stressors allows not only for the diffusion of K^+ and Na^+ between the cytoplasm and the extracellular environment, but also for the release of metabolites like ATP, glutamate, prostaglandin E2, glucose, glutathione (GSH), cAMP, IP3 and NAD^+ that may act as paracrine signals and trigger a response in neighboring cells.[36,40,58,59] An example of this phenomenon is the release of glutamate, ATP and lactate during acute inflammatory responses in the central nervous system that contributes to neuronal stress and loss.[36,40,58,60] Moreover, conditions described to be present during aging like oxidative stress, a

pro-inflammatory environment, and alterations in [Ca^{2+}], more specifically prolonged elevation of intracellular [Ca^{2+}], are major factors for HC opening, the consequent release of different signaling molecules and propagation of cell stress.[38,39,61–63]

This bystander effect, promoted either by HC or GJ, is also a contributor to the alterations observed during aging, namely to the generation of senescent cells via bystander signaling from pre-existing senescent cells. The release of SASP factors, oxidized lipids, reactive oxygen species and EVs by senescent cells is a potent inducer of cell senescence in bystander cells in vitro and in vivo.[27,64] In fact, data shows that transplantation of senescent cells into young mice is sufficient to cause persistent physical dysfunction and increase senescence of tissue-resident cells.[64,65]

Interestingly, co-culture of replicative senescent cells with young cells induces the DNA damage response, characteristic of senescence, in the young neighboring cells. Moreover, this effect was only observable when cells were grown in contact; and conditioned medium from senescent cells or growth of young and aged cells in separate layers, sharing the same medium, had no significant effects in terms of DNA damage foci or proliferation rates of recipient cells (young cells).[27] This data, together with the observation of senescent cell clusters in adult/aged mice tissue, suggests that cell-cell contact contributes to the spreading of senescence. Strikingly, chemical inhibition of GJ channels in the co-culture system (senescent and young cells), blocked the increase of DNA damage foci formation rate in young cells induced by a mechanism that was found to be dependent on ROS, suggesting that this type of cell-cell communication participates actively in senescence/damage spreading during aging.[27]

9.6 Intercellular communication mediated by tunneling nanotubes

Tunneling nanotubes, or TNTs, are thin and long membrane structures, with a transient and dynamic nature, that mediate cell-cell communication. These structures, characterized by being positive for F-actin and detached from the substrate, have a typical diameter of 50 to 700 nm and a length up to 100 μm, and allow the transfer of various materials like proteins, small RNAs, signaling molecules, vesicles, organelles, prions as well as pathogens such as viruses between two connected cells.[34] Additionally, TNTs have also been implicated in the calcium flux and intercellular electrical coupling.[66,67]

Since their first description in 2004,[68] TNTs have been reported in vitro using several cell systems including fibroblasts, neural, immune, epithelial or cardiac cells. In this context TNTs have been associated with a number of biological processes including cancer progression, pathogen spreading, immune defense, development, transdifferentiation, and resistance to stress but also stress spreading/propagation.[69–73] In vivo, structures that resemble and present features of TNTs have also been reported, being associated with electrotonic coupling, cell migration, stem cell differentiation, wound healing, stress resistance, embryogenesis and neurovascular coupling.[74–78] Despite the

formation of TNTs occurring at basal levels, it can be triggered by oxidative stress, viral infection, the presence of protein aggregates, and prion-like proteins and pro-inflammatory stimuli,[72,79–83] suggesting that TNT formation may be increased during aging where many of these stimuli/phenomena are known to be elevated.

Additionally, it was already described that senescent cells can communicate, through TNTs or TNT-like structures called cellular bridges, directly transferring proteins to neighboring cells in vitro and in vivo and that this process may facilitate immune surveillance of senescent cells by natural killer (NK) cells.[84] Moreover, when comparing proliferating and senescent human vascular smooth muscle cells, cellular bridge formation and direct transfer occurs preferentially between senescent cells.[85]

Furthermore, TNTs were also described to influence the expression of senescence markers in mesenchymal stem/stromal cells (MSCs) spheroids, in which, pre-senescent MSCs (high passage MSCs) transfer via TNTs, the cell cycle inhibitor p16 to younger MSCs (low passage cells) maintaining pre-senescent MSCs' regenerative capacity, avoiding stem cell exhaustion and potential senescence.[86] This once again suggests that the hallmarks of aging are not independent entities/phenomena but are interconnected and the study of aging and aging phenomena has to be approached holistically.

Similar to what has been described for GJIC, the communication mediated by TNTs can have a dual effect. In one hand, the dilution of harmful/deleterious components or the transfer of cellular components which are dysfunctional or lacking in the recipient cell may decrease cell damage, promote cell survival avoiding cell senescence and death.[74,87,88] On the other hand, the spreading of unwanted/harmful molecules and other cellular components such as dysfunctional organelles (mitochondria and lysosomes) and protein aggregates, such as α-synuclein, tau and prions, can contribute to the damage spreading.[69,70,80,89]

9.7 Intercellular communication mediated by extracellular vesicles

Another important vector for intercellular communication that has gained attention in the recent years is extracellular vesicles (EVs). Extracellular vesicles are membrane-enclosed nanoscale particles that convey information from the cell of origin at a distance and across the entire organism. Despite initially seen as amorphous cell debris with no biological relevance, EVs are now recognized as important communication entities, released by virtually all prokaryotic and eukaryotic cells, as part of their normal physiology or in response to environmental cues. In fact, EVs can be perceived as a manner of producing cells getting rid of unwanted components, modifying their molecular landscape, and maintaining homeostasis, but also as crucial vehicles for long distance communication. The biological relevance of EVs was already demonstrated in various cellular physiological and pathological processes including modulation of synaptic

activity, immune response, cell differentiation, angiogenesis, apoptosis, cancer progression, infection spreading, cardiovascular and neuronal disease development and progression.[90]

The content of EVs is highly dependent of their cell of origin and the mechanism of biogenesis, and comprises proteins (membrane or cytosolic), lipids, metabolites, and nucleic acids namely DNA, mRNA and noncoding RNA molecules like microRNAs.[91] However, the content profile does not always directly mirror the content of the cell of origin, demonstrating that it is selectively sorted depending on the conditions affecting the producing cell. This fact, together with their presence in biological fluids and consequent easy accessibility, makes EVs attractive entities for diagnosis, monitoring of disease progression and response to therapy as well as therapeutic agents.

According to their biogenesis, EVs can be categorized into two main groups: ectosomes and exosomes.[90] Ectosomes, which include microvesicles, microparticles and apoptotic bodies, originate from the outward budding of the plasma membrane and typically present with a diameter from 50 nm to 1 μm. The biogenesis of this type of vesicles is highly dependent on reorganization of lipids and proteins within the plasma membrane, which includes phosphatidylserine flipping from the inner leaflet to the cell surface and the consequent physical bending of the membrane, supported by actin cytoskeleton reorganization, culminating in membrane budding and subsequent vesicle release. Additionally, the biogenesis of ectosomes is also dependent, at least in part, on ESCRT (endosomal sorting complex required for transport) proteins and the enzymatic conversion of sphingomyelin to ceramide.[92]

The other category of EVs, the exosomes, has endosomal origin, and have a small size ranging from 40 to 160 nm. This type of vesicle forms during multivesicular body (MVB) maturation during which inward invagination of the limiting membrane of the endosome gives rise to intraluminal vesicles. These MVBs then fuse with the plasma membrane, releasing the exosomes to the extracellular environment.[92,93] ESCRT-dependent and independent mechanisms that comprise diverse players have been associated with exosome biogenesis, including ceramide, sphingomyelinases, ESCRT subunits, ALIX (apoptosis-linked gene 2-interacting protein X), TSG101 (tumor susceptibility gene 101), Rab GTPases and tetraspanins like CD9, CD63 and CD81, which also regulate cargo sorting into the vesicles.[92,94] However, due to the lack of specific markers of EV subtypes and the resultant impossibility of assigning an EV to a particular biogenesis pathway, it is recommended to refer to EVs using operational terms such as experimental/environmental conditions, cell of origin, physical characteristics like size or biochemical composition.[95]

After release into the extracellular space, EVs can interact with, and depending on their cargo and composition, potentially trigger a response in the recipient cell. Subsequent to the docking with the recipient cell, EVs can directly activate receptors at the membrane triggering intracellular signals or release their content into the

receptor cell. This content release can occur by the direct fusion of the EVs with the plasma membrane or after its internalization following the endocytic pathway, if not degraded by the lysosome. Internalization of EVs can occur by various mechanisms including caveolae, lipid raft-mediated endocytosis, clathrin-mediated or clathrin-independent endocytosis (phagocytosis and micropinocytosis).[93,96,97] Various mechanisms/players have been described to facilitate the docking and release of EV material into the target cell, including Cx43 channels,[98] however what confers a cell or organ tropism to EVs, remains largely unknown. The clarification of these mechanisms, together with the intrinsic properties of EVs to exchange components and deliver cargo that can triggers a cell response, and possible engineering to make them carriers of diverse agents like peptides, oligonucleotides and drugs, predicts an important role for EVs as potential therapeutic agents.[99]

Various reports have already demonstrated that the amount of circulating EVs and its content changes with aging. In fact, it has been shown that EVs secreted by senescent cells are functionally active in other cells, and are now considered part of the SASP, playing an important role on alterations observed during aging. According to Eitan et al. and others, the amount of plasma EVs decreases during human aging, but more importantly, EVs from older donors induce an increase in MHC-II expression on monocytes, suggesting an increase in their activation.[100,101] However, additional studies performed in mice showed that although old murine plasma has a lower particle count, the number of EVs is significantly elevated.[102] These results can be explained by the use of different methodological approaches to isolate and characterize EVs, that due to their characteristics cannot always distinguish EVs from other particles such as lipoproteins.[103] Strikingly, functional results are similar and show that aging alters circulating EV function, manifested by a decreased macrophage response to inflammatory stimuli, increased phagocytosis, and an impairment of endothelial cell response to pro-angiogenic conditions.[102] These effects can potentially be explained by the higher levels of some miRNAs carried by EVs derived from older animals, namely let-7a, miR-21, miR-146a and miR-223.[102] Moreover, elimination of senescent cells in old mice by the treatment with senolytics led to an altered composition of the plasma EV cargo and a function similar to those observed in young animals.[102,104] This observation suggests that senescent cells are important contributors to circulating EVs that can reach and modulate the cellular function across the entire organism.

Consistently, and despite the broad origin of circulating EVs, it has been described that senescent cells secrete more EVs than young cells.[105–108] The increase in EV production by aged and senescent cells can, at least in part, be explained by another hallmark of aging, the loss of proteostasis. In fact, inhibition of endolysosomal trafficking, decreased autophagy and dysfunctional lysosomal activity present during aging and characteristic of senescent cells, was associated with increased EV release.[109–111]

Additionally, published data suggest that EV secretion can serve as a vehicle for the removal of unwanted molecules like misfolded proteins and fragmented DNA, avoiding apoptotic-like death of senescent cells.[107,110,111] That phenomena can, on one hand, serve as a distress signal and promote a protective reaction from neighboring cells, but on the other hand, contribute as a stressful bystander effect.

Importantly, when compared with EVs of healthy cells, senescent cell-derived EVs present changes in protein and miRNAs that can control activity of other cells. An example of this is the altered levels of IL-1β levels in circulating EVs of aged rats that can be part of the inflammatory environment associated with aging.[112] Moreover, miR-192, which has anti-inflammatory effect on macrophages, was also shown to be significantly increased in EVs from aged mice, in consequence of the hyperinflammatory state of aged mice, suggesting that EVs can also contribute to the attenuation of excessive inflammation.[113]

Additionally, it was shown that osteogenic differentiation of mesenchymal stem cells is promoted by galectin-3 present in plasma EVs from young subjects.[114] However, that capacity is absent in plasma EVs from older subjects that present a reduction in galectin-3 levels, suggesting that reduced EV-mediated secretion of galectin-3 may contribute to the decreased bone formation observed during aging.[114] In accordance, bone marrow stromal cell proliferation and osteogenic differentiation is also diminished upon the stimulation with EVs from aged individuals.[115] Furthermore, miR-31, enriched in EVs derived from senescent endothelial cells and plasma of older subjects, was shown to inhibit ossification and differentiation of mesenchymal stem cells.[116] EVs isolated from aged donors as well as those from senescent endothelial cells were shown to also promote calcification of human aortic smooth muscle cells, demonstrating the role of EVs in the alterations observed during aging.[117]

Importantly, and as suggested by the studies described above, EVs derived from senescent cells also contribute to the induction of cellular senescence in normal cells through a mechanism mediated by IFN-induced transmembrane protein 3 (IFITM3), a protein elevated in EVs from elderly donors in comparison with EVs isolated from young individuals.[118]

In accordance with these findings, various authors had demonstrated that EVs from young animals are able to attenuate or even reverse aging-related effects.[114,119–123] In fact, EVs from the serum of young animals, but not the non-EV supernatant from the same animals, or serum EVs extracted from aged animals, were able to attenuate several markers of inflammaging, namely serum levels of pro-inflammatory IL-6 and IL-1β, in old recipient animals.[119] Moreover, extracellular nicotinamide phosphoribosyl transferase (eNAMPT) present in human and mouse circulating EVs was found to decrease during aging. Interestingly, eNAMPT-containing vesicles that increase NAD^+ in recipient cells, were shown to significantly improve physical activity, delay aging and extend lifespan in mice, unraveling their potential as anti-aging factors.[124]

A better understanding of EV alterations during aging and the players involved in this specific type of intercellular communication, are crucial for our efforts to alleviate senescence spreading and/or delay stem cell exhaustion. Furthermore, capitalizing on the potential of EVs may prove to be a viable strategy to suppress generalized cellular aging and improve stem cell function.

9.8 Concluding remarks

General and unbiased studies of the aging process have shown that abnormal cell-cell communication patterns occur during aging. In fact, mounting evidence demonstrates the importance of intercellular communication in the propagation of deleterious signals released by senescent cells, with impact not only locally at the tissue level, but also globally, with the spreading of "senescence cues" through the entire organism, contributing to interorgan coordination of the aging phenotype. However, given its relevance for organismal homeostasis and function and a coordinated response to external changes, intercellular communication can also have a protective role in the aging context, promoting cellular homeostasis and stemness and avoiding or delaying cellular damage.

An integrated and holistic view of intercellular communication patterns can lead to a better understanding of the causes of multicellular systems aging and the underlying mechanisms paving the way to interventions that could reverse certain aging processes and slow development of age-related diseases.

Although this is a concept that is now in its infancy, the biological and physiological impact of the results gathered in the last few years anticipates a promising future for this field of research.

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CHAPTER 10

Genomic instability and aging

Zhiquan Li, Sharath Anugula and Lene Juel Rasmussen
Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Copenhagen, Denmark

10.1 Introduction

Aging is a complex process of damage accumulation, causing a functional decline. Several hallmarks of aging have been identified, of which genomic instability plays a critical role in aging and age-related diseases and closely interacts with other hallmarks of aging. The genome is constantly challenged by exogenous DNA damaging sources, such as ionizing radiation, ultraviolet (UV), and chemicals. But also, endogenous DNA damage caused by alkylation and hydrolysis can lead to chromosomal aberrations, mutations, and epimutations, eventually causing genomic instability and cellular dysfunction. The stressor-induced alterations include chromosomal (chromosome aneuploidy, chromosomal rearrangements, and fragile sites), genomic (increased genetic variability and mutated nucleic acid sequences), replicative (replication stress), and transcriptional impairments. In response to these damages, a wide range of sophisticated repair mechanisms have evolved to repair different types of damage to preserve genomic integrity, such as proper chromosome segregation, efficient DNA damage repair, and faithful DNA replication, to maintain normal function and promote organismal survival.[1,2] Mammalian DNA damage response systems mainly comprise nonhomologous end joining (NHEJ), homologous recombination (HR), nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), direct reversal repair (DRR), and translesion DNA synthesis (TLS) (Fig. 10.1). To ensure genomic stability, there is crosstalk and redundancy between the different DNA repair pathways.

Genomic instability is commonly associated with a wide range of diseases, such as cancer,[3] immunodeficiency, and age-related diseases, such as amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases.[4,5] Chromosomal aberrations, including chromosomal rearrangements, chromothripsis, etc., are strongly implicated in age-related diseases.[6,7] Mutations in DNA repair genes cause diverse aging phenotypes and diseases, such as the genetic disorders xeroderma pigmentosum (XP) and Cockayne syndrome (CS). The age-related increase in DNA damage is likely caused by an age-dependent decrease

Aging
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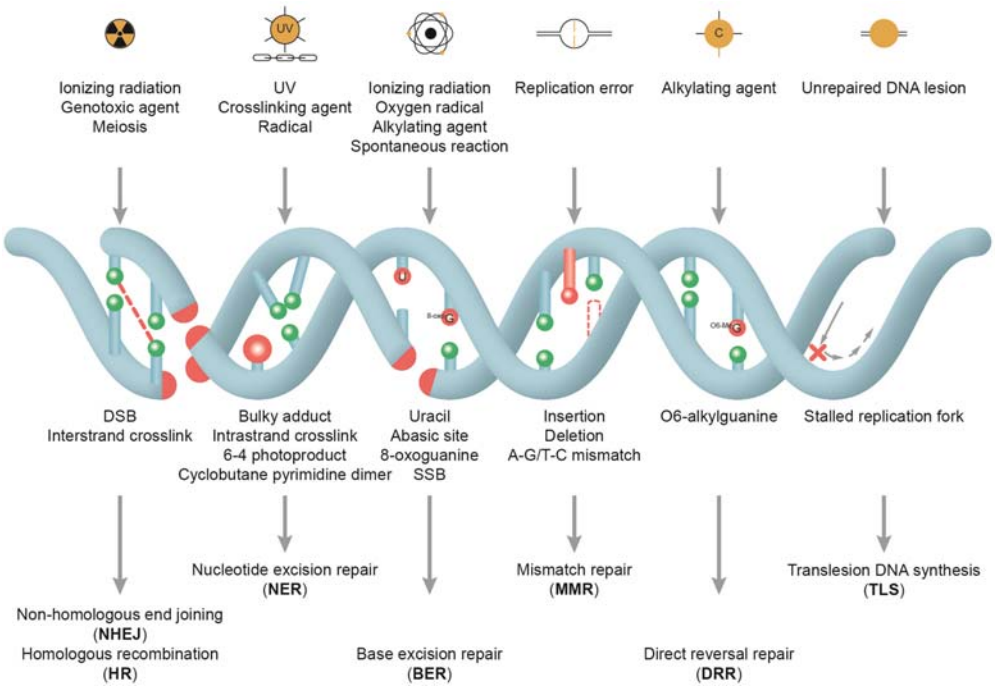

Figure 10.1 Mammalian DNA Damage Response. Main DNA damage repair or tolerance pathways in mammalian cells. Diverse DNA lesions caused by various DNA damaging agents are repaired by distinct but closely related mechanisms. The mammalian DNA damage response consists mainly of non-homologous end joining, homologous recombination, nucleotide excision repair, base excision repair, mismatch repair, direct reversal repair, and translesion DNA synthesis. UV, ultraviolet; DSB, double-strand break; SSB, single-strand break.

in repair capacity.[8,9] The importance of functional DNA repair is illustrated by the often-longer lifespan and healthspan via DNA repair stimulators and/or gene targeting.

The central role of mitochondria and their communication with the nucleus is well documented in the regulation of DNA damage responses and aging in model systems and humans. Genomic instability is also linked with other hallmarks, such as telomere attrition and epigenetic alterations.[10] Furthermore, altered epigenetic factors are also contributing to genomic instability through chromatin remodeling and/or post-translational modifications, such as histone acetylation and methylation.[11] For example, along with others, histone acetyltransferase, TIP60 plays a crucial role in chromatin remodeling by modifying histones and nonhistone proteins in DNA repair as well as other aspects of aging and neurodegeneration.[12]

The highly dynamic DNA damage response in diverse cellular processes as well as compartments involves ingenious mechanisms to cope with different insults and

therefore provides various opportunities for alimental, physiological, and pharmacological interventions or therapy.

10.2 DNA strand breakage-induced genomic instability

Double-strand breaks (DSBs) are the most cytotoxic DNA lesions caused by ionizing radiation and genotoxic agents, and repaired by NHEJ and HR repair. DSBs and mutations in DSB repair genes lead to senescence and premature aging.[13,14] For instance, ataxia telangiectasia mutated (ATM) deficiency leads to an age-related anomaly, ataxia-telangiectasia (A-T). Moreover, expression of HR repair protein BRCA1 and other DSB repair proteins are decreased in mouse models and humans with age.[15] A variety of other age-related diseases are also deficient in DSB repairing. DNA helicase deficiencies, such as mutations in the WRN helicase in Werner syndrome (WS), BLM in Bloom's syndrome (BS), as well as RECQL4 in Rothmund-Thomson syndrome (RTS).[16]

Interestingly, it has been proposed that repair of DSBs can be shifted from NHEJ to HR with age.[17–19] The dynamics of DNA repair pathway choices with age would be an interesting target of DSB repair mechanisms.

Single-strand breaks (SSBs) are the most common lesions caused by diverse sources, such as oxidative stress, and are repaired mainly by single-strand annealing and BER. The persistence of SSB in epithelial cells leads to senescence and carcinogenesis.[20] Defect in the repair of SSBs is associated with the human progressive neuropathy of spinocerebellar ataxia with axonal neuropathy 1 (SCAN1) and ataxia oculomotor apraxia 1 (AOA1).[21]

10.3 Replication-induced genomic instability

DNA repair is also accompanied by chromatin remodeling, replication, transcription, and recombination, etc., which makes it even more complex[22] (Fig. 10.2). Correct DNA replication plays a pivotal role in cellular functions and survival, and imprecise replication can result in mutations and stalling of replication forks, which causes genomic instability.

10.3.1 Replication stress

Replication stress can be caused by multiple factors such as depletion of replication proteins, shortage of nucleotides, hard-to-replicate sites, or alternatively, hyper-replication. Replication stress has been implicated as an important factor driving hematopoietic stem cell aging[23] and also plays a role in AD.[24] Replication stress in a DNA bypass deficient mouse model shortens lifespan.[25] Interestingly, attenuation of replication stress by suppression of growth signaling promotes mitochondrial health, slows aging, and extends longevity.[26] Moreover, sustained proliferation-mediated replication stress promotes escape from apoptosis and leads to genomic instability.[27] Replication stress is a common

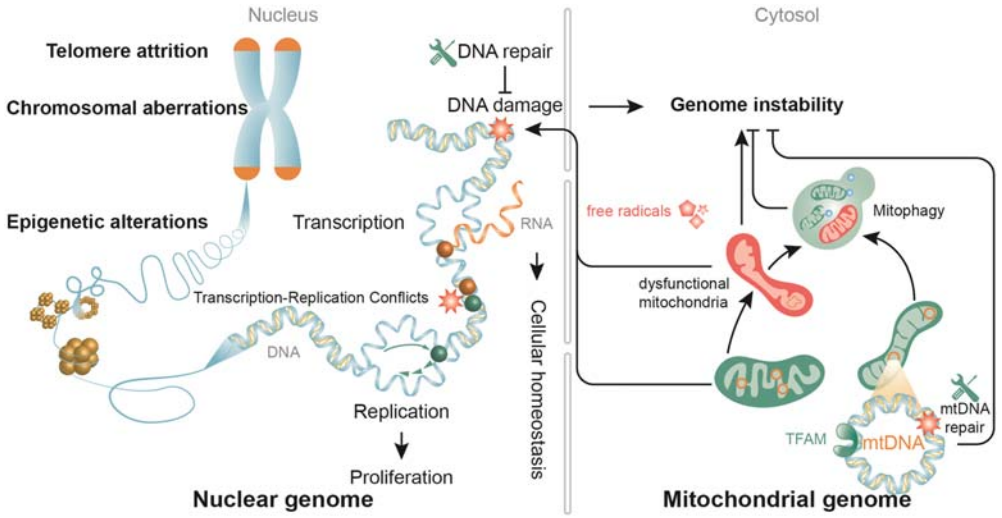

Figure 10.2 The maintenance of genome integrity. The genome is constantly challenged by chromosomal aberrations, telomere attrition, epigenetic alterations, DNA damages, etc., leading to genomic instability. DNA damage is usually caused by exogenous physical, chemical, and/or biological stressors, along with endogenous cellular processes, such as transcription, replication, replication-transcription conflicts, and reactive oxygen species (ROS). Efficient DNA repair attenuates nuclear and mitochondrial DNA damages. Except for mtDNA repair, the mitochondrial transcription factor A (TFAM) also guards mtDNA by physical interactions. Enhanced mitophagy promotes mitochondrial health and reduces ROS production to safeguard genomic integrity.

feature of pre-tumor and tumor cells and failure to resolve this stress is a major driving force to genomic instability.[28] In addition, replication stress can induce genome-damaging R-loops.[29] Therefore it is crucial to understand replication stress at a cellular and molecular level to discern its roles in tumorigenesis and aging.

10.3.2 Translesion DNA synthesis

TLS is a mechanism to bypass DNA lesions that otherwise stall replication. This DNA damage tolerance (DDT) mechanism ensures replication fork stability and progression by switching from DNA polymerases to translesion DNA polymerases and, therefore, favors cellular and organismal survival. It is subcategorized as an error-free and error-prone pathway, depending on translesion polymerases with different fidelities, including Rev1, Pol ζ, Pol κ, Pol η, and Pol ι.[30,31]

Patients harboring the gene variant form of XP-V/*POLH* gene are deficient in the TLS polymerase η and show hypermutability after UV exposure and susceptibility to skin cancer.[32] Besides, TLS is also associated with the induction or treatment of other types of cancers.[33] In addition, TLS replication gap suppression might be a novel hallmark of cancer,

therefore inhibitors targeting TLS seem to be promising for cancer therapy.[34] Interestingly, the deficiency of Rev1, a DNA polymerase involved in TLS, has been shown to induce replication stress, alter NAD + metabolism, disrupt mitochondrial functions, compromise autophagy, and decrease lifespan.[25,35] This suggests a role of TLS-mediated genome maintenance in aging. Moreover, DDT defects-induced DNA damage accumulation has been observed in the hematopoietic system, whereas its roles in mutagenesis and aging in other tissues and models remains to be understood.[36]

10.3.3 Mismatch repair

MMR corrects post-replication base mispairings. MMR also functions in DNA damage surveillance, prevention of recombination between homeologous (nonidentical) sequences, and participation in chromosome pairing in meiosis.[37,38] Further, it repairs some oxidative DNA lesions, such as 8-oxoG[39] and MSH2 deficiency is also determined to be involved in UVA-induced DNA damage.[40] Defects in MMR cause microsatellite instability (MSI), which is implicated in hereditary nonpolyposis colorectal cancer (HNPCC) or Lynch syndrome (LS) but has also been detected in skin cancer.[41] Interestingly, MSI is elevated in the elderly compared to the young, suggesting an age-related decline in MMR.[42] Several mouse models of MMR deficiency have been developed and they primarily suffer from various cancers such as lymphoma, gastrointestinal stromal tumors (GISTs), and skin tumors.[43] Hypermethylation of the *Msh2* promoter region and its reduced gene expression is associated with increasing age in mice.[44] Similarly, MSH2 and MSH6 (MutSα complex) protein expression, as well as MMR activity, are reduced in senescent human colonic fibroblast and embryonic lung fibroblast, while exogenous supplementation of purified MutSα restores MMR activity.[45]

Surprisingly, MMR defects promote cell proliferation in the absence of telomerase in yeast.[46] Meanwhile, MMR protein PMS2 deficiency extends the mean lifespan and median survival of telomerase-deficient mice ($Terc^{-/-}$/$Pms2^{-/-}$ mice) as well as promotes cell proliferation by reducing CDKN1A/p21 expression.[47] Following this, short telomeres show anticancer and pro-aging properties, but MSH2 defects abrogate all these effects.[48] The exact role of MMR in aging is still unclear and the causal links warrant further investigations.

10.4 Transcription-induced genomic instability

Transcription plays an essential role in physiology and aging,[6,7] which can be partially explained by transcription-mediated genomic instability. Transcription damages DNA and transcription rate is positively correlated to the pace of aging in humans and other mammals.[49]

10.4.1 Transcription-coupled repair

NER is repairing UV-induced DNA damage and is divided into two subpathways, global genomic NER (GG-NER) and transcription-coupled repair (TC-NER, TCR), dealing with helix-distorting and transcription-blocking DNA lesions, respectively. Defects in GG-NER are more mutagenic, therefore leading to cancers; while those in TC-NER are less mutagenic but more cytotoxic or cytostatic, sanctioning premature aging.[50] Many proteins involved in TC-NER are implicated in aging and age-related diseases, such as CS. In CS, the CSA and/or CSB proteins-induced TC-NER impairment has little impact on mutagenesis and cancer incidence but displays accelerated aging.[51] The mouse models deficient in NER and TCR show microglia activation-mediated white matter anomalies as well as age-related cumulative neuronal loss, indicating the synergy of both pathways in promoting neuronal survival and plasticity for neuropathology prevention.[52] Despite CSB functions in TC-NER, a recent study also shows that CSB promotes HR while repressing NHEJ, which regulates G2/M checkpoint activation.[53]

10.4.2 Transcription-replication conflicts

Transcription and replication are routine cellular processes that interfere with each other, causing transcription-replication collisions/conflicts that threaten genomic instability (Fig. 10.2), while numerous mechanisms have been evolved to combat such collisions.[54] For the transcription-replication conflicts (TRCs), deleterious head-on encounters on lagging DNA strand and co-directional encounters on leading strand cause replication fork stalling and genomic instability, which might resume by replication restart.[55] Interestingly, the replisome regulates co-transcriptional R-loops in a TRCs orientation-dependent manner by resolving co-directional R-loops but enhancing the formation of head-on R-loops, which causes ATM-Chk2 and ATR-Chk1 pathway activation, respectively.[29] TRCs-induced genomic instability is also observed in cancer and other diseases.[29] Furthermore, common fragile sites (CFSs) can be protected from TRCs by Polycomb proteins to maintain genome stability.[56]

10.5 Nucleotide pools

Balanced cellular deoxynucleoside triphosphates (dNTPs) biosynthesis is pivotal for the normal functionality of cellular activities, such as replication, transcription, and DNA damage repair.[57] dNTPs are mainly synthesized in the cytoplasm via de novo and salvage pathways but they can also be produced in mitochondria for mtDNA synthesis. Altered dNTP pools might affect the DNA replication fidelity by interfering with the DNA polymerases through an intrinsic error rate at the nucleotide insertion step or subsequent editing at the proofreading step.[58] dNTP imbalance is closely related to genome stability, aging, and cancer.[59] Both high and low levels of dNTPs affect mutation frequencies[57,60]

arguing for a tight regulation of purine and pyrimidine biosynthesis. The ribonucleotide reductase (RNR), which converts the ribonucleotides to deoxyribonucleotides is a rate-limiting enzyme in all living organisms and is a critical enzyme in regulating dNTP pools. Its function is tightly regulated by allosteric control and activity, protein expression, and proteolysis as well as the cellular localization of the small RNR subunit.[61–63] Inhibition of RNR leads to elevated ROS, which can be removed by the replisome-associated ROS sensor, peroxiredoxin 2 (PRDX2), that can dissociate from chromatin to slow down replication fork progression to attenuate replication stress,[64] indicating concerted coordination between dNTP biogenesis and replisome progression. The chromatin modulator KAT5/TIP60 regulates aging via dNTP biosynthesis and autophagy.[12] Moreover, the proper dNTP supply for different cellular compartmentations, mitochondria, and nucleus DNA must be coordinated to maintain organismal homeostasis.[65] Mitochondrial DNA (mtDNA) replication defects lead to low and imbalanced cellular dNTP pools and alter the folate-driven one-carbon metabolism.[66]

10.6 Mitochondrial functions in genomic integrity

As the powerhouse of eukaryotic cells, mitochondria produce ATP via oxidative phosphorylation (OXPHOS) by releasing the chemical energy of nutrients. Meanwhile, they are biosynthetic hubs of nucleotides, fatty acids, amino acids, and glucose.[67] Specifically, mitochondria supply cells with nicotinamide adenine dinucleotide (NAD), in the form of NAD^+ and NADH, and many other intermediate metabolites, acting as a chemistry factory. Therefore mitochondria have also been proposed as a chemotherapeutic target in cancer.[68]

10.6.1 Mitochondrial oxidative stress

Mitochondria are the primary source of reactive oxygen and nitrogen species. Among different DNA damaging reagents, oxidative stresses are the most common sources. It is also the major component of the free radical theory of aging[69] and free radicals constantly attack the genome (Fig. 10.2). Oxidative stress can be derived from endogenous and exogenous sources and leads to oxidative DNA lesions. For example, 8-oxoG, the most common oxidative lesion, is repaired by DNA glycosylase OGG1 via the BER pathway.[70] Moreover, HDAC1 also interacts with OGG1 to repair oxidative DNA lesions in the aging brain as well as in AD.[71] It has been shown that oxidative stress can impair MMR,[72] which makes it possible that oxidative stress not only causes different types of DNA damage but also affects their repair. In addition, increased ROS can induce TP53 and CDKN1A/p21, or CDKN2A/p16 and RB1 to induce senescence.[73] This is consistent with the notion that oxidative stress is widely implicated in aging, cancer, and neurodegeneration.[74]

10.6.2 FOXO in oxidative DNA damage response

The forkhead box, subgroup O (FOXO) transcription factors are a subfamily of a conserved FOX protein family which regulates gene expression, consisting of FOXO1, FOXO3, FOXO4, and FOXO6 in mammals. FOXOs play crucial roles in development, immune response, inflammation, tumor suppression, aging, and longevity. In which, FOXOs control cellular homeostasis, such as stress resistance, cell cycle, cell differentiation, apoptosis, proteostasis, intracellular signaling, metabolism, and autophagy.[75–77] FOXOs are well known for their functions in oxidative and redox regulation downstream of the insulin/insulin-like growth factor-1 (IGF-1) signaling (IIS) pathway. Specifically, FOXOs also play critical functions in DNA damage repair.[78] For example, FOXO1 and FOXO3 are essential for the expression of ATM[79] and GADD45A.[80,81] FOXO3 also prevents DSBs and subsequent genome rearrangements and instability.[82] Alterations of FOXOs lead to the accumulation of oxidative DNA damages[83] and are widely associated with many disorders, such as cancer,[84] aging,[78] and neurodegenerations.[85] Pharmacological approaches targeting FOXOs have been reviewed in cancer, neurological diseases, diabetes, and cardiovascular diseases.[86]

10.6.3 Mitochondrial genome maintenance

The mitochondrial genome is a circular DNA molecule that is more vulnerable to damages, such as ROS. Deficits in mtDNA repair are implicated in a multitude of degenerative diseases, cancer, and aging.[87] ROS may reduce mtDNA replication fidelity, therefore, compromising mtDNA integrity.[88] Certain inherited mtDNA variants and somatic mtDNA mutations are widely observed in the human aging brain and the development of neurodegenerative diseases.[89,90] Alongside the roles of mitochondria in energy metabolism, mtDNA damage and repair are major contributors to combat neuronal loss and ensuing vulnerabilities.[91]

To maintain mtDNA integrity, recombinational repair (RER), BER, MMR, DRR, TLS function in mtDNA repair, showing high similarity to nuclear repair systems.[92] BER is mainly used to maintain the mtDNA integrity and defects in BER lead to premature aging and neurodegenerative diseases.[93] Except for generating energy and ROS, mitochondria also impact other pathways, and genetic variants related to mitochondria causes age-related diseases.[94] Mitochondrial degradosome, also known as mitochondrial exoribonuclease complex (mtEXO), is composed of SUV3 and PNPase and prevents the accumulation of dsRNA and that of pathological mtDNA R-loops to protect mitochondrial genomic instability.[95] Moreover, the mtDNA instability and reduced mtDNA copy number are involved in the tumorigenesis of many human cancers.[96] Accumulation of 8-oxoG in neuronal mtDNA due to OGG1 deficiency indicates mtDNA alterations in AD, ALS, and other neurodegenerative disorders.[97] Novel methodologies should also be developed to reconcile the current knowledge regarding the major roles of mtDNA damage in these neurodegenerative disorders.[98]

In addition to mtDNA repair, another route to safeguard the mitochondrial genome is TFAM. As a transcription factor binding to mtDNA, TFAM is essential for mitochondrial genome maintenance by regulating mtDNA replication, transcription, packaging, and stability.[99] TFAM determines the mitochondrial genome abundance (mtDNA copy number), so a reduction of mtDNA copy number as well as TFAM expression levels are linked to a variety of age-associated pathologies.[100]

10.6.4 Mitochondrial dysfunction

Accumulation of mtDNA mutations contributes to the decline of mitochondrial functions, which further promotes aging and age-associated cancer incidence.[101] As a hallmark of aging, mitochondrial dysfunction has been widely investigated in the past few decades. Defects in mitochondrial morphology, biogenesis and the electronic transport chain (ETC) lead to mitochondrial dysfunction, which might also affect the mitochondria-nuclear communications. Interestingly, mtDNA mutations or deletions-mediated mitochondrial dysfunction cause defects in iron-sulfur cluster (ISC) biogenesis which further leads to nuclear genomic instability.[102] Mitochondrial ISC biogenesis is widely implicated in many human diseases.[103] Intriguingly, MMS19 (nucleotide excision repair protein homolog) acts as an adapter in a targeting complex of cytosolic iron-sulfur cluster assembly (CIA) machinery and plays crucial functions in DNA metabolism to maintain genomic integrity.[104,105] Alterations in MMS19 are associated with XP. Meanwhile, MMS19 is also located in mitochondria to protect mitochondria from oxidative stress.[106] In parallel, the ISC defect is also a driving force in mitochondrial heme defects and elevated ROS in Friedreich's ataxia (FRDA).[107] Mutations in the iron-sulfur subunit of mitochondrial succinate dehydrogenase (SDH) lead to superoxide overproduction, premature aging, and tumorigenesis.[108] Mitochondrial dysfunction-mediated ROS accumulation causes telomere loss, chromosomal aberrations, and genomic instability.[109] Also, mtDNA depletion-induced mitochondrial dysfunction causes nuclear genomic instability.[110] Mitochondrial dysfunction concomitant with oxidative stress is implicated in the etiology of a variety of neurodegenerative diseases, such as AD, Parkinson's disease (PD), Huntington's disease (HD), and ALS.[111]

In addition, mitochondrial dysfunction also plays critical roles in nucleotide biosynthesis and degradation, which is required for DNA and RNA homeostasis.[112,113] Mitochondrial dysfunction mediated by mtDNA depletion or mitochondrial ETC inhibition displays alterations in serine and formate biosynthesis which are key components in one-carbon metabolism, a process important for nucleotide metabolism.[114]

10.6.5 Mitophagy

Mitophagy is an evolutionarily-conserved quality control mechanism to recycle excess, dysfunctional, or damaged mitochondria by autophagy, contributing to a healthy mitochondrial turnover. It functions in either a PINK1/Parkin-dependent or -independent manner.

Mutations in mitophagic genes, mitophagic flux decline, and other factors (such as DNA damage and DNA repair deficiency) lead to alterations in mitophagy, resulting in the accumulation of dysfunctional mitochondria that elevates ROS, which ultimately impinges the genome's stability and leads to aging and neurodegeneration.[115] Moreover, alterations in mitophagy also cause some other age-related diseases, such as cancer,[116] inflammation, AD,[117] and PD.[118] Mitochondrial dysfunction and compromised mitophagy are widely observed in AD, which might be ameliorated by fasting and healthy diet, exercise, and mitophagy-inducing compounds.[117] For example, boosting mitophagy by pharmacological application of mitophagy inducers (such as NAD + , urolithin A, and actinonin) has been shown to reinstate the cognitive defects in the Aβ and tau *Caenorhabditis elegans* models and APP/PS1 mouse model.[119] Moreover, urolithin A is safe and capable of enhancing mitochondria function and cellular health in humans.[120] Furthermore, many natural mitophagy inducers have been identified such as resveratrol and spermidine. Various pharmacological approaches have been generated to activate mitophagy: (1) reduce $\Delta\Psi m$ by mitochondrial uncouplers (such as FCCP and CCCP); (2) impair respiration by ETC inhibitors (such as rotenone, antimycin A, and oligomycin); (3) decrease iron by iron chelators; (4) enhance PINK1 by its agonists; and (5) activate sirtuins by its enhancers (such as NAD + precursors).[121]

Interestingly, mitophagy can be evoked by DNA damage and contributes to maintaining mitochondrial functions, and facilitates DNA repair.[122] XP group A (XPA) is caused by XPA gene deficiency and shows mitochondrial and mitophagic dysfunction[123] which is similar to A-T[124] and CS.[125] Besides, PARK2/Parkin physically interacts with mtDNA, stimulates mtDNA repair, and reduces mtDNA oxidative damage.[126]

10.7 Genomic instability in health and disease

Genomic instability is leading to many malfunctions and diseases, such as neurodegeneration, cancer, progeria, as well as other progeroid syndromes,[127] either by inheritance or age-related decline/deficiency (Fig. 10.3). Some signaling pathways have common characteristics in premature aging and age-related diseases, such as DNA repair.[128] Importantly, a variety of drugs have been identified to target DNA repair and metabolism.[127]

10.7.1 Longevity

DNA repair is positively related to extended longevity. Comparison of lifespan and DNA repair gene expression in human, naked mole rats and mice shows that protein-encoding genes involved in the BER, HR, NHEJ, and MMR pathways are generally upregulated in humans.[129] Similarly, NER deficiencies show elevated DNA damage accumulation, accelerated aging, and shortened lifespan.[130] Numerous studies have shown that enhancing DNA repair extends lifespan. For example, overexpression of several repair factors has beneficial effects on lifespan and stress resistance in

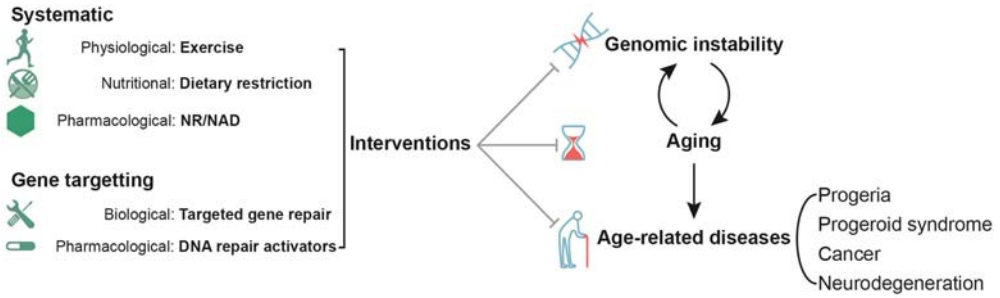

Figure 10.3 Interventions to combat genomic instability and aging. Genomic instability accelerates aging, and vice versa, ultimately leading to age-related diseases. Interestingly, all of these can be attenuated or reversed by systematic interventions, such as physical exercise, dietary restriction (DR), supplementation of nicotinamide riboside (NR), or NAD + , or even by direct correcting DNA repair genes or stimulating DNA repair pathways.

Drosophila.[131] Enhancing DNA integrity via DNA repair holds the promise to extend both longevity and healthspan.

10.7.2 Progeroid syndromes

In addition to deficiencies in NER and RecQ DNA helicases, other DNA damage repair-related deficiencies can also be inherited from the germlines, such as the *LMNA* gene mutations-mediated laminopathy in Hutchinson-Gilford progeria syndrome (HGPS), which is an autosomal dominant syndrome.[132,133] Alterations in LMNA affect nuclear morphology and function, leading to DNA repair defects and genomic instability, and are widely implicated in degenerative diseases and premature aging syndromes, such as HGPS and restrictive dermopathy (RD).[132,134] Mutations in lamin-related (LMNA, ZMPSTE24, BANF1) or DNA functions (POLD1, SPRTN, AKTIP) are also implicated in distinct progeroid syndromes.[135] HGPS shows persistent DNA damage checkpoints activation, and inhibition of ATM and ATR kinases partially rescue the cell cycle arrest, while inhibiting farnesyltransferase attenuates aberrant nuclear morphology but fails to reduce DNA damage and checkpoint signaling, further supporting a close interaction between lamin processing and DNA repair capacities.[136]

Except for the progerin generated by aberrant splicing of the LMNA gene in HGPS, it is also detected in senescent cells and cells from the elderly.[137] Importantly, research into progeroid syndromes plays a critical role in elucidating the causes of these diseases and is also useful to characterize basic mechanisms in physiological aging.[138] Meanwhile, established and developing cellular and animal models would potentiate more therapeutic opportunities.

10.7.3 Cancer

Compromised DNA repair systems are found in most cancers and, therefore, are regarded as a hallmark of cancer.[3] Many genes involved in genome maintenance are also implicated in cancers. For example, p53 mutations show vulnerability to various types of cancers. Meanwhile, p53 also plays key roles at the intersection between cancer and aging.[139] DNA damage and deficiency in DNA repair also increases cancer susceptibility. Therefore maintaining genome integrity is exploited as a strategy for cancer prevention.

On the other hand, tumor cells are featured by high levels of oxidative stress and DNA damage, as well as the loss of tumor suppressors. DNA repair pathways are pivotal for tumor cell survival and they are also targets for developing strategies to kill tumor cells.[140] Inhibitors of DNA damage response-related kinases, such as ATM, ATR, CHK1, and WEE1, synthetic lethality, and chemo-/radio-therapy are exploited to treat cancer. Besides, the DNA repair enzyme poly (ADP-ribose) polymerase (PARP1) is an important protein involved in DSB and SSB repair. Its inhibitors have been widely developed and used in cancer treatment.[141] To this end, DNA repair pathways are suppressed to induce genomic instability to eliminate tumor cells.

10.7.4 Neurodegeneration

Genomic instability is believed to be involved in neurodegenerative diseases, such as AD.[142] DNA repair and related proteins' involvement in premature or accelerated aging and neuropathologic disorders have been under intensive investigation and have gained recognition for their functions and translational implications in disease diagnosis and treatment. However, the preferences of different repair mechanisms in different brain regions, and the ways and the extent they affect neuronal health and function, as well as their cognition maintenance remains to be elucidated.

10.8 Aging interventions activating DNA repair

Numerous interventions have been developed to target aging to alleviate or prevent age-related diseases, such as physical exercise, dietary restriction (DR), NR, and NAD + . Many pharmaceutical targets are identified to improve DNA repair.

10.8.1 Exercise

Regular physical exercise improves body fitness and delays the onset of a variety of age-associated diseases, including type 2 diabetes (T2D) and neurodegenerative disorders. Physical exercise has also been implicated in the maintenance of genome integrity in the past few decades.[143] Although it produces more free radicals, it enhances DNA repair, attenuates DNA damage, and reduces oxidative damage to proteins in rat

skeletal muscle[144] and liver.[145] In T2D patients, exercise training induces longer telomere in patient-derived leukocytes and attenuates the increase of genomic oxidative DNA damage and sensitivity to apoptosis.[146] Exercise is also shown to induce brain-derived neurotrophic factor (BDNF) that is normally reduced in AD, PD, and HD, to activate cAMP responsive element binding protein 1, and to upregulate DNA repair protein apurinic/apyrimidinic endodeoxyribonuclease 1 (APEX1).[147] Strenuous exercise or overtraining exposes our body to oxidative challenge that damages the genome, but exercise with moderate intensity and duration increases muscle mass and resistance to stressors, enhances the immune system, improves cardiovascular function, and may reduce the incidence of AD by inducing neurotrophins and by modulating redox homeostasis.[148] Interestingly, our body also has systemic adaptation to exercise-induced oxidative stress function in a hormesis manner by upregulating antioxidant enzymes and DNA damage repair enzyme activity to orchestrate redox homeostasis, which is on the contrary to the vulnerability of a sedentary lifestyle.[148,149]

10.8.2 Dietary restriction

Dietary restriction (DR) or caloric restriction (CR) is a regimen of reducing food and/or energy intake. It has been extensively investigated for the last few decades. The activity of APEX1, a key protein in BER, is decreased with age in different mice brain regions. DR shows beneficial effects on improving APEX1 activity and reducing oxidative lesions.[150] Short-term DR can also promote DNA repair.[151] DR reduces the transcription rate and extends lifespan.[49] DR has also been tested on *Mlh1* deficient mice, which has modest effects on cancer incidence and lifespan so it is speculated that compromised MMR might be epistatic to DR.[152] It would be very interesting to investigate the effects of DR on DNA repair proteins and DNA damage accumulation.

10.8.3 Nicotinamide adenine dinucleotide

As a substrate of sirtuins (SIRT1-SIRT7) and PARPs, both involved in DNA repair, $NAD+$ pools play pivotal roles in cellular and organismal homeostasis. However, $NAD+$ declines with age.[153] $NAD+$ can target DNA repair as well as mitophagy to slow aging and age-related disorders. For example, A-T is a progressive neurodegenerative disease caused by ATM deficiency (a key gene in DSB repair) and displays mitochondrial dysfunction and defective mitophagy. In its animal models, supplementation of $NAD+$ has been shown to reinstate mitophagy and DNA repair, and even extend lifespan and healthspan.[124] Alternatively, extracellular nicotinamide phosphoribosyltransferase (eNAMPT), also declines with age and its supplementation restores $NAD+$ levels and extends healthspan in mice.[154] Boosting $NAD+$, by $NAD+$ precursors, activates its synthesis, inhibits its degradation, slows aging, reduces disease

susceptibility, and extends healthspan.[155] NAD + precursors include nicotinamide (NAM), nicotinamide mononucleotide (NMN), and NR. Interestingly, when knocking out NADase CD38, NAD + decline is attenuated, which is concomitant with improved mitochondrial function and glucose tolerance.[156] In this regard, drug screening and development might hold the promise to maintain NAD + pools by administrating CD38 inhibitors.

10.9 Conclusion and future perspectives

The well-coordinated DNA repair mechanisms are evolved to protect the genome from substantial DNA damaging agents to counteract genomic instability. Genetic disorders, defective in DNA damage responses, often display characteristics of premature aging, cancer predisposition as well as shortened lifespan. Alterations in DNA repair either elevate abnormal cell death-induced atrophy or neuron loss-mediated neurodegeneration or result in uncontrolled cell proliferation, which contributes to cancer initiation and progression, further exacerbating the overall DNA damage load and disease incidence.

This generates opportunities to develop strategies to enhance the DNA repair system thus maintaining genomic integrity and promoting tissue homeostasis. Changing lifestyles such as exercise, food, and dietary supplements might systematically improve the body's health. Concerning genetic deficiencies, targeted gene therapy might hold the promise for the precise correction of the mutant genes; alternatively, pharmacological approaches will be developed to orchestrate the balance between cell death and proliferation.

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CHAPTER 11

Telomeres and cell homeostasis in aging

Stella Victorelli[1,2] **and João F. Passos**[1,2]
[1]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, United States
[2]Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN, United States

11.1 What is cellular senescence

Cellular senescence is a phenomenon first described by Leonard Hayflick in 1961 characterized by an irreversible loss of the proliferative capacity of somatic cells. Hayflick, observed that cells reached a state of terminal arrest after undergoing a limited number of divisions when cultured in vitro.[1] The senescence phenotype is complex and involves dramatic alterations in multiple features of the cells' biology, including gene expression, organelle composition and function, and chromatin remodeling, among others.[2] Importantly, senescent cells have been shown to secrete several proteins, including pro-inflammatory cytokines, chemokines, and extracellular matrix-degrading proteins, which are collectively known as the senescence-associated secretory phenotype (SASP).[3] The SASP alters the function of neighboring healthy cells, the composition of the extracellular matrix, and orchestrates the recruitment of immune cells.[4–6]

Senescent cells have been shown to accumulate in different tissues during the aging process and in the context of multiple age-related diseases.[7] Over the last ten years, mouse models have been developed that allow for the specific elimination of highly expressed $p16^{Ink4a}$ positive cells, which are presumed to be senescent.[8–10] These studies have shown that clearance of senescent cells, whether genetically or through the application of drugs that can specifically kill senescent cells (senolytics), alleviates several pathologies in aging mice.[9–12] This led to the popular hypothesis that senescent cells are targets for therapies to counteract age-related tissue dysfunction.

11.2 Link between cell senescence and telomeres

Cellular senescence has been linked to the shortening of telomeres, which are repetitive DNA sequences associated with proteins that act as protective caps at chromosome ends. Somatic cells are particularly susceptible to the loss of telomeric repeats because they lack telomerase activity, an enzyme capable of elongating telomeres. Therefore, with each round of cell division, they are faced with the "end-replication problem,"

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whereby DNA polymerases are unable to fully replicate the C-rich lagging strand, as proposed by Olovnikov and Watson in the early 1970s.[13,14] During lagging-strand synthesis, RNA primers are inserted to allow DNA polymerases to initiate DNA replication. However, when the last primer at the 3′ end is removed, the newly synthesized strand is shorter than the template by a few nucleotides, leading to the loss of telomeric sequences. Indeed, early studies demonstrated that telomeres become gradually shorter with cell division, and this correlated with the onset of cellular senescence.[15] Importantly, promoting telomere elongation by ectopic expression of telomerase was shown to extend the replicative lifespan of human cells, demonstrating that telomere shortening plays a causal role in the senescence arrest.[16]

11.3 Critically short telomeres activate a DNA damage response and senescence

Telomeres contain a C-rich lagging strand and a G-rich leading strand comprised of single-stranded nucleotide repeats, which provides an origin to the 3′ overhang.[17] It is believed that the overhang binds to upstream double-stranded telomeric regions, forming a lariat-like structure known as the telomere-loop (t-loop), thereby protecting the exposed ends of linear chromosomes.[18] Telomeres also associate with a set of six proteins: TRF1, TRF2, RAP1, TIN2, TPP1 and POT1, which collectively form a specialized structure known as the shelterin complex and help to stabilize the t-loop.[19]

It has been proposed that extensive telomere shortening that occurs with cell division causes loss of shelterin components from telomeres, consequently destabilizing the t-loop conformation.[18] This in turn leads to exposure of chromosomes ends, which become recognized by the DNA repair machinery as double-stranded breaks (DSBs). In support of that, studies have shown that expressing a dominant-negative allele of TRF2 in human cells, which prevents accumulation of TRF2 at telomeres and leads to uncapping, promoted recruitment and activation of DNA damage response (DDR) proteins such as 53BP1, Mre11 complex, and phosphorylated ATM, H2A.X and Rad17.[20] Additionally, deletion of Pot1a in mice, a shelterin protein that binds to the 3′ overhang, resulted in DDR activation specifically at telomeres and senescence induction.[21] In line with the hypothesis that extensive loss of telomeric repeats leads to exposure of chromosome ends and activates a DDR, human fibroblasts undergoing replicative senescence display accumulation of DDR proteins, such as γH2A.X, 53BP1, MDC1 and NBS1 at telomeres.[22]

Upon activation of a DDR, effector pathways trigger responses such as DNA repair, cell-cycle arrest or apoptosis. One of the major effector molecules activated downstream of a DDR is the transcription factor p53, which in turn promotes expression of the cyclin-dependent kinase p21, a protein responsible for inducing cell-cycle arrest.[2,23] In support of a major role for p21 in telomere damage-induced senescence,

one study demonstrated that deletion of p21 rescued proliferation of intestinal progenitor cells and hematopoietic stem cells in late-generation telomerase-deficient mice, which displays extensive telomere shortening in a number of tissues and premature aging phenotypes.[24] Another important pathway for the induction and maintenance of senescence, is the p16-pRb pathway. However, the extent to which p16 is involved in telomere-induced senescence is less clear. For example, it has been shown that p16 activation can occur independently from telomere dysfunction in human cells.[23] Conversely, p16 deletion in Wrn-deficient mice, which have dysfunctional telomeres, was shown to improve the proliferative capacity of mouse embryonic fibroblasts.[25] In addition, loss of p16 was sufficient to prevent senescence triggered by telomere dysfunction in these cells.[25] Activation of p16 in response to telomere damage has also been shown upon TRF2 deletion.[26] However, p16 deletion in these cells did not fully rescue the growth arrest induced by telomere dysfunction, and proliferation was completely restored only upon simultaneous inhibition of p16 and p53, suggesting that p16 may serve as a secondary barrier to prevent cell cycle progression in response to telomere damage.[26]

11.4 Telomere dysfunction can occur in a length-independent manner

Although telomere erosion is an important contributor to telomere dysfunction, studies have shown that a DDR can be activated at telomeric regions in a length-independent manner, and this also plays a role during senescence. For example, activation of DNA damage signaling irrespectively of shortening has been reported in human fibroblasts in vitro and mouse neurons in vivo following genotoxic stress.[27] Moreover, length-independent telomere damage has been shown in cells undergoing replicative senescence[28] and oncogene-induced senescence, as in the case of melanocytic nevi.[29] Interestingly, some DDR-positive telomeres still contained shelterin proteins, such as TRF2 and RAP1,[28,29] suggesting that uncapping is not a limiting factor for DDR activation at telomeres. Length-independent telomere dysfunction has also been shown to accumulate during organismal aging. Studies report an age-dependent increase in telomere damage, which occurred irrespectively of shortening, in the gut and liver of mice,[30,31] and in the hippocampal neurons and liver of baboons.[27] Furthermore, in the lungs of patients with COPD, where senescence burden is increased, a higher frequency of length-independent telomere-associated damage has been shown in small and large airway epithelial cells.[32,33]

Recent studies suggest that length-independent telomere dysfunction plays an important role in driving senescence of slow dividing and/or terminally-differentiated cells. An age-dependent accumulation of damaged telomeres without significant shortening has been observed in cells with low proliferative capacity, such as human epidermal melanocytes,[34] as well as in non-dividing cells such as neurons[35] and cardiomyocytes.[36] It was

shown that aged cardiomyocytes have increased mitochondrial dysfunction and reactive oxygen species (ROS), and this is a major contributor to the generation of damage at telomeric regions, which drives senescence.[36] Additionally, one study demonstrated that ROS produced in response to mitochondrial dysfunction causes DSBs to accumulate preferentially at telomeres, supporting the hypothesis that oxidative stress derived from dysfunctional mitochondria plays a key role in inducing length-independent telomere damage.[37]

DNA damage that occurs at telomeres differs from the rest of the genome in that they are long-lived, and in fact have been shown to persist for several months both in cells in vitro and in animal models.[27,30] Indeed, in cells undergoing stress-induced senescence, live-cell imaging has demonstrated that long-lived DNA damage foci co-localizes with telomeres.[30] In addition, persistent DDR signaling has been observed at telomeres in hippocampal neurons of mice even three months after exposure to genotoxic stress.[27] It has also been shown that unresolved telomeric DDR foci accumulate in response to oncogene activation in human cells.[29] Moreover, telomere-associated damage is present in cancer precursor lesions, such as human melanocytic nevi, ductal breast hyperplasia, and colonic adenomas, suggesting that persistent telomeric damage plays a role in tumor-suppressor mechanisms in vivo.[29] It has been proposed that oncogene activation leads to replication stress, causing telomere shortening and dysfunction in cells that lack telomerase.[29] As a result, senescence-associated pathways are activated, preventing the progression of pre-malignant cells into malignant cancers.[29] Interestingly, expression of telomerase in oncogene-induced senescent cells, was recently shown to repair existing telomere lesions, as well as to prevent further generation of telomere damage. Therefore, in pre-malignant cells, telomerase can resolve telomeric DDR foci that occurs as a result of replication stress, allowing these cells to bypass senescence, contributing to malignant transformation.[38,39]

Persistent DDR signaling is believed to be a key mechanism involved in the initiation and maintenance of senescence.[40] Given that long-lived telomere dysfunction is present in replicative, stress-, and oncogene-induced senescent cells, it has been proposed that unresolved telomere damage is an important contributor to persistent DNA damage signaling that initiates and stabilizes the senescence phenotype.[27] In support of this, expressing a telomere-specific endonuclease in cardiomyocytes was shown to generate long-lasting telomeric damage and induce senescence in these cells.[36] On the other hand, non-telomeric DNA damage generated by the endonuclease I-PpoI, was rapidly repaired and did not lead to induction of cardiomyocyte senescence.[36] Nonetheless, the contribution of non-telomeric DNA lesions to senescence cannot be completely disregarded. It has been shown that in replicative and stress-induced senescence, short-lived, non-telomeric DNA damage foci are constantly renewed, likely as a result of increased ROS production in senescent cells.[30,41] Therefore, DDR signaling from both telomeric and non-telomeric regions contribute to the senescent phenotype, although determining their relative contribution to senescence is technically challenging (Fig. 11.1).

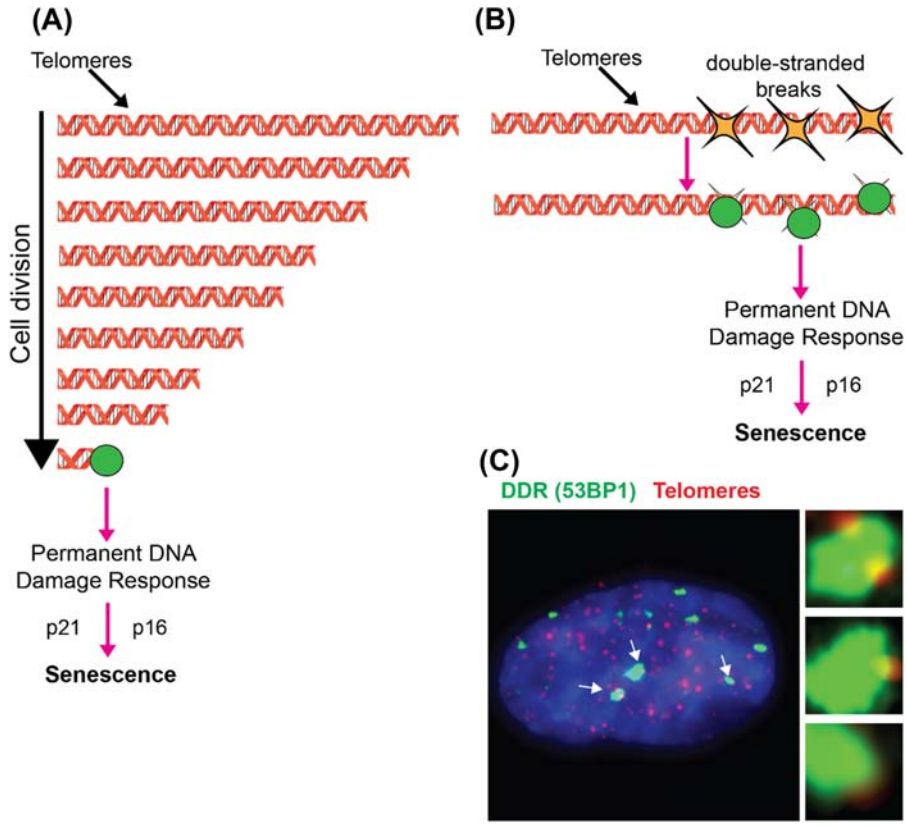

Figure 11.1 Both critically short (A) and long (B) telomeres can activate a permanent DNA damage response resulting in cellular senescence. (C) Representative immuno-FISH for telomere-associated foci (TAF) in a senescent cell. Arrows indicate TAF.

11.5 Mechanisms by which stress accelerates telomere dysfunction

Although repeated rounds of cell division drive telomere shortening, other factors can influence the rate at which telomeres become dysfunctional. Several studies have demonstrated that mild oxidative stress accelerates telomere shortening, reducing proliferative capacity of cells and inducing a phenotype comparable to replicative senescence.[42–44] In support of the role for ROS in telomere dysfunction-induced senescence, it has been demonstrated that reducing ROS levels either by overexpressing the antioxidant enzyme superoxide dismutase or by antioxidant treatment, leads to a slower rate of telomere shortening and extends the proliferative potential of human fibroblasts.[45,46] Furthermore, it has been proposed that the levels of intracellular ROS directly correlates with the rate of telomere attrition, following observations that cells with lower antioxidant capacity and shorter replicative lifespan also display a higher rate of telomere

shortening compared to those that have greater antioxidant capacity.[47] Oxidative stress has also been shown to play a role in telomere dysfunction in vivo. One study suggested that high levels of ROS contribute to demyelination and axonal damage in multiple sclerosis (MS) patients,[48] who also display increased markers of oxidative stress and shorter telomeres compared to healthy participants.[49] While the major source of ROS responsible for telomere dysfunction is still unclear, evidence suggests that mitochondrial-derived oxidative stress is an important contributor to telomere shortening. Treating cells with the mitochondrial-targeted antioxidant, Mito-Q, has been shown to prevent telomere shortening and extend the proliferative potential of fibroblasts under hyperoxic conditions.[42] Others have shown that inducing mild mitochondrial uncoupling, which reduces the level of mitochondrial superoxide, extends the replicative lifespan of cells and reduces the rate of telomere shortening, suggesting that mitochondrial ROS plays an important role in this process.[50] In addition, causing severe mitochondrial depolarization by FCCP treatment, leads to mitochondrial dysfunction, enhances ROS production and leads to telomere shortening, telomere loss and chromosome fusions in mouse embryos.[51] A role for mitochondrial dysfunction in accelerated telomere shortening is also supported by studies conducted in patients with mitochondrial diseases, such as MELAS and LHON. Such conditions are characterized by respiratory chain dysfunction, and white blood cells isolated from these patients contain shorter telomeres when compared to healthy controls.[52] Moreover, accelerated telomere shortening has been reported in many age-related diseases, such as Alzheimer's and cardiovascular disease,[53,54] which are also associated with an increase in mitochondrial dysfunction-induced ROS generation.[55] Telomere damage in cardiomyocytes is thought to be due to oxidative damage, since different mouse models of increased ROS and mitochondrial dysfunction show early onset of age-dependent telomere dysfunction.[36] Additionally, cardiac ischemia-reperfusion injury, known to be associated with high oxidative stress, led to increased telomere dysfunction and senescence.[56] Further supporting a pivotal role for oxidative stress as an inducer of telomere dysfunction, data has shown that ROS produced by neutrophils can lead to telomere dysfunction in neighboring diving cells.[57]

Telomeric regions are highly susceptible to oxidative damage compared to other sites within the genome due to their high content of guanine triplets, which can easily undergo oxidative modifications.[58] In support of this, mild oxidative stress has been shown to cause accumulation of single-stranded breaks specifically at telomeres, which in turn leads to replication fork stalling, incomplete replication of telomeric DNA, and thus accelerated telomere shortening.[46,59] Moreover, it has been recently shown that chronic generation of 8-Oxo-2′-deoxyguanosine (8-OxodG), a common ROS-mediated mutation, specifically at telomeres caused a significant reduction in telomere length and impaired cell proliferation, suggesting that ROS play a major role in telomere dysfunction.[60] Another mechanism by which oxidative stress might contribute to damage at telomeres is by disrupting the binding of shelterin components, TRF1 and

TRF2.[61] However, other studies have shown that stress-induced telomeric DDR activation can still occur even in the presence of TRF2, suggesting that additional mechanisms other than loss of shelterin proteins also play a role in causing telomere dysfunction.[27] As well as being more susceptible to damage, telomeric lesions are also less efficiently repaired when compared to the rest of the genome.[62] This unique feature of telomeres is primarily due to shelterin proteins, such as TRF2 and RAP1, which have been shown to prevent non-homologous end-joining (NHEJ) at telomeric regions by inhibiting DNA-PK, a component of the double-stranded break repair complex, and by preventing ligase-IV-mediated end-joining.[63] Consistent with this, studies in budding yeast have shown that generating DNA double stranded-breaks in regions adjacent to telomeric repeats impairs the recruitment of ligase IV to the site of lesion.[27] Moreover, expression of TRF2 next to a DSB causes persistent DDR signaling in mammalian cells, further demonstrating that telomeric damage is not easily resolved.[27] In addition to contributing to senescence induction by causing telomere dysfunction, ROS are also thought to be an effector mechanism during senescence. Work by our group has demonstrated that generation of mitochondria-derived ROS can be enhanced as a result of a DNA damage response, and this is partly dependent on the activation of p53 and p21, as well as ATM signaling to mTORC1.[41,64] Consistent with this, late generation $TERC^{-/-}$ mice, which contain dysfunctional telomeres, have increased oxidative damage in tissues that can be improved by the deletion of p21.[41] Furthermore, it has been shown that critically short telomeres activate p53, which in turn binds to and inhibits PGC-1α and PGC-1β promoters, and leads to mitochondrial dysfunction.[65] It has been proposed that ROS derived from dysfunctional mitochondria contribute to a positive feedback loop that induces further DNA damage, maintaining a DDR and contributing to the stability of the senescent phenotype.[41,64]

11.6 Telomere-associated DNA damage response foci accumulate during aging and disease

Multiple studies have linked telomere length in peripheral blood with the progression of aging, mortality and morbidity, however, whether telomere length has relevant diagnostic potential is still not clear.[66] The majority of studies rely on the characterization of telomere length in homogenized populations of blood cells and as such, do not provide information regarding the length of individual telomeres which are highly variable within cells. This is an important factor to consider since studies indicate that one or a few critically short telomeres may be sufficient to trigger a DDR and senescence.[28]

With regard to the question of whether telomere shortening is causal in aging, studies in mice have shown that deletion of telomerase (Terc or Tert) in combination with multiple-generation breeding, results in critically short telomeres and the early onset of aging and disease.[67] However, telomere length by itself is unlikely to be the

major determinant of lifespan, as for instance, much shorter-lived species have considerably longer telomeres than humans.[68] For that reason, it has been argued that telomere length alone may not be the culprit, but the rate at which telomeres shorten throughout the lifespan of an organism. Consistent with this hypothesis, recent studies reported a positive correlation between the rate of telomere shortening and lifespan of different species.[69] However, it is not clear whether telomere erosion by itself will result in critically short telomeres capable of destabilizing the t-loop, inducing a DDR and downstream pathways leading to senescence. While mice experience age-dependent telomere shortening, their telomeres at older ages are still considerably longer than the ones found in humans even at a young age. Additionally, while telomere shortening can explain aging in actively diving tissues, this model fails to clarify why quiescent or post-mitotic cells also accumulate DDR signaling at telomeres during aging.

For these reasons, we propose that a more reliable indication of telomere dysfunction is to evaluate if DDR proteins are enriched at telomere regions, rather than assessing telomere length alone. Telomere-associated DDR can be evaluated in bulk populations of cells through methods such as ChIP-seq[22] and at single-cell resolution using Immuno-FISH, a method that allows the microscopic assessment of co-localization between telomeres and DDR proteins such as γH2A.X, 53BP1 and others[30] (Fig. 11.2). Other methods such as the detection of telomeric non-coding RNA allows for the detection of telomeric DDR both in situ (FISH) and in bulk tissues (qPCR).[70]

Data consistently shows that co-localization between DDR proteins and telomeres increase during aging in different tissues and mammalian species (Table 11.1). Telomere-associated DDR foci (TAF) have been shown to increase in multiple tissues in aging mice, such as the liver,[30] intestine,[30] lung,[32] bone,[74] brain[76] and heart.[36] Additionally, TAF have also been shown to increase in the skin and brain of aged baboons[27,73] and in aged human skin.[34] Conditions known to accelerate the aging process such as inflammation,[31,57] obesity,[35,71] mitochondrial dysfunction[36] and impaired autophagy[72] also resulted in increased TAF in vivo.

Interventions targeting senescent cells such as rapamycin,[64,78] ibuprofen,[76] dietary restriction,[71] and 17α-estradiol[79] significantly reduce the frequency of TAF in different tissues. Furthermore, senolytic drugs or genetic clearance of $p16^{Ink4a}$ positive cells was shown to reduce TAF in the medial layer of the aorta,[75] liver,[71] heart,[36] brain,[35] bone[77] and adipose tissue.[35] These data support that TAF are a good marker of cellular senescence.

Further supporting a causal role for telomere dysfunction and DNA damage signaling in the aging process, it was recently shown that selective DDR inhibition at dysfunctional telomeres prevents senescence and can have beneficial effects during aging. Inhibition of DDR at telomeres using telomeric antisense oligonucleotides[70] was able to extend lifespan and healthspan in a mouse model of Hutchinson-Gilford progeria syndrome (HGPS).[80]

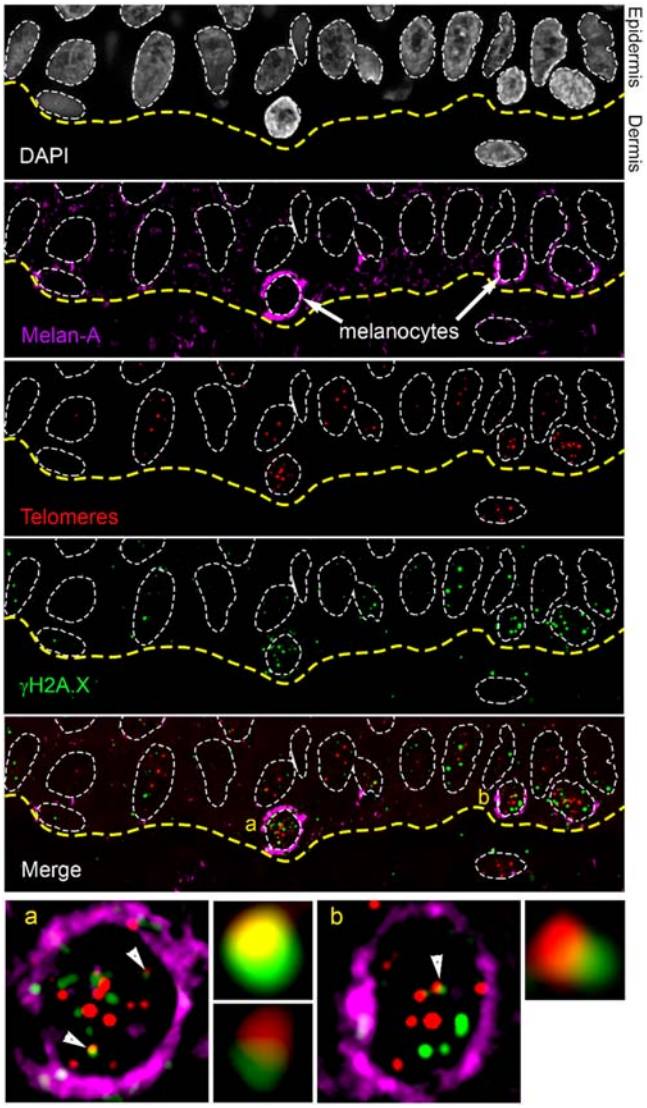

Figure 11.2 Representative immuno-FISH micrographs of human epidermis (using telomere-specific (CCCTAA) peptide nucleic acid probe, anti- γH2A.X antibody and anti-Melan-A). Nuclei were stained with DAPI. Amplification of melanocytes (positive for Melan-A) is shown below. Arrows indicate co-localization between telomeres and γH2A.X. a and b indicate two melanocytes containing TAF which are shown at higher magnification below.

In summary, data consistently show that TAF accumulate during aging and age-related diseases and are good indicators of cellular senescence. Telomeric DNA damage is persistent and irreparable and can occur irrespectively of telomere length in both dividing and non-dividing cells. Importantly, specific inhibition of telomeric DDR

Table 11.1 Publications showing that telomere-associated foci increase during aging and age-related diseases and are reduced by senolytic therapies in different organs.

Aging/age-related diseases	Publications
Liver	[27,30,31,57,71,72]
Intestine	[30,31]
Skin	[34,73]
Bone	[74]
Lung	[32,33]
Aorta	[75]
Heart	[36,56]
Brain	[27,35,76]
Adipose tissue	[11,35]
Senolytics	
Bone	[77]
Heart	[36]
Brain	[35]
Aorta	[75]
Adipose tissue	[11,35]
Liver	[57,71]

Data is from mouse, human and baboon tissues.

prevents premature senescence in the context of HGPS, highlighting the potential therapeutic application of selective DDR inhibition at dysfunctional telomeres to ameliorate aging and age-related disease.

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CHAPTER 12

Cellular senescence during aging

Thomas von Zglinicki
Newcastle University Biosciences Institute, Campus for Ageing and Vitality, Newcastle University, Newcastle upon Tyne, United Kingdom

12.1 Cell senescence is a complex stress response

Replicative cell senescence was first described in 1961 by Len Hayflick as a reproducible, permanent loss of replicative capacity of human primary fibroblasts during in vitro culture[1]; that cells lose the capacity to divide (and change their phenotype) as result of some inherent program-like mechanism rather than due to suboptimal culture conditions, was a remarkable discovery, that was still heavily disputed over most of the previous century. Even more remarkable was the immediate realization that this process represented the cellular equivalent of aging, termed "Aging under Glass,"[2] which recent discoveries have proven as an impressive example of scientific foresight.

It took more than 30 years after Hayflick's initial discovery to understand the molecular mechanisms of replication-driven cellular senescence. Essential for this understanding was the discovery of telomere shortening as part of DNA replication during cell division,[3,4] and of the rescue of senescence by telomerase-mediated telomere lengthening.[5] While these discoveries established telomeres as the "clock" that determines progression towards senescence, it was the discovery of the specific stress-sensitivity of telomeres that linked telomere-driven senescence with (equally stress-sensitive) organismal aging.[6,7] Finally, the finding that short or otherwise uncapped telomeres initiated a canonical DNA damage response (DDR) in senescence,[8] clearly identified cell replicative senescence as a telomere length-driven, DDR-mediated persistent activation of the cell cycle checkpoint machinery.[9]

However, cell senescence is much more than a permanent cell cycle arrest. Phenotypic changes for instance, increased cell size, changed nuclear morphology, lysosomal dysfunction and others, have been noted early on in senescence and frequently been used as senescence markers. Examples of these types of changes are illustrated in Fig. 12.1.

More recently, it became clear that these modifications of the cellular phenotype are not just simple consequences of a persistent cell cycle arrest. Rather, the re-organization of the cellular secretome, their energy metabolism, their epigenome, nutrient signaling, autophagy and mitophagy activities during the establishment of senescence have been recognized as components of interlinked positive feedback loops that stabilize each other and

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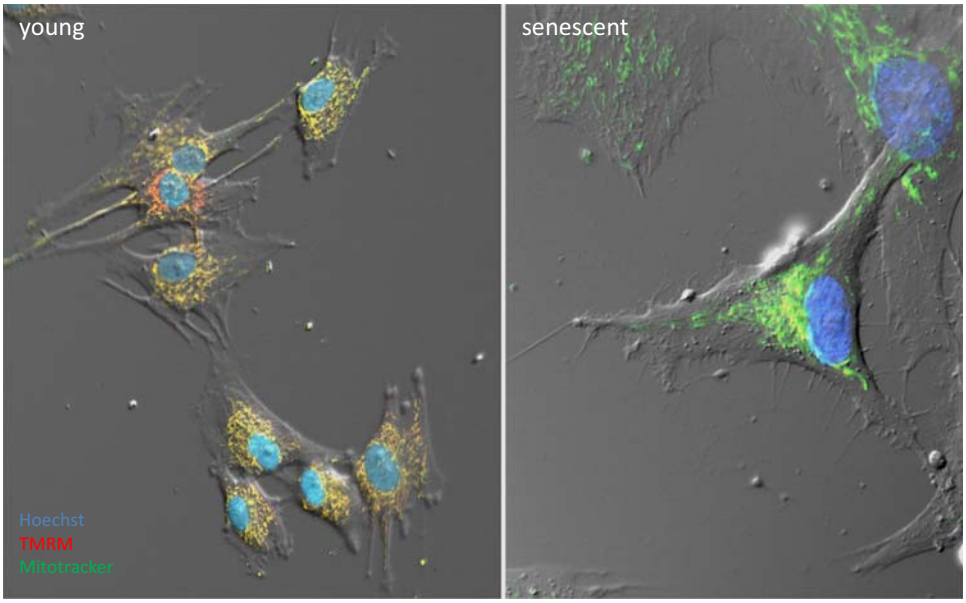

Figure 12.1 ***A comparison between young (left) and replicative senescent (right) human embryonic fibroblasts.*** Both images are at the same magnification. Nuclei are stained with Hoechst (blue) and mitochondria are stained with both Mitotracker (green) and tetramethylrhodamine methyl ester (TMRM) (red). As TMRM binding to mitochondria is membrane potential dependent, a shift from red/yellow to green fluorescence indicates decreased functionality of mitochondria in senescence.

the senescent DDR/growth arrest.[10–12] Moreover, proliferation arrest and senescent phenotype are not always closely associated with each other: tumor cells for instance can not only enter a senescent state (e.g., following DNA-damaging stress) but can also recover from it. If and when they do so, they retain certain epigenetic characteristics which they gained as senescent cells.[13,14] Conversely, postmitotic somatic cells can make the transition towards a senescent phenotype in response to DNA damage at a time long after they have ceased replication.[15,16]

Thus, a persistent cell cycle arrest can no longer be regarded as the sole defining feature of senescence. Instead, senescence may be defined as a cellular stress response composed of a number of interlinked building blocks (Fig. 12.2). These building blocks give rise to phenotypic consequences (i.e., the DDR mediates the cell cycle arrest; autophagy dysfunction causes lysosome build-up and the accumulation of SA-β-Galactosidase; etc.). They are interlinked by a number of connecting signaling pathways that form multiple feedback loops. These feedback loops not only maintain and stabilize the phenotype (see Section 12.2 for a detailed discussion), but they also ensure that very different stresses and interventions always trigger essentially a very similar phenotype. For instance, telomere uncapping, autophagy inhibition or induction of mitochondrial dysfunction all cause cell senescence with involvement of all the six

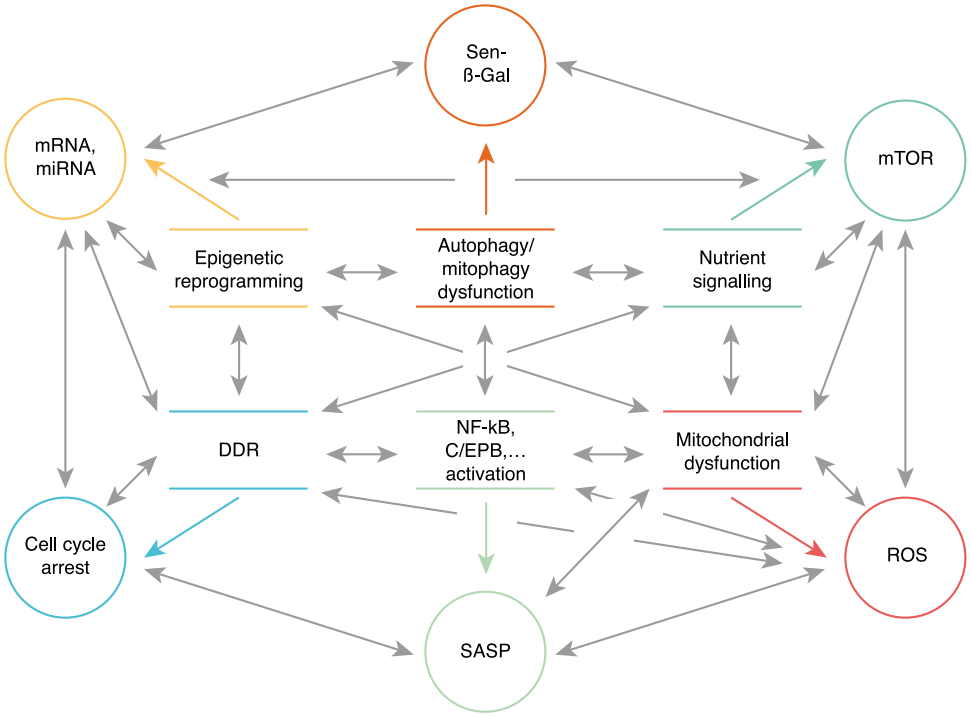

Figure 12.2 ***The senescent phenotype: building blocks, phenotypic consequences and interlinks.*** The scheme shows major building blocks of the senescent phenotype (squares), main phenotypic consequences (circles) and a selection of signaling pathways interlinking them.[20] *With permission from von T. Zglinicki (2021). Mechanisms of cell senescence in ageing. In "Handbook of aging, 9th edition" PJ Hornsby and N Musi, (eds.) Elsevier.*

building blocks depicted in Fig. 12.1. Variable involvement of individual blocks may contribute to the variation of the senescent phenotype between different cell types, in dependence of the initiating stress, or other variables. For instance, the composition of the senescence-associated secretory phenotype (SASP) differs depending on the type of DNA damage[17] or whether a DDR or mitochondrial dysfunction was the primary inducer of senescence.[18] Importantly, we propose that senescence is defined by the involvement of all, or at least the vast majority, of these building blocks (see also[19]).

This definition of senescence implies that it does not prioritize certain features over others. For instance, senescence is frequently identified by reference to DDR/cell cycle arrest and SASP only, with additional features only added as an afterthought. While this may be sufficient for practical operationalization in many cases, it does not cover the full complexity of the senescent stress response and might fall short even of practical utility in more complex cases like tumors or postmitotic cell senescence. The major advantage of defining senescence as interacting building blocks is of course that it easily explains how very different stressors and interventions targeting, for instance,

nuclear DNA, the proteasome, mitochondria, the inflammasome or DNA methylation can all cause essentially the same cellular response.

The list of building blocks and phenotypes presented in Fig. 12.2 is by no means exhaustive. For example, resistance to apoptosis is frequently regarded as an essential feature of senescent cells, especially in the context of anti-senescence interventions,[21] and there might be further features that can be regarded as essential components of the phenotype. However, we will first discuss the building blocks presented in Fig. 12.2 individually as well as some of their interconnecting pathways (see Section 12.2). We will then cover accumulation and detection of senescent cells during aging in vivo (see Section 12.3) and the impact of senolytic and senostatic interventions on the aging process (see Section 12.4). For brevity, a number of aspects of cell senescence will not be covered here. The reader is directed to recent excellent reviews on senescence during development and regeneration,[22–24] on oncogene-induced and therapy-induced senescence[25–28] and on the interaction of senescent cells with the immune system.[29]

12.2 The building blocks of the senescent phenotype

12.2.1 Telomeres and the DNA damage response

In 1990, Cal Harley demonstrated shortening of telomeres with ongoing population doublings in human fibroblasts[4] and a few years later, Bodnar et al. showed that replicative senescence could be bypassed if telomere length was maintained by telomerase overexpression.[5] Originally it was thought that the connection between replication and telomere shortening was solely due to the inability of DNA polymerases to copy the lagging strand to its very end (the so-called "end-replication problem,"[3]). However, it was soon found that relatively mild oxidative stress could contribute significantly to replication-associated telomere shortening because of a telomere-specific single-strand break repair deficiency; indicating that telomeres might not just be passive replication counters but sentinels for cell-autonomous and non-autonomous stress.[6] When it was found mechanistically that short uncapped telomeres in senescent cells induced a classical DDR,[8,30] telomere shortening was firmly established as a driver of replicative senescence in human somatic cells.

In very broad terms, senescence can be induced by replicative exhaustion or by external or internal stressors (e.g., DNA damage, oncogenic stress, mitochondrial dysfunction, and others). To discriminate operationally between replicative senescence (RS) and stress-induced (often termed premature) senescence (SIPS[31]), RS has been frequently equated with "telomere-dependent senescence," which usually meant "senescence induced by telomere shortening due to the end replication problem," while SIPS was considered to be telomere-independent (i.e., telomere length-independent). These are over-simplifications. First of all, telomere shortening, solely due to the end replication problem is an exception rather than the rule, occurring only

under ideal conditions of very low stress and in cells with the most effective anti-stress (especially antioxidant) defenses.[32,33] More frequently, telomere shortening is driven to a significant extent by telomere-specific DNA damage accumulation.[6] Even mild stressors, just sufficient to generate base oxidations or single-strand breaks in telomeres, can accelerate telomere length-dependent senescence.[34] Conversely, SIPS induced by acute stress might not be associated with telomere shortening but typically shows DNA damage foci at telomeres as an indication of telomere uncapping. In fact, shortening is not the only possibility to "uncap" a telomere such that it triggers a DDR. Depletion of shelterin components, a protein complex that binds specifically telomeric DNA and protects it from recognition by the DDR, initiated immediate senescence with long telomeres.[35] Physiologically more important, it was found that apparently intact, long telomeres in the presence of adequate amounts of shelterin proteins could induce a DDR.[36,37] Such telomere-associated DNA damage foci (TAF) were found in both mice and human cells with aging and were associated with stress but not with telomere shortening.[36–39] While they have not been conclusively analyzed mechanistically, it has been suggested that they might resemble stress-induced intra-telomeric double strand breaks.[36] It seems very probable that this type of length-independent but telomere dysfunction-mediated senescence may be a predominant form of senescence in vivo, not only in mouse tissues with very long telomeres[37,39–41] but possibly also in humans, especially in tissues with low cell turnover including the liver,[39] lung,[42] brain[43] and heart.[38] Thus, RS and SIPS are not fully separate entities, rather they overlap significantly with telomere dysfunction playing a central role throughout the whole continuum.

DNA damage foci at non-telomeric sites in the genome have in general short lifespans and are typically resolved with half-lives in the order of 3 h to 10 h[10] following damage repair and proteasomal degradation of the foci components. In contrast, TAF are persistent and remain stable in the nucleus for at least multiple days.[10,37,38] This is assumed to reflect the deficiency of telomeres for multiple modes of DNA repair including nucleotide excision repair,[44] base excision (single strand break) repair[34,45] and double strand break repair.[35] Given the necessity of maintaining a DDR for a couple of days to engage the different components and to secure the stability of the phenotype, it is obvious that TAF are more efficient triggers of senescence than non-telomeric DNA damage.

The DDR is linked intimately to all other aspects of senescence. P53 is the most ubiquitous transcription factor activated by DNA damage. It activates hundreds of genes,[46] controls the SASP directly and indirectly,[47] regulates glucose metabolism, mitochondrial function and autophagy[48] and interacts with multiple chromatin modulators.[46] C/EBPβ and NF-κB activation and SASP have been associated with the DDR both as a consequence of and a contributor to it, thus stabilizing the senescent phenotype.[11,12,49] However, overexpression of the DDR-driven CDKIs p21 or p16 did arrest cell proliferation without inducing a SASP.[50,51] Nevertheless, signaling through p53 and p21 was

found necessary to induce senescence-associated mitochondrial dysfunction (SAMD) through a pathway involving p38MAPK and TGFβ.[10] In turn, SAMD caused hyperproduction of reactive oxygen species (ROS) in senescence, which amplified DNA damage and the DDR, constituting another example of a positive feed-forward loop stabilizing senescence.[10] Further pathways that drive SAMD in response to a DDR in senescence exist. For instance, it was recently shown that the nuclear retinoid X receptor RXRα (and possibly also related receptors) could control senescence by repressing expression of the inositol 1,4,5 triphosphate receptor type 2 (ITPR2), an endoplasmic reticulum (ER) calcium release channel. Suppression of RXRa induced calcium release from the ER, and its uptake by mitochondria followed by enhanced ROS production, mitochondrial dysfunction, nuclear DDR and senescence.[52] Other integrative pathways will be discussed next.

12.2.2 Senescence-associated secretory phenotype

In 2008, two back-to-back papers in Cell showed that in senescence, pro-inflammatory master transcription factors, NF-κB and C/EBPβ, are activated causing production and secretion of cytokines IL-6 and IL-8, which in turn stabilized senescence via paracrine signaling.[11,12] This senescence-associated secretory phenotype was then further characterized and shown to include, in addition to pro-inflammatory cytokines (including Il-6, Il-8, IL-1a, Il-1b), chemokines (CXCL1 and others), matrix-modifiers, specifically matrix-degrading enzymes (PAI-1, MMPs) and other bioactive peptides.[49,50] While these are "typical" components of the SASP, its specific composition may vary depending on the senescence-inducing mechanism, activity and/or presence of DDR components p53, p16 and p21, and cell type.[17,53] More recently, the Narita group showed that senescent cells actually produce at least two mechanistically distinct and kinetically separate forms of SASP. An early response is driven by the Wnt/Notch-1 pathway and contains mainly anti-inflammatory peptides, specifically TGFβ.[54] The NF-κB- and C/EBPβ − dependent pro-inflammatory response develops only after decline of Notch-1 signaling. It is not clear yet whether the first response is necessary for the development of the second in multiple variants of senescence. Both forms of SASP can attract and activate immunosurveillance of senescent cells by monocytes or macrophages.[55] Accumulation of cytoplasmic chromatin fragments activating the cytosolic DNA-sensing cGAS-STING pathway, is a major cause for the pro-inflammatory SASP; deletion of either cGAS[56] or STING[57] abrogates the pro-inflammatory SASP. Both nuclear[57] and mitochondrial[58] DNA are damaged in senescence and may be released into the cytoplasm. Their relative importance for activation of the cGAS-STING pathway in multiple forms of senescence is not clear.

In addition to the "classical," pro-inflammatory SASP and to the anti-inflammatory, TGFβ-centered secretome,[54] senescent cells release ROS, specifically H2O2,[10] oxidized proteins and lipids,[59] specific endosome-encapsulated miRNAs[60] and other factors.

While the terminus SASP is often used (and was originally coined) to describe the NF-κB- and C/EBPβ − driven secretome only, there is now a tendency to include all senescence-specifically released molecules under the same name. As all these secreted factors interact with and stimulate each other, we endorse the use of "SASP" as a generic term and suggest discriminating the different components by sub-terms (pro-inflammatory SASP, pro-oxidant SASP etc.).

12.2.3 Senescence-associated mitochondrial dysfunction

That externally-triggered mitochondrial dysfunction and associated production of ROS can induce senescence, for instance by accelerating telomere shortening, has been known for a long time.[7,31,61–63] Genetically- or pharmacologically-induced mitochondrial dysfunction can also trigger senescence without involvement of ROS, although in this case the SASP appears different, that is less pro-inflammatory.[18] Importantly, mitochondrial dysfunction, phenotypically characterized by increased ROS production, despite reduced electron transport chain coupling and accompanied by increased mitochondrial mass, is an inherent part of the senescent phenotype. SAMD is induced as a downstream consequence of the DDR in both oncogene-induced,[64] IGF-1-mediated,[65] and telomere- or stress-induced senescence.[10,66] Similar to mitochondrial dysfunction during tissue aging, SAMD is primarily associated with complex I dysfunction.[66] A cause of complex I dysfunction in aging is decreasing efficiency of complex assembly, resulting in sub-complexes unable to participate in electron transport but still generating ROS.[67] Whether induction of senescence decreases the efficiency of complex I assembly or not, has not yet been established.

The signaling pathways that cause SAMD in response to a DDR have been partially established to involve activation of p38MAPK and TGFβ.[10] They also involve a shift in the net balance between mitochondrial biogenesis and mitophagy, possibly related to a re-adjustment of nutrient signaling through mTOR ([66], see below). Enhanced ROS production is one consequence of SAMD, and these ROS contribute to continuous DNA damage and DDR in senescence, forming a senescence-stabilizing forward feedback loop.[10] This is one reason why mitochondria are essential for multiple facets of the senescent phenotype. When mitochondria were specifically degraded in cells after induction of senescence, the SASP, p16 and p21 activation, senescent morphology and Sen-β-Gal activity were all abrogated.[68]

SASP and SAMD interact closely with each other. Hyper-activation of NF-κB, a master regulator of the pro-inflammatory SASP, by knock-out of the inhibitory subunit NFKB1, induced senescence with aggravated ROS production in vitro and in vivo.[39] However, pharmacologic or genetic suppression of NF-κB activity did not reduce ROS levels in senescent cells, while conversely, scavenging of ROS in senescence diminished NF-κB activity.[69] Together with the different kinetics of SAMD

and SASP induction in senescence and the abrogation of the SASP by mitochondrial ablation[68] these data suggest senescence-associated ROS production as necessary and sufficient for the pro-inflammatory, NF-κB-dependent SASP.

12.2.4 Nutrient signaling

The mechanistic target of rapamycin (mTOR) kinase complex is the central regulator of the balance between translation, nucleotide biosynthesis, lipogenesis, glycolysis and autophagy in response to nutrient and amino acid availability. Normally, starvation decreases mTOR activity, favoring autophagy.[70] However, in cell senescence mTOR activity remains constitutively high even under amino acid and nutrient starvation.[71,72] It has been suggested that this aberrant activation of mTOR in senescence might be driven by increased oxidative stress caused by SAMD.[73] In fact, nutrient-independent activation of mTOR by oxidants had been shown in HEK293 cells.[74] Moreover, membrane depolarization and a resultant defect in cilia formation contribute to constitutive PI3K/mTORC1 signaling in senescence.[72] Furthermore, autophagic flux is enhanced in senescence,[75] facilitating cell-autonomous replenishment of amino acid pools, which might help to maintain high levels of mTOR activity in the absence of external stimuli.[72]

High mTOR activity is an essential component of the senescent phenotype. Treatment of cells with the mTOR complexI inhibitor rapamycin delayed replicative senescence.[76,77] mTOR activity was positively associated with senescence and PGC1α/β-driven mitochondrial biogenesis and mitochondrial dysfunction in fibroblasts[78] and lung epithelial cells in vitro[79] and in vivo.[80] Mechanistically, both mTORC1 and mTORC2 bind to p53 in competition with MDM2 and phosphorylate it at serine 15, thus enhancing its stability in the absence of DNA damage, leading to increased activation of p21.[81] If rapamycin is applied to senescent cells, it acts as a senostatic, i.e., it suppresses multiple aspects of the senescent phenotype including CDKI activation, SASP and SAMD (but not growth arrest) as efficiently as mitochondrial ablation.[68,82,83]

12.2.5 Autophagy/mitophagy

Autophagy describes several cellular pathways facilitating degradation of intracellular components by lysosomal proteases. Amongst autophagy pathways, macroautophagy (hereafter for simplicity called autophagy) is the best studied process. It begins with the engulfment of surplus or damaged proteins and entire organelles by autophagosomes which eventually fuse with lysosomes. The resulting autolysosome allows degradation of autophagy substrates and the subsequent release of basic components such as amino acids, lipids and nucleotides back into the cytoplasm. Autophagy activity is typically increased in conditions of nutrient deprivation sensed via the mTORC1 pathway and serves as a recycling process necessary for

cell survival during starvation. Importantly, autophagy is also a quality control mechanism by selectively scavenging potentially toxic cellular components.

Autophagy is implicated in cellular senescence in a complex and often contradictory relationship.[66] On one hand, autophagy activation via overexpression of the autophagy gene ULK3 promoted senescence induced by the RAS oncogene in human fibroblasts mediated by a specialized intracellular structure called TOR-autophagy spatial coupling compartment, while knockdown of the essential autophagy genes *Atg5* and *Atg7* was found to decrease SASP and bypass senescence.[84,85] Likewise, overexpression of the inhibitors of cyclin-dependent kinases p16(INK4A), p19(ARF) or p21(WAF1/CIP1) induced not only senescence as expected but also autophagy.[86] Furthermore, autophagy induction by a proteolytic Cyclin E fragment (p18-CycE) was shown to facilitate DNA-damage-induced senescence.[87] In contrast to these results, autophagy can also act as a mechanism to bypass OIS.[88] Moreover, inhibition of autophagy in young proliferating fibroblasts led to senescence,[89] while increased expression of autophagy genes *LC3B*, *ATG5* and *ATG12* and enhanced mitophagy postponed senescence and enhanced replicative lifespan.[90,91] Timing and intensity of changes in the autophagic flux might explain these differential impacts on the senescence process.

Mitophagy describes the selective autophagy of (dysfunctional) mitochondria. While general autophagy may become elevated or reduced depending on the model of senescence, mitophagy activity is reduced in senescent cells in vitro and in vivo.[78,92] This might be a consequence of constitutive activation of mTORC1 in senescence (see above). Likewise, lysosomal overload, as shown by the accumulation of lipofuscin,[93] occurs in senescence despite an extensive expansion of the lysosomal compartment that gives rise to a greatly enhanced β-galactosidase activity even at suboptimal pH.[94] This will disrupt the terminal events of substrate degradation in both general autophagy and mitophagy pathways.[95] Moreover, mitophagy may become impaired due to mechanisms independent of general autophagy. Mitophagy is governed by the PINK1 (PTEN induced putative protein kinase 1)-PRKN (parkin E3 ubiquitin protein ligase) pathway. Cytoplasmic p53, which accumulates in senescence, interacts with Parkin and prevents its translocation to dysfunctional mitochondria, thus suppressing mitophagy and stabilizing senescence.[96] Knockout of PRKN or inhibition of PRKN-mediated mitophagy resulted in mitochondrial dysfunction and senescence.[80,97] Similarly, expression of PINK1 is low in aging,[98] which suppresses the process of mitochondrial fission that is important for mitophagy by facilitating the separation of dysfunctional portions from the mitochondrial network. PINK1 knockdown again accelerated senescence.[99] Reduced dynamics and increased fusion of the mitochondrial network in senescent cells has been proposed to result in a failure to sequester defective components of the mitochondrial network for degradation via mitophagy.[78] As a result, "old" mitochondria avoid degradation while newly synthesized isolated mitochondria are turned over by mitophagy. The extent to which insufficient mitophagy might be a cause of SAMD remains to be established.

12.2.6 Epigenetic reprogramming

Cell senescence is associated with large scale changes in chromatin organization which overlaps significantly with those seen during tissue aging (for review see[100,101]) but also with those seen during transition to a more stem cell-like phenotype.[102] In human cell senescence, more than 30% of the chromatin is reorganized, including formation of large-scale domains (mesas) of H3K4me3 and H3K27me3 over lamin-associated domains and large-scale losses (canyons) of H3K27me3 outside these areas.[13] These changes are associated with transcriptional downregulation and autophagic degradation of lamin B1.[103,104] Senescent cells also show enrichment of H4K16ac at promoters of active genes overlapping with the histone chaperone HIRA.[105] DNA methylation, H3K9me3 and H3K27me3 levels are globally reduced[106] but heterochromatic foci (Senescence-Associated Heterochromatin foci SAHF) are formed in otherwise euchromatic regions containing H3K9me3, macroH2A and DDR proteins like γH2AX.[107] These chromatin changes are characteristic for the late-stage, fully developed senescent phenotype (Fig. 12.2).

There is strong evidence showing that epigenetic reprograming is both driven by changes in the other building blocks of senescence and contributes to the stabilization of the phenotype. For instance, changes in mitochondrial function trigger gene expression changes in the nucleus (retrograde response,[108]), which is active in cell senescence.[61] At least in yeast, the retrograde response to mitochondrial stress involves large-scale changes in heterochromatin and H3K9 methylation.[109] Sirtuins are histone deacetylases that affect both mitochondrial biogenesis and function via a decline in NAD + levels and increased HIF-1alpha transcription factor activity,[110] as well as DNA damage repair and telomere maintenance.[111,112] Availability of NAD + also interconnects epigenomic regulation with nutrient sensing.[113] Epigenetic changes, specifically the loss of H3K27me3, was strongly correlated with the upregulation of SASP genes in senescent cells,[13] and the inhibition of the H3K4 methyltransferase MLL1 reduced pro-proliferative cell cycle and DNA response genes, also resulting in reduced SASP.[114]

12.3 Senescence during aging in vivo

Since the first description of cell senescence[1] there has been a longstanding discussion on whether senescence is in fact "aging under glass" as suggested by Hayflick[1,2] or rather a cell culture artifact with no or very limited importance for aging. This discussion has firmly been settled in favor of Hayflick's insightful prediction by showing that targeted ablation of senescent cells can postpone and/or relieve a vast multitude of degenerative phenotypes in mice[115] (see Section 12.4). This may not be surprising, given that senescent cells have been found to accumulate in practically all mammalian tissues during aging. Instead of summarizing all this evidence, this chapter will focus

on senescence in postmitotic tissues/cell types and on one mechanism that contributes to senescent cell accumulation in vivo – the bystander effect. For a recent summary of methods to detect senescent cells in vivo, refer to Gorgoulis et al.[19]

12.3.1 Senescence in postmitotic cells

In 2012, we discovered that the senescent cell response was not restricted to proliferation-competent cells. We found that DNA damage accumulation during mouse aging triggered the all the senescence building blocks also in postmitotic neurons, both in the CNS and in the periphery. As in replication-competent cells,[10] p21 was essential for signal transfer between an extended DNA damage response and additional markers of the senescent phenotype.[16] More recently, a senescent phenotype was observed in neurons bearing neurofibrillary tangles from both human patients and transgenic mice, and it was shown that a senolytic intervention improved neuropathology in these mice.[116]

Among postmitotic cells, the ability to mount a senescence-like stress response is not limited to neurons. Multiple markers of senescence are induced in retinal cells in response to ischemic stress,[117] in skeletal muscle fibers[41] and in cardiomyocytes[38] during aging (for review see[15]). Similarly, significant senescence was found in slowly dividing cells like hepatocytes in vivo in both humans and mice.[39,40,118] Senolytic or senostatic interventions resulted in therapeutic benefits for all of these tissues.[21,40,115–117,119] Thus, senescence in postmitotic cells is as much a cause of aging phenotypes and a target for "anti-aging" interventions as senescence which is directly coupled to cell cycle arrest.

12.3.2 Senescent cell bystander effects

With the myriad of bioactive (SASP) molecules secreted by senescent cells, strong paracrine effects of senescence are no surprise. In fact, Campisi's group showed already in 2001, that senescent somatic cells stimulated growth and invasion of tumor cells,[120] and this was confirmed by multiple groups.[121,122] We discovered in 2012 that senescent cells also had a paracrine effect on normal, somatic cells - they induced senescence as a bystander effect.[123] This was soon confirmed by others.[124] Not unlike the situation with radiation-induced bystander effects in cancer therapy,[125] the quest to identify specific, individual components of the secretome as mediators of the senescent bystander effect has been elusive. Multiple interventions targeting ROS, NF-κB activity, mTORC1 signaling or p38MAPK activity were all capable of suppressing the bystander effect, but SAMD-derived ROS appeared to be upstream of the NF-κB-mediated pro-inflammatory SASP.[69] Weak bystander effects seemed dependent on cell-cell contacts via gap junctions, as they could efficiently be blocked by octanol, a disruptor of such contacts.[123]

Recently, the role of bystander-induced senescence for the accumulation of senescent cells and their functional consequences in vivo has been delineated. After transplanting very low numbers of senescent cells into either muscle or skin of mice, enhanced frequencies of tissue-resident senescent cells were detected adjacent to the transplanted cells.[41] When slightly higher numbers of senescent cells were transplanted into fat depots, senescence was spread into peripheral organs, persistent physical dysfunction was induced and both mean lifespan and health span was reduced. These effects could be blocked by senolytic treatment following transplantation, indicating the causal role of the senescence-induced by the bystander effect.[126]

Senescent cells accumulate in many tissues with age, and the rate of accumulation, at least in some tissues, predicts lifespan in mice.[39] Decreasing efficiency of immunosurveillance is one of the causes for age-associated accumulation of senescent cells,[127] while the spread of senescence via bystander signaling is another. This was tested by comparing accumulation rates of senescent cells in wt and severely immunodeficient NSG mice under both ad libitum and dietary restricted feeding.[41] As expected, senescent cells accumulated much faster in NSG mice fed ad libitum in agreement with the absence of a functional immune system. Suppressing the SASP (and thus the bystander effect) by dietary restriction slowed down the accumulation of senescent cells in the NSG mice, but resulted in a net loss (a senolytic effect) in the immunocompetent wt mice.[41] These data suggest that immunosurveillance and the bystander effect are the two major partners that determine the rate of accumulation of senescent cells in vivo in a dynamic equilibrium.

12.4 Senolytics and senostatics as anti-aging interventions

12.4.1 Senolytics

Around 2008, Jim Kirkland developed the concept to use a p16 promoter-driven suicide gene for the selective ablation of p16-positive, presumably senescent cells in mice. This led to a fruitful collaboration with Jan van Deursen showing that this approach not only reduced senescence markers in a mouse model of p16-driven accelerated aging, but importantly postponed multiple degenerative aging phenotypes in these animals.[115] This success was confirmed in normally aging mice using two similar approaches with different suicide gene constructs.[128,129] In parallel, drugs were developed that targeted antiapoptotic pathways which are specifically active in senescence, thus achieving preferential induction of apoptosis in (at least some types of) senescent cells.[21,130–134] Around a dozen or so of these senolytic drugs have been described,[135] of which the combination of the tyrosine kinase-inhibitor, dasatinib with the flavonoid quercetin (D + Q), the BCL2 family-inhibitor ABT263 (navitoclax) and the flavonoid fisetin have been tested in multiple applications. Altogether, this was a remarkable success story: by early 2019, there were at least 20 publications all reporting significant improvements in a wide range

of age-associated degenerative conditions by pharmacogenetic and/or pharmacologic senolytis in mice. These conditions included muscle weakness, cataracts, lipodystrophy, cardiovascular dysfunction and low stress tolerance, tumor incidence, atherosclerosis, pulmonary fibrosis, liver steatosis, osteoarthritis and osteoporosis, chemotherapy-induced multimorbidity, frailty, neurodegeneration, obesity-induced anxiety and median lifespan.[26] Thus, even allowing for publication bias and taking major unresolved questions in the senolytics field into account, these results collectively very strongly supported the notion that cellular senescence is a major and malleable driver of mammalian aging in many, if not all tissues and organs.

Mechanistically, there are multiple pathways by which a reduction of senescent cell load may improve systemic and tissue function. Many studies have shown that senolytic interventions reduce markers of systemic inflammation in the blood. Chronic, systemic inflammation has long been recognized as a driver of aging.[136] More evidence for systemic effects comes from experiments in which senescent cells were implanted in a single tissue (fat), leading to functional deterioration in a distant tissue (muscle weakness).[126] However, unambiguous identification of a mechanistic pathogenetic role of senescence-dependent inflammation was not often successful. For instance, senolytic interventions in obese mice reduced levels of multiple pro-inflammatory cytokines and chemokines in blood and relieved obesity-dependent anxiety. However, manipulating systemic cytokine levels independent of senescence did not change anxiety.[43] SASP factors including pro-inflammatory cytokines might however be pathogenetically important mediators of more local cell-cell interactions. For instance, in the obesity-driven anxiety model, senescence was observed in certain brain areas including glial cells in proximity to the lateral ventricle, an area responsible for neurogenesis, but not in lateral ventricle neurons themselves. Neurogenesis was reduced in obesity but restored following senescent cell ablation,[43] pointing to the importance of local interactions between senescent and non-senescent cells. Moreover, in a transgenic mouse model for human tau-mediated neurodegenerative disease, progressive senescence was observed in astrocytes and microglia but not in neurons. However, senolytic interventions improved the capability of neurons to handle mutated tau and neurofibrillary tangle deposition and maintained cognitive function, indicating the importance of cross-talk between neurons and senescent non-neuronal cells.[137] However, a direct role for neuron senescence in tau-mediated neurodegeneration should not be excluded. Neurons from Alzheimer patients bearing neurofibrillary tangles as well as neurons from tau transgenic mice were found positive for senescence markers, and treatment with senolytics (D + Q) resulted in a reduction in total NFT density, neuron loss, and ventricular enlargement.[116]

Senolytic interventions can also improve energy metabolism by reducing SAMD. For various types of senescent cells including fibroblasts, hepatocytes and brain cells[40,43] a reduced ability to metabolize fat is an important part of the SAMD,

resulting in intracellular lipid accumulation. This way, hepatocyte senescence can cell-autonomously cause liver steatosis. Moreover, lipid accumulation in senescent cells might be sufficient to induce a SASP via GAS-STING activation, which might mediate local pathogenetic cell-cell interactions.[43] In general, the role of these different mechanistic pathways and their interaction during senolytic intervention is not well understood. Cell-type specific transgenic approaches are eagerly awaited for better clarification.

Another area of insufficient understanding is the question of specificity of senolytics. Most senolytic drugs described so far have only been tested in few cell types. As testament to the complexity of the senescent phenotype and the variability in the engagement of individual senescence building blocks and pathways between cells of different origin, senolytics typically show a clear differential in their apoptotic efficiency between senescent and non-senescent cells for one or few cell types, but not in others. Although senolytics have broad beneficial effects throughout many different organ systems, this is not sufficiently informative regarding their specificity. While the possibility of cell-autonomous effects has been shown in some studies (for instance[40]), a significant impact for systemic effects has so far neither been proven nor ruled out. It still remains possible that many beneficial effects of senolytics may be mediated through the reduction of senescence in a single major tissue like fat or muscle, which in turn then improves function in the target tissue(s) via reducing systemic inflammation.

Related to the issue of specificity is the question of side effects of senolytics. Two different types of side effects need to be considered, resulting from the effects of the drugs on either non-senescent or senescent cells. Side effects of the cancer drugs navitoclax and dasatinib on non-senescent cells have been well documented and are dose-limiting in cancer therapy. They include anemia and thrombocytopenia and, for dasatinib, pulmonary edema and heart failure. It is assumed that these risks might be minimized by short-term treatment, which should be sufficient for long-term senescent cell ablation. However, more data are needed to establish the long-term efficiency of short senolytic treatments in a pre-clinical setting. While there are data indicating that a single dose of a senolytic can result in long-lasting physical improvement, these data were generated in mice transplanted with senescent cells[126] and strong evidence for a curative effect of a single course of senolytic intervention is still missing.

Even less is known about the second potential type of side effects arising from ablation of senescent cells. Senescence plays an important role for tissue remodeling in development and regeneration,[22–24] especially for the resolution of the fibrotic response.[138,139] Accordingly, it has been shown that acute ablation of senescent cells postponed wound healing,[138] but thus far, no other 'negative' effects of senescent cell ablation seem to have been noted.

In summary, the impressive efficiency of senolytic interventions to relieve a wide range of age-related disabilities and degenerative conditions calls for major efforts to

address the issues of specificity, mechanism of action and side effects preclinically and to translate the results into clinical trials. First clinical trials are under way, and encouraging results have been reported.

12.4.2 Senostatics

Senostatics (sometimes also called senomorphics) are interventions that do not directly kill senescent cells but suppress the senescent phenotype by modifying one or more of the buildings blocks shown in Fig. 12.2. Ideally, they should not interfere with the DDR and leave the senescent growth arrest and thus the tumor suppressor function of senescence intact. Accordingly, typical senostatics will suppress nutrient signaling, improve auto/mitophagy and/or mitochondrial function. Thus, dietary restriction[41,140] and dietary restriction mimetics including mTORC1 inhibitors like rapamycin[68,141] or Torin-1,[78] mild mitochondrial uncouplers like metformin[64] and 2,4-dinitrophenol[61] or monoamine oxidase-A inhibitors[97] are potential or proven senostatics. Moreover, antioxidants or inhibitors of NF-κB can be efficient senostatics,[69,123] and there is evidence that multiple flavonoids, polyphenols and other phytochemicals may have senostatic activity.[142,143] Significant beneficial effects on health span in mice[144–147] and humans[148,149] have been demonstrated, however, it is not clear to what extent these were caused by their impact on senescence.

Cell-autonomous effects of senostatics on the senescent phenotype are reversible. Therefore, it is generally assumed that senostatics would have to be given continuously to have lasting effects. However, this might not be true: short-term interventions with, for instance, dietary restriction[150,151] or rapamycin[152] have resulted in long-term beneficial health span effects. There is an important difference between the effects of senostatic interventions in vitro and in vivo. None of these interventions ablates senescent cells in in vitro assays. However, short-term (2 to 4 months) treatment of mice with either rapamycin,[153] metformin (Miwa et al., in prep) or dietary restriction[40,41,140] decreased frequencies of cells positive for multiple senescence markers below the levels measured before the intervention. This was dependent on intact immunosurveillance: in contrast to wild-type mice, dietary restriction of severely immunocompromised NSD mice resulted only in a slowing of accumulation of senescent hepatocytes in the liver, but not an actual decrease of their numbers.[41] Importantly, reduction of senescent cell frequencies under dietary restriction remained irreversible (at least for three months) when animals were returned to ad libitum feeding.[40] This suggested that senostatic drugs might actually exert a net senolytic effect in immunocompetent hosts. By suppressing the senescent phenotype, they might shift the balance between senescent cell biogenesis via the bystander effect and destruction via immunosurveillance towards the latter.[41] If this is true, one might expect similar beneficial effects of relatively short senostatic interventions as with senolytics.

This would be advantageous given the, on average, better safety profiles of senostatic drugs. However, essential experiments are still lacking; the persistence of reduced frequencies of senescent cells after short or medium term senostatic interventions other than dietary restriction has not been shown yet.

12.5 Conclusion

Senescence is a complex stress response phenotype involving a complete reprogramming of core cellular functions. Senescent cells accumulate with age in the vast majority of tissues including slowly dividing and postmitotic cell types. They are among the causes for many age-associated diseases and disabilities. Specific ablation of senescent cells or suppression of their phenotype, and thus suppression of the signaling pathways that spread senescence inter-cellularly, can postpone and potentially relieve age-associated multi-morbidity and frailty.

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CHAPTER 13

The epigenetics of aging

Athanasios Metaxakis[1], **Ilias Gkikas**[1,2] **and Nektarios Tavernarakis**[1,3]
[1]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Greece
[2]Department of Biology, University of Crete, Heraklion, Greece
[3]Department of Basic Sciences, Faculty of Medicine, University of Crete, Heraklion, Greece

13.1 Introduction

After three decades of extensive research on the molecular biology of aging, we can now specify several molecular and cellular processes that accelerate or delay aging in animal model systems. Molecular mechanisms that affect metabolism, caloric and dietary consumption, genomic stability, telomere attrition, autophagy and epigenetic alterations are the major anti-aging interventions shown so far to extend longevity, increase healthspan and delay the onset of age-related pathologies in animals.[1] Among them, the most complicated and least investigated are the epigenetic alterations that progress with aging. These are described as reversible alterations of chromatin that are heritable, but do not affect underlying DNA sequences and, consequently, permanent genetic information. Several causes, such as diet, genes, environmental and lifestyle factors influence epigenetic alterations, which, together with genetic information, preordain lifespan in animals. Consequently, differential epigenetic regulation of genetic information can sufficiently explain differences in longevity of identical twins and animals with the same genetic background. Several studies have shown that epigenetic information changes through aging and, most importantly, that these changes are associated with age-related progressive physiological deterioration and the development of age-related diseases, such as cancer, neurodegeneration and cardiovascular diseases.[2–4] Although the mechanisms underlying both the impact of aging on epigenetic alterations and of the latest age-related physiological decline are not fully understood, research on epigenetic phenomena that occur with age can provide novel anti-aging therapeutic approaches. This is due to the reversibility of epigenetic changes via the administration of drugs that correct these chemical modifications on proteins and nucleic acids.

Epigenetic alterations include various modifications of chromatin components, histones and DNA. These changes are described as the "epigenome" and can be even passed down to the offspring and impact their health in a transgenerational manner.[5] Histone modifications, chromatin remodeling, DNA methylation and altered expression of non-coding RNA molecules (ncRNAs) constitute epigenetic alterations.

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Through altering chromatin accessibility and genomic activity, the epigenome imposes various effects on cellular function. Chromatin activity, level of protein expression, the activity of transposable elements, integrity of telomeres and the stability of the genome have been suggested to mediate the effects of the epigenome on health and lifespan.

In this review, we will briefly describe the nature of the major epigenetic alterations and their relevance to longevity determination. Furthermore, we will present the primary findings that correlate epigenetic changes with the development of the major age-related diseases, cancer, neurodegenerative and cardiovascular diseases.

13.2 Epigenetic alterations and aging

Genetic activity is largely dependent on the accessibility of transcription factors to DNA. DNA is tightly bound by histone proteins, to compose chromatin. Depending on its flexibility, chromatin can be found in either two forms, euchromatin and heterochromatin. The former consists of a decondensed, highly transcriptable structure, while in the latter, the strong DNA-histone binding does not allow transcription factors to access DNA and ignite transcription. Consequently, genetic and environmental factors that alter chromatin tightness can affect transcription activity.[6] This is achievable through quantitative changes in the expression of histone proteins, expression of histone variants, histone post-translational modifications, such as acetylation and methylation, ATP-dependent remodeling, and DNA methylation. These modifications affect longevity via deregulation of genetic activity and genomic stability. Moreover, altered expression of ncRNAs has a regulatory role on protein translation.

13.2.1 Histone depletion

Several studies show that canonical histone levels are reduced through aging, while ectopic upregulation of histone biosynthesis increases lifespan.[7–11] Age-related changes in telomeres, histone chaperones, and lysosomal activity are suggested to cause histone depletion.[9,10] Strong evidence suggests that the effect of histone depletion on lifespan depends on the nature of the depleted histonic genetic area, and also on the degree of the depletion.[10,11] On the other hand, in mouse tissues and neural stem cells, the expression levels of H3 histone is not significantly changed with aging, but depending on the genomic location, the occupancy of H3 histone is differently affected.[12] As a result, chromatin at pro-inflammatory genes is more accessible and active; an observation that suggests differential nucleosome occupancy as a mechanism for reprogramming genetic expression through aging.

13.2.2 Non-canonical histone variants

With the exception of gradual histone depletion with age, non-canonical histone isoforms are increasingly expressed with age, such as the histone variants H3.3 and H2A.Z, accompanied by the downregulation of canonical histones.[13–17] For example, the H2A histone variant, H2A.J, accumulates with aging in mouse tissues and human skin.[18] H2A.J overexpression activates inflammatory genes, induces senescent-associated phenotypes and is suspected to contribute to the development of age-related chronic inflammation and diseases. Hence, not only quantitative, but also qualitative age-dependent alterations in histone expression affect healthspan.

13.2.3 Histone acetylation

A major chemical modification that alters the histone-DNA binding strength is acetylation of histonic lysine domains. The positively charged lysine domains significantly contribute to the attachment of histones on DNA. As a result, any chemical change that reduces the positive charge of lysine, weakens the interaction between histones and DNA. Such a chemical modification is the addition of acetyl moiety on the ε-amino groups of lysine, which neutralizes its positive charge and reduces histone-DNA interactions. Transfer of acetyl moiety is catalyzed by histone acetyltransferases (HATs), while deacetylation is catalyzed by histone deacetylases (HDACs). Activity of the HATs loosens histone-DNA interactions and increases transcription, while activity of HDACs has the opposite effect. Several reports indicate the importance of histone acetylation on longevity. Loss of HATs Gcn5, CREB-binding protein (CBP), and RTT109 in yeast, *Caenorhabditis elegans* and *Drosophila melanogaster* reduces longevity, while loss of members of the *sirtuin* genes, coding for evolutionary conserved NAD^+-dependent deacetylases, are associated with longevity extension in invertebrates and vertebrates.[19–25] CBP activity is reduced through aging and correlated with lifespan in several mice strains. In support, lifespan extension by dietary restriction (DR) in *C. elegans* is inhibited by the loss of the *cbp-1* gene, thus linking DR-induced longevity enhancement with increased acetylation. In addition, loss of acetyltransferase Gcn5 in yeast decreases lifespan through impeding interplay of metabolism and stress responses, chromatin-dependent gene regulation and genome stability. Contrarily, downregulation of the histone H4K12-specific acetyltransferase Chameau extends longevity in flies, through uncoupling age-related metabolic alterations from transcriptional regulation.[26]

Maybe the most remarkable examples that highlight the importance of histone acetylation on longevity determination come from studies on the activity of the Sirtuin deacetylases.[27] Sirtuins are involved in the regulation of cell metabolism, DNA repair, inflammation and apoptosis.[28] In yeast, deletion of the histone deacetylase gene *rpd3* and upregulation of the *Sir2* gene, which is activated by caloric restriction, extend lifespan.[27]

Similarly, downregulation of histone acetyltransferase Sas2 increases lifespan in yeast.[29] Similar effects have been described in worms, flies, mice and cells, thus showing that these findings are evolutionarily conserved.[19,20,22–24,30–32] Mechanistically, Sir2 maintains chromatin silencing through deacetylation of the residues H4K16 and H4K56 and recruitment of other silencing proteins. Sir2 protein levels decrease with aging, while H4K16 acetylation increases and histone abundance diminishes at subtelomeric regions. The above are suggestive for an abnormal upregulation of transcription at these loci, which is associated with the development of aging phenotypes.[29,33]

Some histone acetylation sites have been reported to be more important for lifespan determination, such as the H4K16. Sas2 targets H4K16 sites at the boundaries of euchromatin with telomeric regions and H4K16 hypoacetylation has been associated with defective DNA repair and premature senescence in mice.[34–36] Lifespan extension in flies via Chameau downregulation has been attributed to H4K12 hypoacetylation; deficiency of SIRT6 deacetylase promotes aging in mice via altered acetylation at H3K9 and H3K56, which cause telomeres dysfunction. H3K56 acetylation levels are critical for longevity in yeast, as in H4 N-terminal acetylation, which is regulated by caloric restriction.[7,23,26,29,37–39]

13.2.4 Histone methylation

Another type of histone modification that occurs with aging is histone methylation (HMT). Similar to histone acetylation, HMT is catalyzed by the addition of a methyl group by histone methyltransferases, while removal of methyl groups is catalyzed by histone demethylases. Depending on the histonic site, methylation can lead to enhanced or reduced transcription.[40] According to the heterochromatin loss model, transcriptionally inactive areas of chromatin become activated through aging, resulting in disparate profiles of gene activity and promoting aging.[41–44] Highly methylated histonic sites, such as H3K9, H4K20 and H3K64, are associated with transcriptional inactivity of heterochromatin.[45–47] Tight interconnection of histone hypomethylation and aging phenotypes is further supported by research in premature aging diseases. Patients with progeria syndromes have decreased expression of histone methyltransferases, reduced methylation at H3K9 and H3K27, loss of heterochromatin and changes in heterochromatin architecture.[44,48] Furthermore, mild mitochondria damage in *C. elegans* and mice induces activity of histone demethylases jmjd-1.2/PHF8 and jmjd-3.1/JMJD3, which delay aging through mitochondrial unfolded protein response (UPRmt).[49] On the other hand, in a mouse progeria model, inhibition of methyltransferase gene Suv39h1 improved DNA repair and increased longevity.[50]

Recent studies suggest a role for specific methylation patterns on longevity. In worms, trimethylation of H3K4 (H3K4me3) increases with aging. Reduction of the ASH-2 Trithorax complex proteins, which activate transcription by inducing

trimethylation of H3K4, decreases H3K4me3 and increases lifespan, while reduction of H3K4 demethylase RBR-2 decreases lifespan.[40,51] Similar results have been observed after downregulation of the ortholog of RBR-2 in flies, the demethylase Lid.[52] Inhibition of another demethylase in flies, the Dmel\Kdm4A H3K9me3 demethylase, reduces lifespan.[53] Trimethylation of H3K9 is abundant in heterochromatin, thus suggesting that alterations in the transcriptional activity of heterochromatin affect lifespan. In support, expression of H3K9me3 methyltransferase SUV39H1 is reduced through aging in mouse and human cells, which causes the reduction of H3K9me3 trimethylation, perturbs heterochromatin function and induces loss of B cell potential.[54] Trimethylation of H3K27 is increased with age and catalyzed by the transcription repressor Polycomb Repressive Complex-2 (PRC2).[55,56] Mutations in subunits of PRC2 in flies reduce H3K27me3, by increasing glycolysis and healthspan.[57,58] On the other hand, in human cells and *C. elegans*, trimethylation of H3K27 is reduced with age.[59–61] Reduction of the UTX-1 H3K27 demethylase in *C. elegans* extends lifespan by affecting the insulin pathway.[59] Accordingly, the link between H3K27me3 and aging is complex and cell type and/or animal model specific. Another methylation site, H3K36, is highly methylated proximally to the 3' end of actively transcribed genes, which is suggestive for a role in transcriptional termination and RNA processing.[62] Loss of H3K36 methyltransferase and mutations at the H3K36 site decrease lifespan in yeast, while loss of the Rph1 H3K36 demethylase increases H3K36me3 and enhances longevity.[63] In this study, the authors concluded that increased methylation at H3K36 suppresses cryptic transcript initiation and promotes longevity through recovering transcriptional fidelity in old yeast. The role of H3K36 methylation in the maintenance of transcriptional stability and longevity is presumably evolutionary conserved, since low levels of H3K36me3 are associated with altered length of 3′ untranslated regions (3′UTR) and shortened lifespan in worms and flies.[64,65]

Histone acetylation and methylation comprise the major and better described histone modifications. However, histones can be also modified through phosphorylation, ubiquitination and sumoylation. Although the biological importance of these modifications on cellular homeostasis and longevity are not yet elucidated, several reports suggest a modulatory role for histone phosphorylation on transcription regulation, DNA repair and chromatin compaction.[66] Ubiquitination is involved in transcription activity, inflammation signaling and HMT.[67] Sumoylation is involved in inflammation signaling and the epithelial–mesenchymal transition, which is related to cancer progression.[68]

13.2.5 ATP-dependent chromatin remodeling

The above described chemical histone modifications alter chromatin compactness and regulate transcriptional activity. Often these modifications function in concert with, or through activation of ATP-dependent chromatin remodeling factors to alter the

nucleosomes positions along DNA and modulate its accessibility to transcription factors and DNA replication machinery components.[69,70] For example, acetylation of the histone H3 N-terminal tail recruits and increases the affinity of the ATP-dependent chromatin remodelers SWI/SNF and RSC, which leads to nucleosome mobilization and chromatin remodeling.[71] The major groups of ATP-dependent chromatin-remodeling enzymes are the SWI/SNF, ISWI, Nurd/Mi/CHD, SWR1 and INO80, and recent studies prove their importance for lifespan determination.[72,73] In worms, chromatin remodeler SWI/SNF activates transcription at specific promoters in collaboration with the longevity promoting DAF-16/FOXO transcription factor. Inactivation of SWI/SNF decreases longevity and DAF-16/FOXO-mediated stress responses.[74] Moreover, loss of LET-418/Mi2, the catalytic subunit of the nucleosome remodeling and histone deacetylase complex (NuRD), increases longevity and environmental stress resistance in *C. elegans*, *Drosophila* and *Arabidopsis*.[75] In yeast, deletion of Isw2 increases response to genotoxic stress and extends yeast replicative lifespan, while deletion of components of the ortholog chromatin-remodeling complex in worms also extends lifespan.[76] PRC2, which is able to remodel chromatin and silence genes, has been implicated in the transcriptional dysregulation that the progeria primary fibroblasts exhibit.[77] These findings provide strong evidence for the evolutionarily-conserved role of ATP-dependent chromatin remodeling in facilitating stress responses and aging.

13.2.6 DNA methylation

Histone modifications are the primary targets of factors that affect epigenetics, such as diet, metabolism, environmental pollutants, drugs, etc. Their binding on DNA protects it from chemical modifications and restricts the accessibility of transcription factors. Nevertheless, epigenetic factors can directly chemically modify DNA, via methylation at cytosine residues which are mainly placed 5′ of guanine (CpG dinucleotide), located predominantly at intergenic, intronic and repetitive sequences. The latest are often generated by transposable elements and the increased methylation they exhibit might be related to the necessity of cells to inactivate such mobile DNA sequences and avoid genomic instability.[78] On the other hand, hypomethylated CpG dinucleotides are frequently located at promoters and first exons of the majority of genes (CpG islands). Transfer of a methyl group to cytosine is catalyzed by DNA methyltransferases (DNMTs), thus generating 5-methylcytosine (5mC). When DNA methylation occurs in promoters, it leads to transcriptional repression and causes gene silencing.[79] Although DNA methylation levels during the first years of life are similar between monozygotic twins, significant tissue-specific differences on DNA methylation appear with age, starting from childhood (epigenetic drift).[80–82] In animals and humans, a reduction of DNA methylation occurs with age both globally and tissue-specifically.[83–87] Methylation patterns of CD4 + T cells from newborn and centenarian individuals showed that DNA

methylation levels decrease with age. Likewise, CpGs dinucleotides are less methylated throughout the genome of centenarians, which is characterized by highly heterogeneous DNA methylation.[88] Age-dependent changes in DNA hypomethylation can lead to pathologies, through aberrant transcription. Progressive DNA hypomethylation at specific gene promoters has been implicated in the development of autoimmune responses.[89,90] On the other hand, age-related hypermethylation in promoters of genes that code for transcription and translation regulating factors can severely impact various cellular functions.[91–93] Contrarily, epigenomic analysis of pancreatic β cells revealed age-related differences in methylation patterns that were associated with the repression of proliferation and activation of metabolic regulators. B cell function was improved in old mice, suggesting that epigenetic alterations through aging do not necessarily lead to pathologies and physiological decline.[94]

Age-related changes in DNA methylation can be attributed to altered expressions of methyltransferases, demethylases and environmental factors. The importance of inadequate DNA methylation on health and lifespan has been clearly proven in animal models. In flies, functional dDnmt2, the gene expressing for DNA methyltransferase, is required for the maintenance of the normal lifespan of fruit flies, while its upregulation extends lifespan.[95] Enhanced DNMT2-induced longevity is achieved via retrotransposons silencing in *Drosophila* somatic cells and maintenance of telomeres' integrity.[96] In support of a beneficial role for DNA methyltransferases on health and longevity, mice with mutations in the gene coding for DNA methyltransferase 1 (Dnmt1) have decreased DNA methylation, decreased bone mineral density and body weight, impaired learning and memory functions in an age-dependent manner, but with canonical survival.[97] Additionally, mutations in the DNA methyltransferase 3 gene (Dnmt3a) cause premature neurodegeneration and death.[98] In honey bees, pharmacological demethylation enhances lifespan.[99] In mice and monkeys, age-related methylation drift was found to be associated with longevity, while caloric restriction diminished age-related methylation drift.[100] Furthermore, DNA methylation is implicated in transgenerational effects that regulate lifespan in offspring. In mice, old father offspring mice lived less and experienced stronger aging phenotypes compared to young father offspring mice. Genome-wide epigenetic analyses revealed differentially methylated promoters of genes expressing components of the lifespan regulator mTORC1 signaling pathway.[101] Interestingly, DNA methylation seems to cooperate with other epigenetic alterations, such as HMT, to regulate transcriptional activity at specific genomic areas, thus suggesting a strong interconnection between different epigenetic modifications.[102]

DNA methylation patterns at CpGs have been associated with aging and diseases such as cancer, obesity, and cardiovascular disease.[103–110] Clinical epigenetics aims to decipher such patterns and use them to predict the biological age of individuals, improving diagnostics and therapies.[111,112] The epigenome is formed by the co-action of genes, age, environmental factors and lifestyle. Hence, epigenetic profiles are very

informative regarding the depiction of the health status of an organism.[113] This has challenged the design of supervised machine learning approaches to analyze epigenetic profiles, with several studies having used machine learning to diagnose diseases.[114] There is a great deal of progress in the development of "epigenetic clocks," aging biomarkers made of DNA methylation profiles, which enable accurate age estimates.[115] However, only a few DNA methylation patterns of CpG sites can allow precise age prediction[116] and more studies are required to further advance this approach.

13.2.7 Non-coding RNA molecules

Non-coding RNA molecules are small or long RNAs that, despite not having a code for proteins, they regulate cellular function. They are classified into transfer RNAs, ribosomal RNAs, microRNAs (miRNAs), small interfering RNAs, piwi-interacting RNAs, small nucleolar RNAs (snoRNAs), small Cajal body-specific RNAs, extracellular RNAs and long non-coding RNAs (lncRNAs). Through their regulatory role on gene silencing, ncRNAs, especially miRNAs and lncRNAs, exert various effects on chromatin architecture, cell cycle, metabolism, etc., and their dysregulation is relevant to the progression of cellular senescence, cancer, cardiovascular, neuronal and immune pathologies.[117] In yeast, lifespan maintenance is regulated by the repression of rDNA non-coding transcription, which is achieved through Sir2. Mutations that reduce ncRNAs expression extend lifespan.[118] In human stem cells, ncRNAs expression from Alu sequences increases with age and causes senescence. Knockdown of ncRNAs expression reverses this effect.[119]

MiRNAs play a crucial role on cellular senescence and aging.[120] Through binding with the 3′UTR sequence of mRNA molecules, they inhibit translation and negatively modulate gene function. They cause heritable changes without directly altering the DNA sequence or chromatin structure, and their expression is differentially regulated through aging in mice and humans.[121–124] An essential pathway for health and lifespan determination, regulated by miRNAs, is the insulin pathway.[125] In *C. elegans*, several miRNAs regulate longevity and stress responses.[126,127] The miRNAs *lin-4* and *lin-14* have opposite roles on longevity, with *lin-14* serving as the target for *lin-4*. Reduction in *lin-14* activity is dependent on the DAF-16 and HSF-1 transcription factors, which are the mediators of the insulin pathway effects on healthspan.[128] In flies, a well-established longevity promoting intervention, caloric restriction (CR), is shown to alter expression of more than 100 lncRNAs, which serve as mediators of CR on healthspan.[129] In mice, the H19 lncRNA participates in a complex that interacts with histone lysine methyltransferases and facilitates repression of several genes, among which is the Igf2 (insulin-like growth factor 2).[130] Loss of this regulation occurs through aging in mice and the human prostate.[131] Hence, ncRNAs can regulate longevity via interfering with well-established metabolic pathways. Furthermore, ncRNAs are

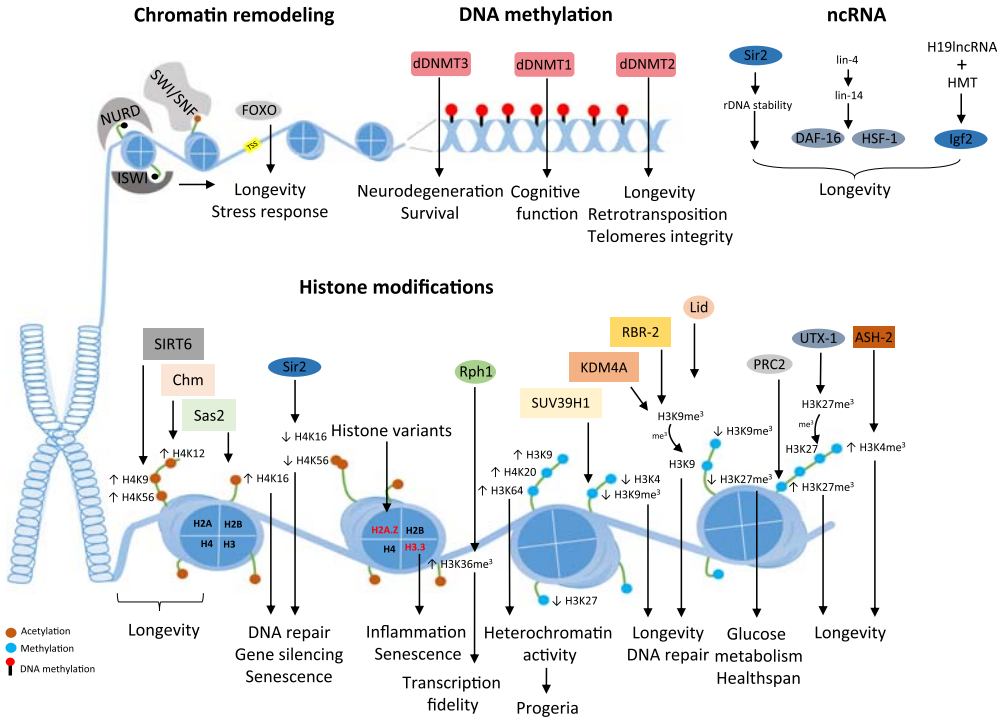

Figure 13.1 Epigenetic alterations that regulate health and lifespan.

also involved in forming the boundaries of heterochromatin.[132] The major epigenetic changes that affect health- and lifespan are summarized in Fig. 13.1.

13.3 Epigenetic alterations and age-related diseases

13.3.1 Cancer and epigenetics

Several studies show similarities between epigenetic changes that occur with aging and in cancer development.[68] Several histone modifications are prevalent in distinct cancer types. Hypoacetylation at H2BK12 is prevalent in osteosarcoma, hyperacetylated histone H3 is common in colorectal cancer, extensive hypoacetylation at H3K4 and H3K9 accompanies oral squamous cell carcinoma and ovarian tumors, and invasive colon cancer and glioma are characterized by upregulated H3K27ac, which has been shown to induce lncRNAs secretion in colon cancer cells.[133–137] Acetylation of lysine residues at histone 4 is also correlated with cancer. Acetylated H4K16, as also H4K20me3 are downregulated in breast, renal, colon and ovarian cancer, while acetylated H3K18 and H3K4me2 are upregulated in prostate, pancreatic, lung and kidney cancers.[138–145] Several other examples establish a causative relation between epigenetic alterations and

cancer. Demethylation of H3K9 has been associated with derepression of genes involved in breast and esophageal cancers.[146,147] P300 and CBP HATs suppress tumors and several cancers are characterized by their dysfunction.[148] Moreover, deacetylation of several non-histone proteins, including p53 and STAT3 transcriptional activator, is associated with cancer.[149] Expression of the polycomb group protein enhancer of zeste homolog 2 (EZH2) is higher in metastatic prostate cancer, while its downregulation inhibits cell proliferation in vitro. EZH2 regulates hypermethylation at H3K27 and represses gene activity in prostate cells, an effect that is mediated through histone deacetylase activity.[150] EZH2 is a marker for breast cancer and glioblastoma.[151,152] Several studies suggest a role for enhanced secretion of exosomes carrying lncRNAs in cancer development, through mediating intercellular communication in tumor microenvironments.[153] Interestingly, 25% of all cancers harbor mutations in genes encoding subunits of the SWI/SNF complexes. Novel findings support an anti-cancer role for SWI/SNF via repressing transcription and the facilitation of DNA damage repair.[154] Moreover, mutations in the genes that encode H1 isoforms B−E are causative to the development of B cell lymphomas, through inducing chromatin relaxation, upregulation of H3K36me2 and loss of repressive H3K27me3, which leads to derepression of developmentally-silenced genes.[155]

Recent findings suggest a role for miRNAs in cancer development. MiR-205 regulates differentiation and morphogenesis in epithelial cells and its aberrant expression is frequently detected in human cancers. Depending on the tumor type, it has been suggested to act as tumor-suppressor or as oncogene.[156] MiR-34a is shown to repress tumor progression through synergizing with p53 and transcription factors, via inhibition of the transition from epithelial cells to mesenchymal cells.[157] Also, a significant association between the expression of miR-181 and miR-200 family members and colorectal cancer has been observed.[158] Members of the miR-181 family have been suggested to perform their anti-cancerous function through downregulation of the hepatic transcriptional regulators, CDX2 and GATA6, and the Wnt signaling inhibitor NLK.[159] On the other hand, overexpression of miR-145 is shown to be carcinogenic, through altering methylation patterns and reducing activity of genes that regulate DNA damage response and apoptosis, consequently leading to overproliferation and enhanced epithelial to mesenchymal cells transition.[160]

Aging is a risk factor for cancer development and the retrotransposition is upregulated with aging, as in cancers, thus raising the possibility for a role of epigenetic drift on cancer development via age-related enhanced retrotransposition.[8,161,162] In yeast, age-related histone loss leads to increased retrotransposition, which causes genomic instability and disruption of cellular homeostasis, an age-related event which can be reverted via CR in mice.[8,163] Hypomethylation at repetitive regions such as Alu and long interspersed element-1 increases genomic instability and is associated with cancer.[164,165] On the other hand, cancer is induced by CpG dinucleotides hypermethylation at promoters of tumor suppressors and esophageal cells of individuals with a long smoking history and high methylation levels.[166−168] Moreover, carcinogenic factors

such as chronic inflammation, *Helicobacter pylori* and hepatitis B or C infections, as also with alcoholism, induce aberrant DNA methylation, which forms tissue- and carcinogenic factors-specific patterning and specificity.[137,169–174] Interestingly, the methylation degree can be indicative of exposure to carcinogens.[175]

13.3.2 Neuronal diseases and epigenetics

Epigenetic changes comprise a molecular link between aging and neurodegeneration, with etiology and symptomatology of neurodegeneration being, in many cases, linked to epigenetic effects.[176] Increased retrotransposition has been associated with neurodegeneration and reduced levels of DNA methyltransferases is a common feature in aging, Alzheimer's disease (AD) and Parkinson's diseases (PD).[177–179] In support, a deficiency in 5-hydroxymethylcytosine was found in a mouse model of Huntington's disease (HD).[180] On the other hand, several studies demonstrated increased DNA methylation in post-mortem tissues from cohorts of patients with AD.[181,182] Histone acetylation at the repetitive DNA sequences decreases with age in mice brains and altered histone acetylation has a causative role on age-dependent memory impairment.[183,184] Histone acetylation at certain residues is high in memory regulating brain areas, such as the hippocampus, with these residues being frequently affected in neurodegeneration.[185] Hypomethylation at neuronal enhancers in patients with AD is related to synapse degeneration.[186] Reduced PRC2 activity causes the upregulation of genes activated in HD and of genes that are known to induce neuronal cell death and neurodegeneration.[187]

MiRNAs also play various roles on neuroprotection and neurodegeneration, via non-elucidated mechanisms, with their concentration being dramatically decreased with age in the human brain.[188,189] In flies, expression of miR-34 is altered through aging and its loss causes brain degeneration and lifespan reduction. Its upregulation extends lifespan and inhibits human pathogenic polyglutamine disease protein-induced neurodegeneration. This is partially mediated via translation inhibition of Eip74EF.[190] On the other hand, samples from humans with AD and from mice with modeled AD, have different patterns of miRNAs expression compared to controls, as also with elevated levels of miR-34.[191,192] MiR-34 targets and decreases pro-survival factor Bcl2 and antiaging deacetylase SIRT1 and is suspected to play a causative role on neurodegeneration onset.[121,193] Levels of lncRNAs have been correlated with the expression of mutant alpha synuclein in presymptomatic PD.[194] Several lncRNA molecules are dysregulated in brains of patients with HD. Some of these have been suggested to target the neuroprotective transcriptional repressor, REST, a key mediator of transcriptional changes in neurodegenerative diseases.[195,196] Levels of another ncRNA, the miR-181c is decreased in the brains of AD patients, while its loss increases the levels of the amyloid precursor protein (Aβ).[197] The major epigenetic alterations that are involved in the development of cancer and neurodegeneration are depicted in Fig. 13.2.

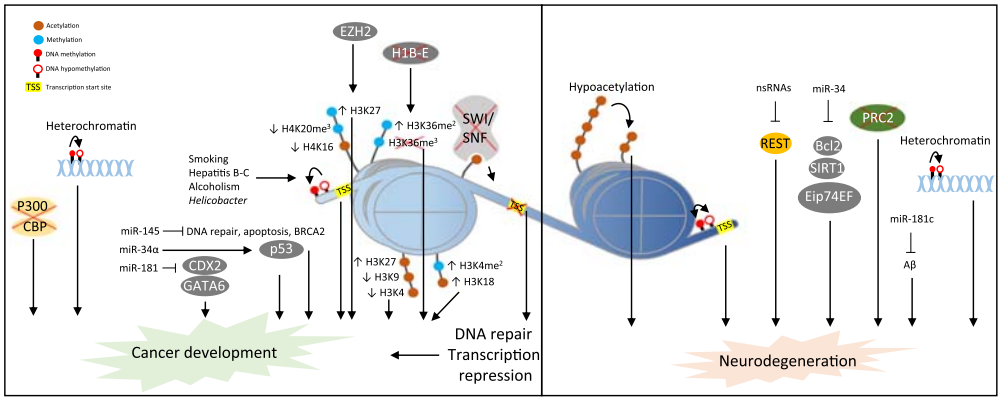

Figure 13.2 Epigenetic alterations implicated in cancer and neurodegeneration development.

13.3.3 Cardiovascular disease and epigenetics

One of the main risk factors of cardiovascular disease (CVD) is age. Several studies prove the major impact of epigenetic alterations in vascular function and arteriosclerosis, while histone deacetylase inhibitors are promising drugs to treat vascular diseases and arteriosclerosis.[198] When biological age is measured with the Horvath DNA methylation-based method, for each year of additional biological age, the risk for CVD occurrence increases by 4%.[199] Many studies reveal associations between epigenetic alterations through aging and CVD.[200] Hypermethylation of genes coding for superoxide dismutase-2 (SOD2), for histone 3 and for angiotensin I converting enzyme 2 promoter increases the risk of essential hypertension. Reduced global DNA methylation, hypomethylation of H3K79 and hyperacetylation at the promoter of the endothelial oxide synthetase gene (eNOS) are associated with hypertension.[201–203]

Epigenetic changes are also implicated in the development of hypercholesterolemia and atherosclerotic lesions. Patients with dyslipidemia have different methylation profiles in genes regulating mitochondrial function and lipid metabolism. Patients with hypercholesterolemia have hypermethylated promoters in genes that regulate transfer of cholesterol and formation of atherosclerotic lesions is associated with enhanced histone acetylation on H3K9 and H3K27 in the smooth muscle cells, as also altered methylation of several genes.[92,204–209] Methylation status of specific residues, such as H3K9, and the activity of the SWI/SNF chromatin remodeler have been causative to cardiomyocytes pathologies.[210,211] Finally, several ncRNAs are involved in age-related CVD.[212] Although there are not experimental proofs to establish a causative relationship between age-dependent epigenetic changes and CVD, these and several other findings suggest a strong correlation between epigenetic alterations and the development of CVD with age.[213]

With the exception of cancer, neurodegeneration and CVD, increasing evidence suggests that more age-related diseases, such as age-related renal, immune and metabolic diseases are correlated with age-related epigenetic changes.[214,215]

13.4 Conclusions

Age-dependent epigenetic changes constitute a longevity denominator that promotes age−related decline and pathologies. With age, several genetic, environmental and lifestyle agents alter epigenetic identity of individuals, leading to epigenetic drift, which can serve as a biomarker for "biological age" and functions as a regulator of physiology and lifespan, even of next generations. Epigenetic alterations mainly impact transcription regulation and proteins translation, which affect activity of genes involved in healthspan and lifespan determinations, such as genes participating in insulin signaling and responses to diet, genomic stability, telomeres attrition, cellular differentiation, senescence, stress responses and genes that are implicated in the onset and progress of age-dependent diseases. Although the same type of epigenetic alterations can impact cellular homeostasis and longevity in an opposite manner, dependent on the afflicted genomic areas, several studies have attributed epigenetic changes on specific genomic areas to distinct phenotypes and the onset of pathologies. However, research findings suggest that epigenetic alterations do not exclusively lead to pathologies and physiological decline, but they can even be beneficial for age-related physiological adaptations.[94]

Some difficulties impede the elucidation of the role of epigenetic mechanisms on aging and the development of age-related diseases. The same type of epigenetic alterations can have contradictory effects on health, depending on the specific histonic or genomic residue affected. Also, the same residual modifications can have opposite effects on cellular function in different animal model systems, thus making interpretation of research findings in humans' physiology puzzling. Moreover, the strong interconnection between different epigenetic alterations hinders causative relationships between such alterations and specific phenotypes. Nevertheless, in a simplistic, but solid, speculation, age-related epigenetic changes observed in humans possibly impacts aging phenotypes through the same mechanisms that laboratory-induced epigenetic alterations use to modulate cellular physiology in animal model systems.

The importance of clinical epigenetics for human medical treatment lies on the reversibility of epigenetic modifications. Adoption of a certain lifestyle, including increased physical activity, consumption of low-caloric foods and dietary polyphenols, changes in habits such as tobacco smoking and alcohol consumption can reduce the effects of epigenetic drift on physiological decline.[100,216–219] Moreover, a group of chemicals that enhance longevity through altering the epigenome has been described, which can potentially alleviate age-related deterioration and pathologies in humans (Table 13.1). Furthermore, large arsenals of drugs that target specific disease-related epigenetic modifications exist and can potentially confront the development and symptomatology of age-related human diseases[220] In the future, the usage of such drugs, combined with the analysis of the epigenome "fingerprint" of individuals, have the potential to revolutionize the contribution of clinical epigenetics in geriatrics, through improving both diagnostics and treatments of human diseases.

Table 13.1 Drugs and biomolecules related to both longevity regulation and epigenetics alterations.

Epigenetic mechanism	Interventions	Targets	Aging (Chronological or replicative)	Organism	References
DNA methyltransferases (DNMTs)	Decitabine	AID	Extension	*Mus musculus*	221
	Hydralazine	NRF2, PKA/SIRT1	Extension	*Caenorhabditis elegans*	222,223
	RG108	Vg	Extension	*Apis mellifera*	99
	EGCG	AMPK/SIRT1/ FOXO, glucose metabolism	Extension	*C. elegans, Drosophila melanogaster*	224,225
	Curcumin	REDOX signaling	Extension	*C. elegans, Saccharomyces cerevisiae, Rattus rattus*	226–228
	Genistein	SOD-3, HSP-16.2	Inconsistent extension	*C. elegans, M. musculus, D. melanogaster*	229–231
	Ursolic acid	Srl/PGC1α JNK	Extension	*D. melanogaster, C. elegans*	232,233
	Ascorbic acid	NA	Inconsistent extension	*M. musculus, D. melanogaster, C. elegans*	234–236
	Metformin	mTOR/AMPK KDM6A/UTX	Inconsistent extension	*D. melanogaster, C. elegans*	237–239
Histone modification (HDACs, HMTs, HDMs and HATs)	Resveratrol	SIRTs	Inconsistent extension	*C. elegans, D. melanogaster M. musculus*	240–243
	SB	FOXO/DAF-16, NRF2/SKN-1HSPs	Extension	*C. elegans, D. melanogaster, M. musculus*	26,36,244–246
	PBA	NA	Extension	*D. melanogaster,*	247
	TSA	HSP22	Extension	*D. melanogaster, C. elegans,*	248,249
	Quercetin	PaMTH1, MPF	Extension	*M. musculus, Podospora anserina, C. elegans*	250–252
	Spermidine	Autophagy	Extension	*M. musculus, S. cerevisiae, D. melanogaster C. elegans*	253,254
	Rapamycin	mTOR	Inconsistent extension	*M. musculus, D. melanogaster C. elegans*	255–259

EGCG, epigallocatechin-3-gallate; Vg, hemolymp vitellogenin; SB, sodium butyrate; AID, activation-induced cytidine deaminase; PBA, phenylbutyrate; TSA, trichostain A; NA, no information available.

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Conflict of interest

The authors declare no conflict of interest.

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CHAPTER 14

Disrupted cellular quality control mechanisms in aging

Cristina Carvalho[1,2,3], Joana F. Pires[4], Paula I. Moreira[1,3,5] and Nuno Raimundo[4,6]
[1]CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[2]IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal
[3]CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[4]Multidisciplinary Institute of Ageing, University of Coimbra, Coimbra, Portugal
[5]Laboratory of Physiology – Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[6]Penn State University College of Medicine, Department of Cellular and Molecular Physiology, Hershey, PA, United States

14.1 Aging: is it a programmed fate and/or an error accumulation?

Aging is a biological process that affects cells, organs and organisms, mainly characterized by a time-related deterioration of the physiological functions necessary for fertility and survival.[1] Aging affects all the individuals of a species at variable rates and is unavoidable. Therefore, it is important to elucidate the mechanisms underlying this process. When lay audiences refer to aging, they usually refer to senior people and the highlighted features are typically: a decline in motor abilities, cognitive performance and "external" visual signs of age, such as wrinkles. However, aging is much more complex than that and engages the accumulation of detrimental modifications in deoxyribonucleic acid (DNA), proteins and lipids, as well as the appearance of cellular protein aggregates and lysosomal lipofuscin, a specific hallmark of senescent, post-mitotic cells.[2] Generally, this process is accompanied by a progressive decrease in the capacity to properly react to stress conditions, which occurs in association with the homeostatic failure and accumulation of molecular damage.[3]

Until a few decades ago, it was believed that aging was not dependent on adaptive changes and the maximum lifespan was steady and dependent on genetic traits. However, in the 1900s, several studies showed that a maximum lifespan does exist, and it depends on a sequence of factors that ensure an individual's survival to a certain point until biological aging eventually causes death (for further details see[4–6]).

In the last century, scientists carried out a battle against aging, trying to decipher the mechanisms underlying this biological process, and how to harness those findings against some of the more complex age-related human pathologies. So far, a broad spectrum of theories was brought to light as an attempt to explain what aging is. Aging theories can be broadly divided in two main categories: (1) genetic and (2) non-genetic theories. The first group, where we can include the programmed

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longevity, the endocrine and the immunological theories, relies on the hypothesis that lifespan of a cell or organism is genetically predetermined, in a similar way as eye color is determined, and proponents of this theory justify it with several studies showing, for instance, that people with parents who have lived long lives are likely to live long themselves.[7–10] On the other side, the non-genetic theories, or so-called the "damage or error theories," are composed of wear-and-tear, rate of living, reactive oxygen or nitrogen species, and the somatic DNA damage theories, which posit that aging is the result of a breakdown in the control mechanisms that are required in a complex performance.[11–18] These theories hypothesize that aging is the result of an accumulation of damaged molecules and toxic byproducts of recurrent cellular activities, such as cellular respiration.[12,15,17] Nevertheless, based on present knowledge, none of these theories properly or completely explains the complex biological process of aging. Evidence leads us to think that indeed, both types of theories can have some legitimacy and are not necessarily mutually exclusive; they can actually complement each other.[9,19] Significant recent progress in the field of biology of aging focused on the understanding of the cellular and molecular processes underlying aging and on the biological factors associated with extended longevity in humans and animal models. Furthermore, researchers believe that identifying ways to slow down age-related changes will open new venues for the development of new therapeutic interventions aimed at maintaining health quality in the elderly, for as long as possible. Indeed, targeting the aging biological process may bring new insights for a novel and potentially broadly effective treatment against diseases associated with old age, including neurodegenerative diseases, cardiovascular diseases, cancers, and type 2 diabetes mellitus.[20] Despite the recent advances, the truth behind the mysteries of human lifespan remains elusive. However, evidence from the last decades suggests the existence of a convergent route of a plethora of signaling pathways in the main degradative pathways (lysosomal and proteasomal), that seem to exert a pivotal role in the balance between aging and longevity.[21] In fact, the idea that aging is a result of an inevitable accumulation of toxic metabolic waste products or damage caused by them is actually sustained, directly or indirectly, by almost all of the aging theories.[19,22] In this context, in 2009, a stealthy chance brought into the spotlight, a pivotal role for autophagy in the regulation of aging.[23] In the next sections we will discuss autophagy and how it can be the key to decipher the aging process. Moreover, we will give an overview about recent advances in the field of autophagic inducers that can represent the future of the "youth elixir."

14.2 Autophagy: an evolutionarily conserved process

Autophagy, a name derived from the Greek, "eating of self," is a widespread and evolutionary process that was first described in 1967.[24] The name was attributed due to the observation of an autodegradation process of cellular components inside the

lysosomal compartment in eukaryotic cells.[25] It was described that cytoplasm and organelles were encapsulated into bilayer membrane vesicles called autophagosomes, which were transported to vacuoles/lysosomes for decomposition and recycling (Fig. 14.1).[26,27] Further studies demonstrate that autophagy is a process that appeared early during the evolution and homologous genes essential to the autophagic process can be found in all eukaryotic model organisms investigated so far.[28–30]

In the last two decades, the role of autophagy in health and disease was revived and a remarkable understanding of this process was achieved.[31] Indeed, the initial studies by Deter and De Duve showed that autophagy is a general bulk recycling process involving the sequestration and transport of intracellular material to the lysosome for degradation. More recently, a panoply of metabolic and proteostatic signaling roles,

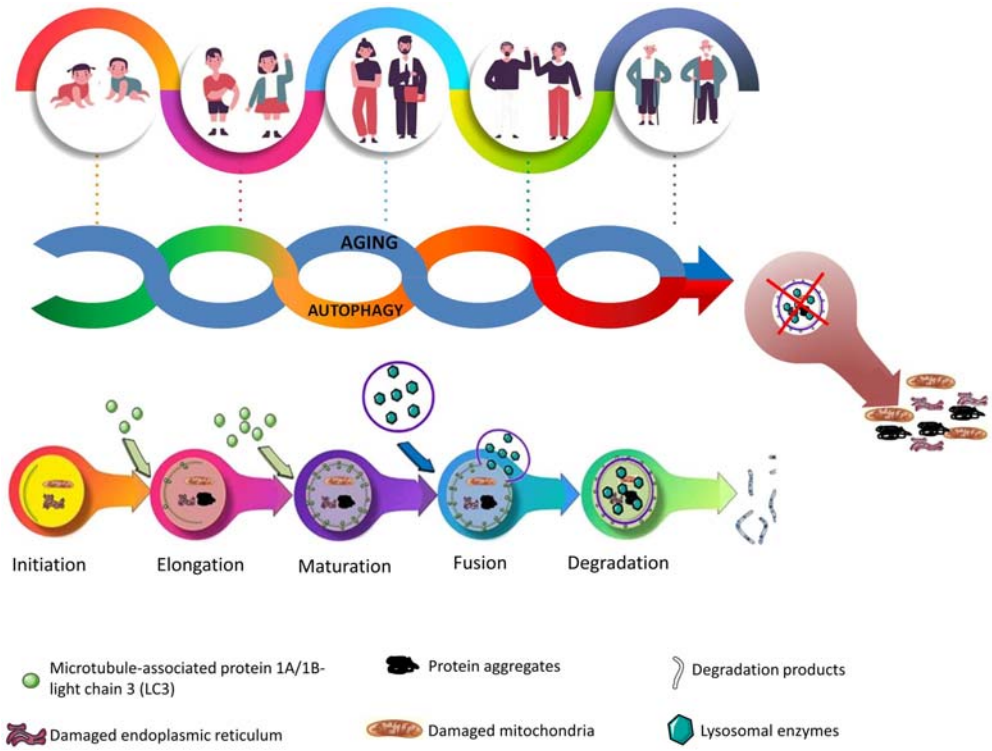

Figure 14.1 Aging and autophagy: an intertwined relationship. The biological process of aging is one of the more complex and unsolved puzzles of humanity. Recent studies highlight a pivotal role for autophagy in aging process and it is presently described as a "longevity determinant." Evidence clearly show that during aging the quality control efficacy of autophagy decreases leading to an accumulation of damaged organelles (e.g., mitochondria, endoplasmic reticulum) and protein aggregates, among others, which affects cells homeostasis leading to cell degeneration and, eventually, death.

determinant for key physiological processes, ranging from cell fate to organism lifespan, were attributed to autophagy.[31] Besides its self-eating role of intracellular misfolded proteins, damaged organelles or invading microorganisms in cells, autophagy was also recently described as an adaptative response able to provide nutrients under different stress conditions.[32]

There are two different kinds of autophagy, defined according to the targets involved in the process: the non-selective and selective autophagy. In non-selective autophagy, the cargo is total cytosol and its degradation in the lysosomes generates nutrients essential during starvation. Selective autophagy includes a cytoplasm-to-vacuole targeting pathway, mitochondrial autophagy (mitophagy), and pexophagy (peroxisomes).[33] More recently, also aggrephagy (protein aggregates), lysophagy (lysosomes), ribophagy (ribosomes) and ERphagy (endoplasmic reticulum) were described under the umbrella of selective autophagy.[31,34] Furthermore, autophagy can also be divided in macroautophagy (generally called autophagy), microautophagy and chaperone-mediated autophagy (CMA), based on the selective uptake of cargo destined for degradation.[21,22] Macroautophagy involves the formation of double-membrane structures (autophagosomes) that engulf portions of cytoplasm and complete organelles that, then, fuse with lysosomes to form autophagolysosomes where the cargo will be degraded and resulting elements can then return to cytosol to undergo metabolic processing.[21,35] Concerning microautophagy, it is mainly characterized by the lack of autophagossomes where engulfment occurs directly through the lysosomal membrane.[36–38] Although microautophagy is generally very similar to macroautophagy, it does not seem to exhibit adaptation to nutritional deprivation; instead, a continuous protein degradation occurs even under normal conditions.[39] Studies about microautophagy are scarce and generally limited to yeast; few studies can be found in mammalian cells, in which more investigation is necessary.[38,40] Lastly, there is the CMA, a third complementary autophagic route.[41] In this specific case, single soluble cytosolic proteins are targeted with a specific lysosome motif (KFERQ-related sequences) that is recognized by a complex of chaperone proteins and delivered to the lysosomal membranes for degradation.[42–45] Docking of this substrate/chaperone complex to lysosome is mediated by specific binding to the cytosolic tail of the lysosome-associated membrane protein 2A (LAMP2A).[46] Yet, the role of CMA in the turnover of long-lived proteins and intracellular quality control, as well as its role in different diseases, seem similar to other types of autophagy.[21] Recently, the identification of expressed sequences displaying high-sequence homology with the mammalian LAMP2A in several fish species, bring the hypothesis of CMA existing from earlier times during evolution than initially thought[32,47]; although the process is more intricate in higher organisms.[48]

Usually, all forms of autophagy are considered as housekeeping processes responsible for recycling cellular components with major roles in normal development and

physiology. Nevertheless, autophagy functions go far beyond what is considered "normal" conditions to maintain cellular homeodynamics, specifically in non-dividing differentiated cells such as neurons, that cannot dilute and eliminate waste by cell division.[48] Indeed, an essential role for autophagy has been described under distinct stress conditions such as hypoxia, endoplasmic reticulum stress, DNA damage and invasive pathogens[49,50] where autophagy is the key process responsible for the degradation of damaged proteins and organelles, oxidized lipids or intracellular pathogens.

Autophagy regulation is very complex and includes a wide variety of signaling cascades and regulatory mechanisms. Recent evidence revealed a key role for sirtuin 1 (SIRT1) in the process: it was shown that SIRT1 interacts with several autophagy-related (ATG) proteins, essential regulators of the autophagic process.[51,52] Apparently, SIRT1 is able to deacetylate ATG proteins (including the homolog microtubule-associated protein 1A/1B-light chain 3 (LC3) in mammalian cells) in a NAD^+-dependent manner,[51] stimulating the autophagic uptake of cellular proteins and damaged organelles for degradation and thus, maintaining cellular homeostasis.[53] Additionally, interaction of SIRT1 with the family of forkhead box O (FoxO) transcription factors and p53 pathway also seems to exert a pivotal role in autophagic regulation in different stress resistance and aging models.[12,53–55]

Likewise, probably the most conserved autophagy inducer known so far is adenosine monophosphate-activated protein kinase (AMPK). It is well known that physiologically, nutrient deprivation and/or energy scarcity is one of the major autophagy inducers and, at which point AMPK importance raises due to its key role in the control of several metabolic processes, working as energy sensor and connecting the dots between cellular metabolism and energy availability.[56] Indeed, AMPK is able to inhibit the mechanistic target of rapamycin (mTOR), the main autophagy repressor in mammalian cells that is physiologically active in situations of high energy levels, and it is responsible for a decrease in the rate of autophagosomes formation.[57–59] In short, AMPK senses decreases in cells' energy availability and suppresses mTOR activation, stimulating autophagy in order to enhance energy levels. However, the role of AMPK is not fully understood and recent evidence shows that AMPK can also regulate autophagy through a direct association with an autophagy-initiating Unc-51 Like Autophagy Activating Kinase 1 (Ulk1), which is an orthologue of yeast ATG1,[60] and by directly modulating lysosomal function.[61] Further studies are necessary to fully understand the complexity of AMPK-dependent autophagosome maturation and lysosome fusion.[62]

Knowing that different highly conserved signaling pathways converge in autophagy, which seem to regulate aging and lifespan in multiple model organisms, including worms, flies and mice,[63] it is of utmost importance to reinforce the fact that a strict balance in autophagy activation/suppression is of enormous significance not only due to its pivotal role in energy balance but also because it can be a double-edged sword protecting cells when it is moderately activated or inducing cell death when excessively activated.

In the next section, we will further exploit this issue trying to summarize the consequences of autophagic deregulation in the aging process.

14.3 Role of autophagy in aging: what can go wrong?

Hitherto, the molecular mechanisms underlying the aging process remain one of the greatest scientific mysteries of humankind and with it, the unsolved basis of why aging comprises the major risk factor for some of the most complex age-associated diseases. In the last several decades, researchers tried to decipher the biological process of aging and clearly demonstrated that autophagy has a crucial role as a "longevity determinant" since its proper regulation can determine lifespan in different species.[48,53] As a matter of fact, it has been shown that autophagy failure or mutations in autophagy genes promotes premature aging and increased age-associated diseases.[64–66] One of the major threats of aging, is the accumulation of misfolded or cross-linked aggregated macromolecules and defective organelles that affect cell functions,[67–69] supporting the vital role of autophagy in this process (Fig. 14.1). However, the complex interplay between autophagy and aging is a question that remains enigmatic. Despite the uncertainty entailing this issue, it is already known that both aging and autophagy regulation are driven by shared signaling pathways.[70] This strict relationship seems to rely on the fact that autophagy is a biological process occurring downstream of different longevity pathways, such as insulin/insulin growth factor 1 (IGF-1) and mTOR signaling, calorie restriction (CR) and mitochondrial activity.[48] These pathways are deeply involved in a coordinated program[71–73] where the aging process seems to downregulate some of the most crucial survival protein cascades (e.g., phosphatidylinositol 3-kinase (PI3K), B cell CLL/Lymphoma-2 (Bcl-2) and/or SIRT-FoxO signaling pathways),[53,74,75] alterations that will also compromise their pivotal role in the regulation of autophagy. So, it can be stated that autophagy is a biological process inseparable from aging. Several studies clearly demonstrated that the activation of autophagy due to the inhibition of mTOR is responsible for the increased lifespan of several species.[76–79] Additionally, unbalanced regulation of the mTOR and AMPK signaling pathways impairs autophagy contributing to organismal aging.[80] Such alterations can contribute to the development of cancer, metabolic diseases and neurodegeneration due to an increase in cellular vulnerabilities.[41,81–83] In 2001, a study performed in *Saccharomyces cerevisiae* showed for the first time the mTOR involvement in aging,[84] and this finding later was confirmed and extended to other species such as *Caenorhabditis elegans* and *Drosophila*.[76,85] Moreover, evidence from the literature shows that the depletion of autophagy genes (e.g., Atg6, Beclin 1, S6K1) shorten lifespan in long-lived nematodes and mammals.[86–88] Recent genetic studies also indicate that autophagy genes are required for lifespan extension in various long-lived nematode mutants.[89] Likewise, the existence of two hypomorphic alleles that reduce mTOR

expression by 25% in mice resulted in a ~20% increase in lifespan of these animals when compared with wild-type counterparts.[90] Nevertheless, modulation of mTOR signaling can be a double edged sword in longevity since its complete inhibition in early life stages can induce premature lethality.[91–93] Thus healthy aging should go through a tight regulation of mTOR activation in order to maintain cellular homeostasis and health.[94,95]

Presently, one of the major hypothesis under debate is based on the role of autophagy in cellular defense against damage. Indeed, evidence from a wide variety of organisms indicates that autophagy plays a key role in protecting cells from reactive oxygen species (ROS) generated primarily during mitochondrial respiration (for further details please see[96–98]). The overproduction of ROS by mitochondria, particularly superoxide anion ($O_2^{\bullet -}$) that dismutates to form hydrogen peroxide (H_2O_2) that can further react to form the highly reactive hydroxyl ion ($HO^{\bullet}$), with the consequent accumulation of damaged organelles,[99] supports the idea that aging is associated with a loss of autophagy effectiveness.[48] Generally, a strict balance between the production and the scavenging of ROS is necessary to maintain homeostasis. However, with advancing age, several inherited or acquired (through extrinsic factors such as lifestyle, stress, metabolic diseases, among others) defects in redox-mediated signaling pathways[100] are responsible for a decline in the efficiency of several antioxidant defensive mechanisms, which result in a shift in the balance towards the formation of ROS contributing to accumulated cell damage over time.[100–104] Moreover, it is well known that the unbalanced production of mitochondrial ROS also affects other cellular compartments[12] threatening cellular homeostasis and playing a pivotal role in the acceleration of aging and age-associated pathologies.[19,105]

Under physiological conditions, the loss of mitochondrial function during early age activates mitophagy to eliminate defective mitochondria and slows aging through a balance between mitochondrial quantity and quality.[106,107] Impairments in mitophagy result in the accumulation of dysfunctional mitochondria, leading to a decrease in lifespan, as previously demonstrated by the knockdown of several mitophagy-related genes such as dct-1, pink-1, and pdr-1 (the nematode Parkin homolog).[108] In addition, the deficient removal of damaged mitochondria triggers oxidative injury and protein misfolding,[109,110] compromising vital cellular processes including oxidative metabolism and calcium buffering, favoring cell death.[111]

Together with mitochondria, peroxisomes also play an essential role in ROS production within cells. Their normal function is critical for the maintenance of a healthy cellular redox balance, which influences the aging process.[112] Therefore, for the maintenance of cell homeostasis, damaged peroxisomes must be removed by autophagy. For decades, the importance of peroxisomal ROS production in aging has been hidden behind mitochondrial ROS; nevertheless, their importance in the aging process has been highlighted by several studies that showed a positive correlation between

peroxisomes function, decline and accumulation of damaged peroxisomes, suggesting an age-related decline in pexophagy.[113–115] Nevertheless, although the major role of peroxisomes dysfunction in aging and age-related diseases is already established, pexophagy is still a field under development and further studies are necessary to fully elucidate their role in the aging process.

Aging is considered a major risk factor for several neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington disease (HD), frontotemporal degeneration and prion protein (PrP) disease.[116] These diseases are characterized by an accumulation of protein aggregates (e.g., amyloid β plaques, neurofibrillary tangles of hyperphosphorylated tau, α-synuclein aggregates, huntingtin aggregates) indicating a defective clearance by aggrephagy,[117] as demonstrated by several studies performed in animal models of disease.[21,118–127]

A deregulation in CMA also seems to occur during the aging process.[128] Studies performed in aged animals showed a significant reduction in LAMP2A levels as well as an altered localization of the protein in the lysosomal membranes. These alterations cause a decrease in CMA activity and, consequently, an increased vulnerability to age-associated damage.[128–130] There is also evidence that CMA defects contribute to the development of age-associated diseases, namely neurodegenerative diseases.[131,132]

Despite the recent advances in the field of autophagy, there are many aspects that need further clarifications. For instance, the role of ERphagy, lysophagy and ribophagy in the aging process is underexplored. It is widely known, the importance of healthy pools of ER, ribosomes and lysosomes, in the maintenance of cellular homeostasis. Therefore, we anticipate that impaired autophagic degradation of damaged ER, ribosomes and lysosomes is involved in the aging process.[133] In fact, ERphagy seems to be regulated in a similar way to mitophagy.[107] Most studies regarding aging and associated diseases are mostly focused on "general autophagy," or mitophagy due to the pivotal role of mitochondrial dysfunction in such situations.

At this point, one can ask how scientists have been using the knowledge discussed above in the fight against aging and related diseases. In the next section we will briefly discuss some of the advances in anti-aging therapeutics that were developed under the assumption that autophagy is a central player in aging.

14.4 The chase for "eternal youth": can autophagy-directed interventions be the much-desired youth elixir?

The last decades brought a burst in the development of therapeutic approaches that have been determinants in healthcare improvement contributing to the dramatic increase in life expectancy worldwide. Nevertheless, the biological process of aging still comprises one of the major health threats because it is the major risk factor for several human pathologies.[134] Dissecting the core mechanisms of aging became one of the

biggest missions in medicine. Based on the previously mentioned evidence, several autophagy-directed approaches underlie the quest for "youth and immortality fountain" trying to answer properly the three big promises of this mission: health span, rejuvenation, and longevity, in a step forward, allowing humans to live far beyond their natural span while preserving their health status (Fig. 14.2). In this line, several studies aimed to answer the question: can autophagy promotion prevent aging in humans and thus protect against age-associated diseases? So far, the effectiveness of the proposed approaches remains uncertain.

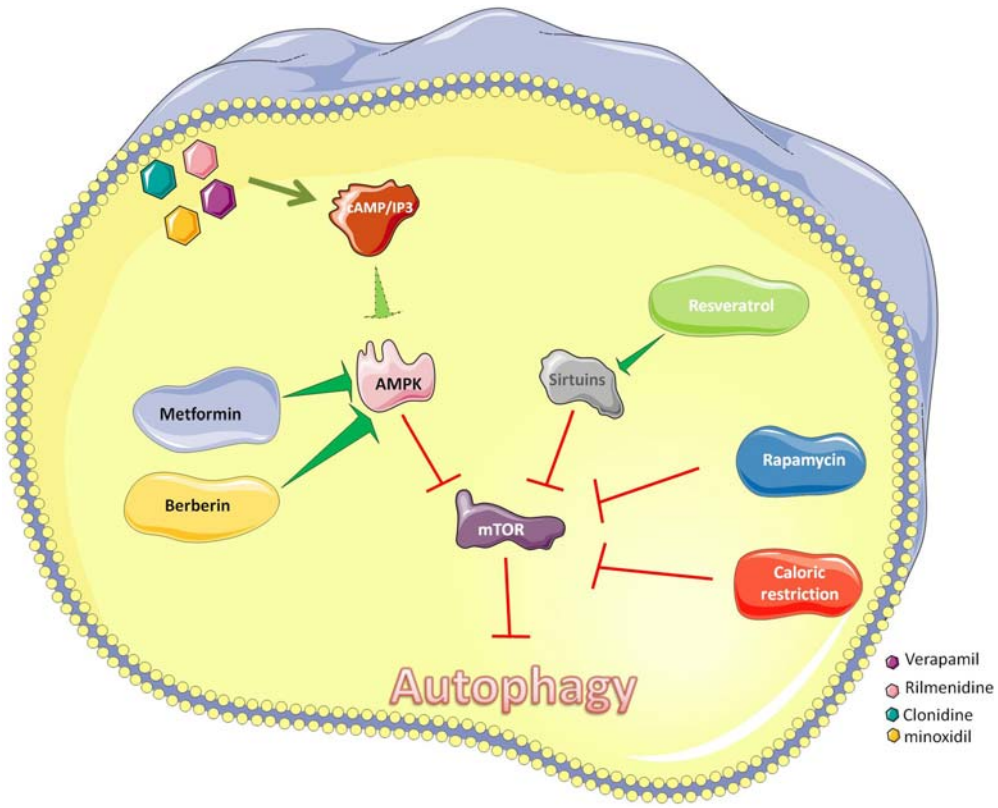

Figure 14.2 Autophagy-targeted therapies as potential anti-aging drugs. Aging is the major risk factor for some of the more intricate and challenging diseases affecting humankind. Thus, researchers in the field are devoting special attention in deciphering the mechanisms underlying the aging process to lay the groundwork for the development of new promising strategies to delay aging avoid or reduce age-related diseases. Due to the key role of autophagy in cells homeostasis, several autophagy-directed therapies are under study including AMPK inducers, sirtuins activators, mTOR inhibitors and modulators of cAMP/IP3 signaling. *AMPK*, adenosine monophosphate-activated protein kinase; *mTOR*, mechanistic target of rapamycin; *cAMP*, cyclic Adenosine Monophosphate; *IP3*, inositol triphosphate.

Thus far, the first line of action builds on non-pharmacological strategies including physical exercise.[135] Several studies showed that physical exercise, adapted to the age and health condition of the individual, can be a powerful ally in aging and age-related conditions.[135] And, physical exercise is considered a major autophagy inducer since it decreases the ATP/AMP ratio which, in turn, activates AMPK stimulating autophagy.[136,137] Indeed, AMPK activation by physical exercise inhibits mTOR signaling and activates PI3K signaling pathway that, in turn, stimulates the expression of ATG and LC3-II proteins.[135,138] It was previously reported that treadmill exercise can modulate several autophagy-associated proteins, such as Beclin-1, improving autophagy during aging.[139] Nevertheless, the potential of physical exercise to act as an anti-aging intervention, via its capacity to activate autophagy, is dependent on the exercise type, duration and/or intensity and, more studies are needed to fully clarify the effects of different exercise modalities in the aging process.[137]

Together with physical exercise, nutrient availability also plays a role in the regulation of autophagy during the aging process. Evidence from the literature shows that autophagy induction via CR is a very robust way to extend the lifespan, slowing the pace of aging by retarding its comorbidities, through improved mitochondrial respiration.[140,141] It has also been reported that a selective reduction of protein intake, especially amino acids, mimics the beneficial effects of CR.[142] The theory of CR as an anti-aging intervention was first described in 1935 by McCay and coworkers.[143] The authors observed that a 40% reduction in calorie intake was responsible for a 50% increase in rat lifespan. The pro-longevity effects promoted by CR are mediated by the signaling pathways involving the activation of AMPK, sirtuin 1 and IGF-1 and inhibition of mTOR[144–147] (Fig. 14.2). In short, CR effects result from the modulation of major autophagy regulators, as previously described. Nevertheless, it is important to emphasize that in the elderly, the risks of this approach can overlay its benefits leading to immunodeficiency and malnutrition. Moreover, some human studies showed detrimental secondary effects such as osteoporosis, loss of libido, inability to regulate body temperature, and loss of strength and stamina.[148] Based on these observations, researchers started focusing on pharmacological compounds capable of mimicking CR-mediated autophagy induction. So far, rapamycin, an inhibitor of mTOR signaling, leads the studies in the field (Fig. 14.2). Rapamycin is the only known pharmacological substance with proven efficacy in lifespan extension in all model organisms studied[149] such as yeast,[78,150] nematodes,[76] *Drosophila*[85,151] and mice.[23] All these studies support the conserved role of autophagy in the aging process.[48,151] Rapamycin works as a CR mimetic providing the same benefits as those provided by CR, without reducting calorie intake.[152] It has been shown that rapamycin was responsible for an increase in lifespan of ~14% in adults and of ~28%–38% in young mice.[153] According to the National Institute on Aging (NIA) Intervention Testing Program, rapamycin was able to prolong the lifespan in mice either when administered at early

adult or elder mice (9 and 18 months of age).[23] Others reported significant benefits of rapamycin against cardiac aging[154] and age-related neurodegeneration.[155] Interestingly, the majority of studies showed that rapamycin has consistently more benefits in females than males.[134] Nevertheless, contradictory results can be found in both mice and humans and several side effects have been reported after chronic treatment with rapamycin such as decreased insulin sensitivity, glucose intolerance, increased risk of new-onset diabetes, increased incidence of viral and fungal infections including pneumonia, chronic edema, painful oral aphthous ulceration, and hair loss,[156–158] which affects rapamycin wide-scale use as an anti-aging compound.[159–161] These side effects lead researchers to propose an intermittent rapamycin treatment, allowing it to be washed out of the human body, a phenomenon that seems to occur within a few weeks.[162–164] In fact, in female 129/Sv mice, rapamycin intermittent treatment (administered at a dosage of 1.5 mg/kg, subcutaneously, three times a week for a period of 2 weeks, followed by 2 weeks without rapamycin, starting from the age of 2 months) inhibited age-related weight gain, decreased aging rate, increased lifespan and delayed spontaneous cancer.[165] Despite these observations, limited effects were observed in the aging process itself, raising important questions about the efficacy of rapamycin: is it worth living longer but, as the "Tithonus Error," decrepit and increasingly crippled by age-related conditions? Further studies are necessary to fully elucidate the potential benefits of rapamycin usage as an anti-aging therapeutic.

Several studies support the therapeutic potential of autophagy-directed drugs in age-associated diseases, particularly in neurodegenerative diseases.[166] It is now known that protein aggregates that characterize neurodegenerative diseases are autophagy substrates (e.g., mutant huntingtin, α-synuclein, and phosphorylated tau).[118–121] Studies in an HD mice model showed that rapamycin increased animals' lifespan through reduction in the toxic aggregates formation.[21,120] Rapamycin and its analogs have also shown benefits in animal models of AD, PD, frontotemporal degeneration and PrP disease.[122–127]

Besides rapamycin, also nicotinamide derivatives such as metformin, urolithin A and spermidine showed efficacy in delaying aging and in extending lifespan (Fig. 14.2).[167] Indeed, metformin (*N*, *N*-dimethylbiguanide), an AMPK-dependent autophagy inducer, widely used in type 2 diabetes, has beneficial effects on healthspan and lifespan on both clinical and model organisms. In fact, it has been demonstrated, that metformin is able to increase lifespan in both *C. elegans* and mice and, when combined with rapamycin, longevity increased in male C5BL/6 mice.[165,168,169] It has also been studied how metformin treatment influences and modulates the biology of aging and its associated diseases in humans, by focusing on gene expression profiles and insulin sensitivity and secretion, in a diabetes-independent mode, as an attempt to open new venues for the design of new anti-aging drugs.[170] Several other AMPK-inducers (e.g., berberin) have proven efficacious against age-associated pathologies through AMPK and/or sirtuin 1 inhibition of mTOR and consequent autophagy induction (Fig. 14.2). Positive effects

were already reported in cardiac remodeling, collagen deposition, apoptosis and fibrosis, resulting in senescence retardation,[171] improved cardiac function,[172,173] and cognition in aging animal models.[174,175] Concerning, Urolithin A, the specific mechanism of action remains elusive however, it is known that this natural compound is able to enhance mitophagy preserving mitochondrial health and to extend lifespan in both *C. elegans* and rodents.[176]

Besides AMPK- and mTOR- directed drugs, also sirtuin activators are emerging as promising anti-aging tools. For instance, resveratrol, a sirtuin activator, has gained the interest of researchers in recent years due to its ability to upregulate ATG genes in different experimental models such as yeast, drosophila and nematodes, promoting an increase in health span and lifespan.[177–179] However, resveratrol efficacy in mammals is yet to be confirmed since, so far, several studies in mice showed that although it promotes significant benefits in motor performance, bone health, cataracts and cardiovascular issues, as well as, an increase in survival rate in high-calorie fed mice, there is no conspicuous increase in mean or median lifespan.[180–184] Nevertheless, the study of sirtuin activators is still a field under development and is presently in the focus of some of the best laboratories in the world, and could in the near future highlight an effective and safe anti-aging therapeutic approach.

A growing number of autophagy inducers are under development with different mechanisms of action. For instance, the literature shows a wide variety of compounds capable of inducing autophagy through the modulation of cyclic AMP (cAMP)/inositol triphosphate (IP3) signaling pathways, such as rilmenidine, clonidine, minoxidil, and verapamil[185,186] (Fig. 14.2). Interestingly, independently of their mechanism of action, all of these molecules modulate, directly or indirectly, mTOR activity and, subsequently, they regulate autophagy.

Despite the substantial knowledge acquired in recent years regarding autophagy involvement in aging and its associated diseases, the challenge of finding a safe and effective anti-aging therapy is far from the end. Certainly, further studies are urgent to clarify the pros and cons of autophagy-directed therapies to fight aging and age-related diseases. We believe that these changes occur not only due to continuous oxidative stress and damage, but also because of the inherent inability of cells to completely remove damaged structures (biological garbage).

14.5 Going down the rabbit hole: how lysosomes modulate longevity pathways

As noted above, autophagy has a key role in longevity and defects in autophagy are associated with age-related diseases. The different modes of autophagy have a common aspect: the lysosome as the terminal degradation organelle. Lysosomes have been known for decades as the cellular recycling organelle, which degrades macromolecules

into building blocks that can be reused to generate new cellular components. In the last ten years, it became clear that in addition to their hydrolytic role, the lysosomes also carry out key signaling roles, mostly related to nutrient sensing, and metabolic roles, associated with the distribution of metabolites such as cholesterol and metals, for example, to other cellular compartments.[187]

The discovery that a group of transcription factors (the microphtalmia family composed by the TFEB, MITF, TFE3, TFEC) can trigger the coordinated expression of genes whose protein products are located in lysosomes, and in net terms promote the formation of new lysosomes, revealed not only the existence of a transcriptional mechanism to maintain lysosomal biogenesis, but also the ability to modulate lysosomal biogenesis in response to the intra/extra-cellular environment.[187,188] Importantly, the activity of the microphatalmia transcription factors is regulated by mTORC1 (among other pathways): mTORC1 inhibits the microphtalmia transcription factors,[189] thus coordinating lysosomal biogenesis to nutrient sensing, and putting lysosomal biogenesis as another branch of autophagy downstream of mTORC1-mediated lysosomal signaling.

Lysosomal biogenesis requires not only the expression of lysosomal genes and the translocation of the respective protein products to the lysosomal membranes, but also the generation of new lysosomal particles. This is achieved through a process known as autophagic lysosome reformation (ALR) (Fig. 14.3). After autophagosomes fuse with lysosomes, and the lysosomal hydrolytic machinery digests the autophagic contents, the lysosome initiates a process of tubulation, which will culminate in the fission of those tubules and generation of new lysosomal particles.[190,191] The tubulation is dependent on the activity of mTORC1.[190] The activation/deactivation of the mTORC1 complex occurs at the lysosomal membrane and depends on the activity of the lysosomal v-ATPase as well as the efflux of several nutrients from the lysosomes, such as amino acids and cholesterol.[192,193] Tubulation usually occurs about 12 h after autophagy was initiated, thus allowing for the temporal regulation of different phases of lysosomal biogenesis. Starvation results in mTORC1 down-regulation, and the consequent activation of TFEB. As the digestion of autophagosome starts occurring in the lysosome, the efflux nutrients such as amino acids and cholesterol from the lysosomes lead to activation of mTORC1. By now, the lysosomal proteins are translated, and being translocated to the lysosomes, and the activation of mTORC1 shuts down TFEB activity. The detailed mechanisms that underlie formation of lysosomal tubules remains poorly understood, but requires the involvement of kinesin KIF5B, the membrane-binding proteins spastizin, spatacsin and clathrin, the sugar transporter spinster1, the actin nucleation promoting factor WHAMM, and the signaling phospholipid PtdIns(4,5)P_2.[187,194] Loss of these proteins results in impaired lysosomal reformation, and accumulation of lysosomes with undigested contents, as illustrated in Fig. 14.3. Importantly, the activity of the lysosomal v−ATPase is also necessary for tubulation.[190]

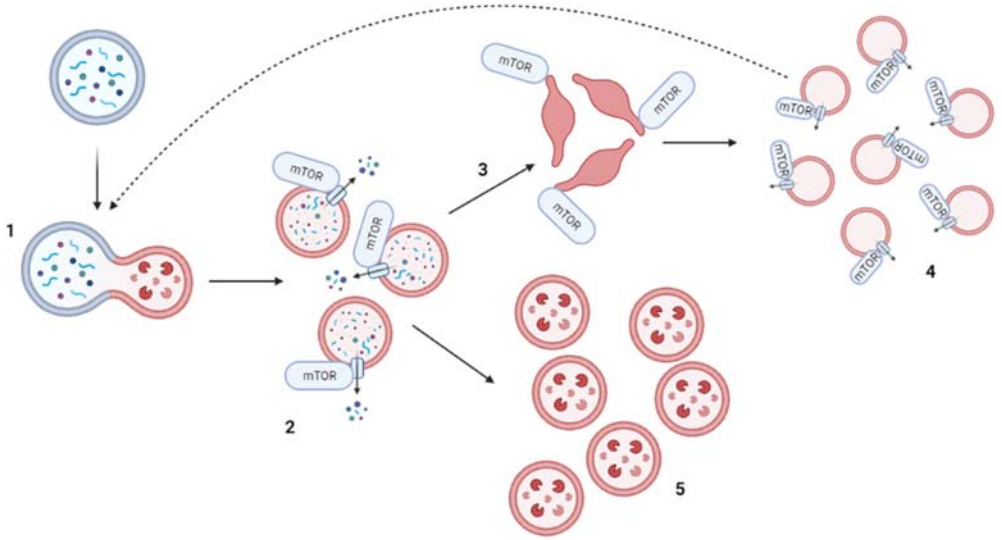

Figure 14.3 Representation of the life cycle of lysosomes, focused on autophagic lysosomal reformation. *mTOR*, mechanistic target of rapamycin. Autophagosomes are represented with blue membranes, and lysosomes with red membranes. The fusion of autophagosomes to lysosomes (step 1) is represented by part of the structure with blue membranes and part with red membranes. After the autophagosomes fuse with the lysosomes, the catabolic machinery of the lysosomes degrades the contents of the autophagosomes and releases recycled building blocks, including amino acids, which activate mTORC1 (step 2). At a later step, the lysosomes start tubulating (step 3), from which new lysosomes will arise (step 4). However, if the lysosomes are not functional, undigested contents accumulate, and this results in the inability of the lysosome to tubulate (step 5), becoming a terminal storage organelle.

Thus, the described progressive loss of v-ATPase activity during aging may trigger a feed-forward cycle in which partly dysfunctional lysosomes cannot undergo ALR, thus resulting in the progressive accumulation of undigested material, and of dysfunctional lysosomes without the capacity to generate new functional lysosomal particles.

The progressive loss of v-ATPase activity has been linked to several neurodegenerative diseases, although the most definitive evidence that v-ATPase is involved in aging was obtained in studies using yeast.[195] Essentially, impaired lysosomal acidification will result in decreased autophagic capacity, with an accumulation of autophagosomes. This is particularly important, in the context of aging, due to the decreased capacity of carrying out mitophagy; mitophagy is a key process in the maintenance of a healthy mitochondrial network. Research in mitophagy is an intense area of work, and we won't be addressing specific aspects of this pathway in this chapter as recent reviews cover the topic, for example.[196] Indeed, it was shown both in lower eukaryotes and in mammals that loss of v-ATPase activity results in impaired mitochondrial function, triggered by a loss of homeostasis in intracellular iron metabolism, for which

the v-ATPase activity is a key component.[195,197–199] These experiments brought further light to the interdependence between lysosomes and mitochondria, which is a key aspect of quality control for both organelles and will be discussed in more detail below.

14.6 Partners in crime: mitochondria and lysosomes need each other

The metabolic roles of mitochondria and lysosomes are well established for half a century, and major inroads were made on their dynamics and signaling roles during the last twenty years. However, these organelles have predominantly been studied using reductionist approaches. Only recently had it started to emerge that mitochondria and lysosomes are well coordinated, and that perturbations in one (due to, for example, genetic mutations) result in secondary malfunction of the other.[200] The mechanisms underlying the coordination between mitochondria and lysosomes can involve signaling pathways, contact sites, and exchange of contents.[200] For the purpose of this chapter, the mechanisms of mitochondria-lysosomal crosstalk that are involved in quality control of these organelles are: delivery of mitochondrial contents to the lysosome (through mitophagy or mitochondria-derived vesicles),[201] delivery of lysosomal contents to mitochondria,[199,202] regulation of the lysosomal signaling phospholipid PtdIns(3,5)P2 by a signaling network controlled by mitochondria,[61] regulation of lysosomal biogenesis by mitochondria,[203] and regulation of mitochondrial biogenesis by lysosomes.[204]

The function of an organelle depends on the amount of that organelle, and on the proportion that is healthy and dysfunctional. Therefore, cells have several strategies to maintain homeostatic levels of mitochondria and lysosomes, such as increasing biogenesis or removing the damaged organelles. Furthermore, dysfunctional organelles can be separated from the healthy ones: impaired parts of the mitochondrial network can be separated by fission, and marked for mitophagy, and impaired lysosomes can be set aside in the cytoplasm as terminal storage organelles. Notably, the biogenesis of mitochondria is strongly repressed in multiple lysosomal storage diseases as well as when lysosomal acidification is impaired,[199,204] and mitochondrial stress can also modulate the biogenesis of lysosomes.[61,203,205,206] Furthermore, chronic defects in mitochondria, akin to what would be expected during an age-related decrease in mitochondrial performance, can result in the inhibition of lysosomal hydrolytic capacity, resulting in the accumulation of undigested lysosomal contents and loss of functional lysosomes.[61,206] It is noteworthy to point out that the loss of lysosomal function in cells with chronic mitochondrial defects is not due to excessive delivery of mitochondria to lysosomes, but rather due to the inability of the lysosomes to generate the phospholipid PtdIns $(3,5)P_2$, a key regulator of many lysosomal membrane proteins.[61] These studies underline that multiple mechanisms are in place to ensure that mitochondria and lysosomes are coordinated; these organelles represent not only key metabolic machineries and

signaling hubs, but also have the capacity to coordinate anabolism and catabolism, hence the importance of ensuring that their functions are in sync.

Of course, mitochondria and lysosomes are not isolated in the cells. The existence of selective autophagy for different components in the cells was already discussed, and both mitochondria and lysosomes communicate with other organelles, via physical contact sites and signaling pathways.[207] For example, perturbations of mitochondrial function are known to trigger endoplasmic reticulum stress.[208] On the other hand, the inability of lysosomes to deliver cholesterol to the endoplasmic reticulum can result in their compensatory association to mitochondria via contact sites.[209] Peroxisomes are intrinsically associated with mitochondria in terms of biogenesis, metabolism and dynamics, and require the lysosomes for pexophagy. The near future is likely to bring increased attention to holistic approaches that enlighten the role of organelle networks as key players in the quality control of organelle function and of multiple cellular roles.

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CHAPTER 15

Stem cells, fitness, and aging

Kristina Kitaeva[1], Andrey Kiyasov[1], Albert Rizvanov[1], Catrin Rutland[1] and Valeriya Solovyeva[2]
[1]University of Nottingham, School of Veterinary Medicine and Science, Nottingham, United Kingdom
[2]Kazan Federal University, Kazan, Russia

15.1 Introduction

Normal development of organisms is impossible without constant renewal of cells and tissues. Continuous division can lead to the accumulation of unwanted changes in cells, the number of which increases with age and reduces the functionality of organs. The elimination of defective or abnormal cells, and regeneration of healthy cells, is necessary for proper tissue homeostasis in an adult. It is known that multipotent stem cells (MSCs) are a regenerative pool in the body. They can differentiate into adipocyte, bone, cartilage, muscle, nerve and the other cell types as necessary,[1,2] therefore, they are point of great interest in the context of regenerative material in medical research. Mesenchymal stem cells (MSCs) are found in adipose tissue, skin, placental tissue, the endometrium, menstrual blood, liver tissue, synovial fluid, dental pulp, muscles, and many other parts of the body.[3–5] The presence of these cells in a large number of body tissues ensures they function normally. As MSCs age, they lose regenerative potential, which may be associated with age-related diseases in the elderly, therefore these cells are potential therapeutic targets.[6–8] Senescence of MSCs is characterized by irreversible cycle arrest in the G0 / G1 phase, increased expression of proteins associated with aging (in particular, p16, p21, p53) and higher β-galactosidase (SA-β-gal) activity.[9] Senescence of MSCs leads to functionality decrease, phenotypic changes, formation of senescence-associated secretory phenotype (SASP), impaired immunomodulatory function and the ability to undergo aberrant differentiation.[10] One of the main characteristics of cellular aging, a turning point in the transition from early to complete aging, is considered to be an irreversible halt in proliferation. In the 1960s, it was discovered that in aging human fibroblasts cultures, once they had reached their maximum number of divisions, their cell cycles stopped, this phenomenon was called the "Hayflick Limit."[11] This aging-related permanent arrest of proliferation, telomere shortening, nontelomeric DNA damage, extreme mitogenic signals, and altered chromatin organization are some of the defining characteristics of aging.[12] However, when a cell reaches the Hayflick Limit or replicative aging, this does not necessarily lead to cell death, loss of functionality, or decreased viability.[13] The senescent cells can still be

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viable and live in a culture, in contrast to the apoptotic cells that undergo programmed cell death.[14] A number of factors, such as oxidative damage, telomere contraction, hyperproliferation, expression of oncogenes, ultraviolet and γ-ray irradiation, and chemical agents can trigger DNA damage response (DDR) in cells.[15,16] Weak DNA injury usually causes cell cycle arrest, while severe damage can activate a program or program the aging of death, such as apoptosis, necrosis, or autophagy.[17] At the same time, a chronically senescent cell acquires various phenotypic changes, including morphological changes, chromatin remodeling, metabolic reprogramming, and the secretion of factors that form the so-called SASP.[18] In this chapter, we will review the latest data on stem cell aging, focusing in on the aging of MSCs, the reasons why cells lose their functionality during aging, and investigate possible strategies to prevent aging and improve cell fitness.

15.2 Cell fitness and cell competition

Studying the mechanisms involved in removing unfit cells is an urgent task. Over the last few years, there has been growing evidence that a mechanism of intercellular competition, called cell competition, helps eliminate these damaged cells precisely because they have a lower fitness in comparison to other cells in the vicinity. During competition and through this determination of relative fitness, cells that are deemed less fit than their neighbors are removed (that is, become "losers cells"), even if they would be classed as viable in a different context[19] (Fig. 15.1).

Although a large number of the studies on cell competition have been conducted in the *Drosophila* fruit fly model, similar mechanisms have also been observed in mammalian cells.[20] Maintaining a high-fitness stem cell throughout life probably also prevents the onset of the tumorigenesis process, because due to competition, high-fitness cells displace damaged stem cells, which pose a potential danger in the form of malignant transformation.[21] However, it has been shown that Mll5 (candidate tumor suppressor gene) mutant mice demonstrate a 30% reduction in the average representation of hematopoetic stem cells and decreased fitness, but inactivation of this gene did not induce spontaneously developed hematologic cancers.[22] Interestingly, other studies of epithelial cells have shown that cell competition drives out cells with potentially deleterious mutations.[23] For example, experiments on mice with constitutional trisomy or chromosomal instability caused by a hypomorphic mutation in the gene Bub1b, showed that despite the presence of nonregenerating aneuploid adult tissues, hematopoietic stem progenitor cells (HSCs) and other regenerative adult tissues are largely euploid.[24] This may indicate that, in vivo, mechanisms exist to select against mutant, unfit cells. It is probably that the aging process affects the cellular competition processes and reduces the selection of cells with higher fitness properties and rejects the mutated cells. For example, the incidence of clonal hematopoiesis of cells with somatic mutations increases in people aged over 40 years old.[25] In a study by Martincorena et al., sequencing of esophageal epithelial cells showed that middle-aged and elderly donors had a high content of cells with

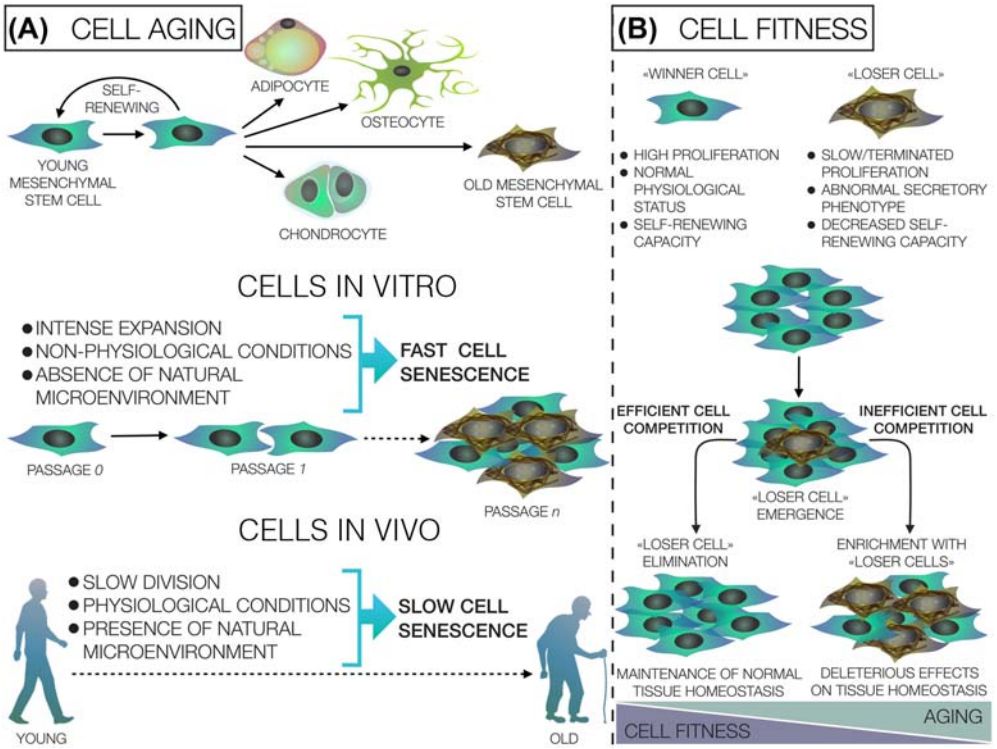

Figure 15.1 Cell aging and cell fitness. (A) As a result of the cumulative influence of senescence inducing factors and the life process on mesenchymal stem cells (MSCs), their differentiation potential and their ability to self-renew are reduced, thus the cell begins the aging process. At the same time, under in vitro conditions, cell aging occurs at a higher rate, due to a number of factors associated with the cultivation conditions. In vivo aging of MSC cells occurs more slowly during the life of the organism. (B) The theory of cellular competition states that "winner cells" displace "loser cells" from tissues, which is part of the process of maintaining normal tissue homeostasis. However, with aging, there is an accumulation of "loser cells" and a decrease in tissue functionality, which is probably associated with a decrease in cellular competition.

mutations in 14 different cancer genes, in particular, NOTCH1 and TP53 (12%–80% and 2%–37% of cells, respectively). An interesting point was that the prevalence of the NOTCH1 mutation was several times higher in the healthy esophagus tissue in comparison to cancers.[26] These data may indicate, on the one hand, a positive selection of cells with high oncogenic risk increases with age, but on the other hand, it may show a decrease in the competition between healthy and potentially degenerated cells within the body.

15.3 Senescent mesenchymal stem cell phenotype

The criteria for the definition of MSCs were proposed in 2006, and included: adhesion to plastic, positive expression of the surface markers CD90, CD73 and CD105,

and negative expression of CD11b, CD14, CD19, CD34, CD45, CD79α and HLA-Dr, as well as the ability to differentiate into adipo-, osteo- and chondrocytes.[27,28] Some sources show that these markers exert stable expression in MSCs regardless of the age status of donors or passage number.[8] However, there are investigations demonstrating that the expression of these conditionally "stable" markers can change. For example, expression of the cell surface marker CD73 in synovium-derived stem cells changed from P1 (49.3%) to P4 (27.9%).[29] Also, it was found that the expression of CD105 decreased in passages 3 to 5 in canine umbilical cord-derived MSCs (UC-MSCs).[30] In addition, the expression of classic MSC markers CD90, CD73 and CD105 in general may vary depending on the site of origin. For example, CD105 expression varies from 4% in liver-derived MSCs to 58% in amniotic-derived MSCs [31] (Table 15.1).

The source of MSCs can also influence the rate of aging. For example, adipose tissue-derived stem cells (hASC) and Wharton's Jelly-derived MSCs (hWJ-MSC) have shown different proliferation profiles over time, and hASCs underwent aging faster during cultivation.[32] However, replacement of CD90 + MSCs by CD31 + can occur in the hearts of aging mice, which affects the cells' abilities in undergoing differentiation into epithelial cells.[33] Some markers can also serve as identifiers of aging.[29,33–39] For example, Stro-1, CD106/VCAM-1 (vascular cell adhesion molecule 1) and CD146/MCAM (melanoma cell adhesion molecule), show suppressed expression during long-term cultivation, whereas expression of the CD295 marker increases.[38,40] Although the source of this research[8] demonstrated the absence of a direct correlation between the expression levels of CD106/VCAM-1 and the number of passages,

Table 15.1 Changes in CD marker expression in senescent and young mesenchymal stem cells (MSCs).

Marker	Young MSCs	Senescent MSCs
CD31	Decrease	Increase
CD54	Increase	Decrease
CD59	Increase	Decrease
CD71	Increase	Decrease
CD73	Increase	Decrease
CD106	Increase	Decrease
CD90	Increase	Decrease
CD146	Increase	Decrease
CD178	Increase	Decrease
CD205	Increase	Decrease
CD264	Decrease	Increase
CD271	Increase	Decrease
CD273	Increase	Decrease
CD295	Decrease	Increase

Moravcikova et al. showed that late passage bone marrow–derived mesenchymal stem cells (BM-MSCs) are characterized by a decreased expression of this marker.[39] The same source studied the level of expression of a number of other markers including CD178/FasL (Fas ligand), CD205/Ly75 (lymphocyte antigen 75), CD273/PD-L2 (programmed cell death 1 ligand 2), all of which showed pronounced expression decreases at 8–10 passages, additionally the expression levels of CD54/ICAM1 (intercellular adhesion molecule 1), CD71, and CD59 also decreased.[39] Decreased expression of the marker CD146 correlated with the aging of human UC-MSCs, decreases in the expression of mRNA stem cell-related octamer-4 (Oct4), Nanog, (sex determining region Y) -box 2 (Sox2), as well as Bmi-1, Id1 and Twist-related protein 1 (Twist1), probably via the transcriptional regulation of CD146 which may delay cellular senescence during in vitro expansion.[41] CD264 may become a promising marker for determining cell aging in heterogeneous MSCs cultures. In a study by Madsen et al., conducted on bone marrow–derived MSCs isolated from different age donors, it was shown that although the expression levels of CD264 do not correlate with the chronological age of the donor, it does increase during long-term cultivation and is associated with cell aging, which was identified as changes in morphology, differentiation and colony-forming potential.[36,37] It should be noted that extensive cultivation of MSCs in vitro significantly accelerates cell aging.[8] Hyperoxygenation, differences in the composition of the sera and media used, and suboptimal cultivation conditions lead to erosion of telomeres, a change in the DNA methylation landscape, and adjusting of the cell membrane composition, which ultimately leads to aging[42,43] (Fig. 15.1). In addition, during aging, MSCs change their morphology, and accumulate cell debris and excess actin fibers, which contributes to the formation of a granular cytoplasm and enhancement of cell autofluorescence. In the article composed by Bertolo et al., the phenomenon of MSCs autofluorescence is described as a promising marker for determining the level of cell aging in culture in a noninvasive manner.[44]

15.4 The functionality changes in senescent mesenchymal stem cells

Cells cultivated under artificial conditions are subject to accelerated aging, the cause of which may also lie in the absence of a natural cell microenvironment and, as a consequence, result in high replicative stress.[45] Phenotypic changes within senescent cells are accompanied by a progressive loss of activity in terms of their reduced ability to undertake homing, migration and differentiation.[46,47] Experiments using BM-MSCs from C57BL/6J mice showed that the level of autophagy in BM-MSCs of aging animals was lower than in MSCs isolated from young mice, while the use of the autophagy inhibitor 3-methyladenine reduced their osteogenic abilities for differentiation and proliferation, it also increased their abilities in relation to adipogenic differentiation.[48] Recent evidence suggests that autophagic activity declines with age, probably contributing to the accumulation of damaged macromolecules and organelles during

aging. Damaged/dysfunctional proteins that accumulate with age and are not removed in a timely manner, disrupt normal cell function.[49] It has been shown that during cell aging, vacuolar H^+ ATPase activity changes and lysosome pH regulation is impaired, which leads to decreased functionality. It has also been shown that 3-butyl-1-chloro imidazo [1, 5-a] pyridine-7-carboxylic acid (SGJ) is able to protect lysosomes from alkalization via increased expression of lysosomal-associated membrane proteins (LAMP) 1 and 2.[50] The study of functional changes in MSCs during aging occurs in vitro at specific stages. As mentioned above, ex vivo cell expansion accelerates aging for a number of reasons associated with culture conditions. However, despite the inevitable aging of cells ex vivo, numerous data have been published showing that there are initially significant differences in viability, proliferation and differentiation capacity between MSCs isolated from elderly and young donors. At the same time, allogeneic transplantation of AD-MSCs from older donor mice to younger mice significantly reduced the walking speed, grip strength, endurance and daily activity of the recipient mice post-transplantation, when compared to mice transplanted with the same number of AD-MSCs from younger donors.[51] UC-MSCs at passage 5 exhibited stronger inhibitory effects on lymphocyte proliferation in co-culture than passage 2 cells. Higher expression of cyclooxygenase 2 (COX-2) and transforming growth factor β1 (TGF-β1) was also seen, leading to suppressed immune cell proliferation and function.[52]

15.5 The role of factors associated with aging

15.5.1 Oxidative stress

Oxidative stress is one of the main causes of cell aging (oxidative stress-induced premature senescence). Oxidative stress can result from chronic, low-grade inflammation associated with aging and many degenerative diseases.[53] At the same time, normal levels of reactive oxygen species (ROS) help maintain MSC function.[54,55] However, abnormally high levels of endogenous ROS and exogenous oxidants damage cell structures and contribute to premature cell aging. Imbalance of ROS and antioxidants, such as superoxide dismutase (SOD), initiates growth arrest regulated by a complex network of molecular signaling pathways.[56] Oxidative stress has a detrimental effect on the osteogenesis capacities of BM-MSCs. However, during the late stages of osteogenic differentiation, intracellular antioxidant enzymes, SOD2, catalase, and glutathione peroxidase 1, can be activated in MSCs, and spontaneous activation of sirtuin 1 (SIRT1) can occur.[57] The generation of ROS can be associated with an increase in D-galactose. However, ascorbic acid has the potential to inhibit MSC aging caused by D-galactose, by activating the RAC-alpha serine/threonine-protein kinase/mammalian target of rapamycin (Akt/mTOR) signaling pathways.[58] The protein kinase C (PKC) isoform, PKCβII (serine/threonine kinase family), increases oxidative stress in muscle

pericytes following activation by growth factors and ROS, through the regulation of various pathways involved in the phosphorylation cascade.[59] Adding serum from patients with ankylosing spondylitis, characterized by a high content of ROS, contributed to the aging of MSCs in vitro, and resulted in decreased mitochondrial membrane potential.[10] It has also been shown that Sirt3 deacetylase promotes decreased levels of cellular ROS through SOD 2 and regulates the balance of intracellular oxidation and antioxidation.[60] Hydrogen peroxide-induced aging of rat MSCs activated miR-206, while targeting Alpl, miR-206 actually improved cell functionality.[61] Interestingly, miR-17 miRNA, miR-20−5p and miR-106-5p, are rapidly and stably suppressed during hydrogen peroxide-induced oxidative stress. At the same time, overexpression of miR-20b-5p/miR-106a-5p saved cells from growth arrest, which facilitated the G1/S transition and DNA synthesis.[62] Overexpression of B cell-specific Moloney murine leukemia virus integration site 1 (Bmi1) in MSCs has antiaging and antiosteoporotic effects by inactivating p16/p19 signaling and inhibiting oxidative stress in a mouse model.[63]

15.6 Genetic and epigenetic aspects

Cellular senescence, which is induced in response to stressors, causes cells to stop dividing.[64] Of the tumor suppressor markers, p21, p16 and p53, are among the most commonly used cell aging markers.[65] High expression of both p21 and p16 was confirmed in AD-MSCs isolated from elderly mice, which was also associated with a higher content of senescence-associated beta-galactosidase (SA-β-gal)$^{+}$ cells compared to cells from young donors; this was consistent with the increased number of senescent cells among the isolated cells. In addition, in natural cells with high p21 expression, the same signaling pathways are activated as seen in the "artificially" senescent cells created in vitro.[51] An interesting article was published in Nature Methods investigating exome sequencing of iPSCs isolated from peripheral blood mononuclear cells from donors of different ages. The work showed that the number of mutations in gene regions was doubled in iPSCs from donors who were 80 years old, compared to those aged 20 years. It is therefore assumed that these mutations were in cells even before reprogramming.[66] During in vitro cultivation of MSCs, decreases in FGF21 expression have been observed, which leads to increased mitochondrial ROS production. Moreover, siRNA treatment with mitofusin2-(Mfn2-) inhibited FGF21-depletion-induced aging of MSCs, which is probably associated with the AMPK signaling pathway. It has also been shown that overexpression of FGF21 in old MSCs inhibits aging.[67] The DDR in cells appears to exist as a result of internal or external stimulation. DDR often initiates replication, leading to the accumulation of DNA damage, mutations and subsequent aging.[68] Senescent retinal pigment epithelium stem cells (RPESCs) show a high SA-β-gal content and increased regulation of p21 and p53 proteins compared to young cells. Senescent RPESCs also express higher mRNA

levels of senescence-associated genes (p21, insulin-like growth factor-binding protein 3 (IGFBP3), plasminogen activator inhibitors SERPINE1, and SERPINB2) when compared to their younger counterparts.[69] In response to DDR, an increase in the transcription factor Zscan4 (zinc finger and scan domain containing 4) occurs; it increases ATM-TRAF6-TAK1 (ataxia-telangiectasia mutated protein/tumor necrosis factor (TNF) receptor associated factor 6/TGF-β-activated kinase 1) axis formation, which ensures the formation of long-term SASP in human stromal cells. TAK1, in turn, activates p38 and phosphoinositide 3-kinases (PI3K)/Akt/mTOR to support continuous SASP signaling.[70] Mutations in the liver/bone/kidney alkaline phosphatase (Alpl) gene cause pathological changes in bone metabolism. Liu et al. found that Alpl removal induces premature bone aging, including bone loss and increased bone marrow fat, coupled with increased expression of p16 and p53 due to aging and impaired differentiation of MSCs.[71] Senescence associated beta galactosidase (SA-β-gal) is one of the most widely used markers of cell aging. SA-β-gal activity depends on the levels of expression of the galactosidase β1 (GLB1) gene, which encodes lysosomal beta-D-galactosidase, and whose activity is usually high at an acidic pH of 4.5.[72] However, with the expansion of the lysosomal compartment and an increase in the expression associated with aging isoform GLB1, activation of SA-β-gal occurs at suboptimal pH values (pH = 6).[73] Cells isolated from adult donors showed decreased cell proliferation and differentiation, stage-specific embryonic antigen-4 expression and ATP content, as well as increased cell size and increased expression of β-gal, intracellular ROS, and annexin-V. In addition, after treatment of young MSCs with conditioned medium from MSCs of elderly donors, decreased proliferation was observed, likely due to the increased levels of SASP cytokines [in particular, interleukin (IL) -6] contained in the conditioned medium.[74] In an investigation by Franzen et al., six CpG sites associated with genes are mentioned: glutamate metabotropic receptor 7, calcium-sensing receptor, selectin P (SELP), caspase (CASP) 14, keratin-associated protein 13−3 (KRTAP13−3), and PRAME family member 2 (PRAMEF2), whose methylation may be associated with aging.[75] Lysine demethylases KDM3A and KDM4C are involved in the regulation of heterochromatin reorganization through the transcriptional activation of the non-SMC condensin I complex (NCAP) subunit D2 and NCAPG2 during MSC senescence. Suppression of KDM3A or KDM4C results in a sustained response to DDR and aggravates cellular senescence.[76] Interestingly, the methylation profile in induced pluripotent cells of different age donors showed a principally linear, but relatively weak relationship with donor age; in cells obtained from older donors, the methylation level was only 5% higher than those recorded from younger donors cells.[66] Epigenetic activation of the INK4/ADP-ribosylation factor (ARF) locus by Polycomb (PcG) and Trithorax (TrxG) group proteins is a decisive mechanism in the establishment of cellular senescence.[77] Overexpression of PcG proteins has been shown to delay the onset of replicative senescence; DPY30 is an important regulator of cell proliferation. Depletion of DPY30 in cells led to serious changes which were characteristic

of aging, cell flattening and increased size, an increase in ROS levels, activation of DDR pathways, increased activity of SA-β-galactosidase, and increased levels of p16.[78]

15.7 Senescence-associated secretory phenotype and the microenvironment

Senescent cells secrete a large number of cytokines and chemokines called SASPs, which affect cellular processes such as proliferation, migration, differentiation and remodeling of tissues, which can eventually lead to the onset of malignant neoplasms.[79] Senescent RPESCs secreted higher concentrations of IL-6, IL-12, IL-17, interferon (IFN)-γ, TNF-α, and TGF-β in comparison to young RPESCs.[69] MSCs from adult donors (over 40 years old) were larger by the 3rd passage and also showed a lower spreading rate compared to cells isolated from young donors (who were just over 16 years old). A SASP study also showed that adult MSCs produced significantly higher levels of the aging-related cytokines IL-6 and IL-8 when compared to MSCs from younger donors.[80] Older MSCs also produced a significantly greater amount of TGF-β2 in their cultures as compared to young MSCs.[35] Therefore, it has been hypothesized that TGF-β2 significantly suppresses growth of young MSCs and holds potential for stimulating osteogenic differentiation of both young and old MSCs. Another interesting component of SASPs and the aging process relates to their interactions with extracellular vesicles (EVs). Research shows that senescent cells produce and secrete EVs that differ in number and composition from nonsenescent cells, and play key roles in the deleterious effects of aging (Fig. 15.2).

In addition, the EVs released from senescent MSCs differ in phenotype - the average size of vesicles decreases and the number of secreted EVs increases.[81] Telomere depletion or DNA damage caused by aging or disease can cause a p53-dependent increase in EV biogenesis.[82] EVs are loaded with microRNAs, which have recently been shown to be part of the SASP and are specifically produced by senescent cells. Weilner et al. found that EVs from senescent MSCs contain decreased levels of galectin-3, which has a positive effect on the ability of MSCs to undergo osteogenic differentiation. Such EVs, with reduced levels of galectin-3, may contribute toward the negative effects of age-related loss of MSC osteogenic differentiation.[83] EVs from aged mice are highly enriched in miR-183 (miR-96/-182/-183). In vitro assays have revealed that old EVs are endocytosed by primary BM-MSCs, resulting in inhibition of osteogenic differentiation in young BM-MSCs. Oxidative stress increases the amount of miR-183−5p in BM-MSC-derived EVs.[84] Endothelial miR-31 is secreted by senescent cell microvesicles, and inhibits osteogenic differentiation of MSCs. Researchers suggest that miR-31 may be a valuable plasma biomarker of aging, which may in turn help decipher the appropriate therapeutic strategy for osteoporosis.[85] Growth differentiation factor 6 (Gdf6) is a regenerative factor secreted by young MSCs. The expression of specific secretory factors, including Gdf6, is regulated

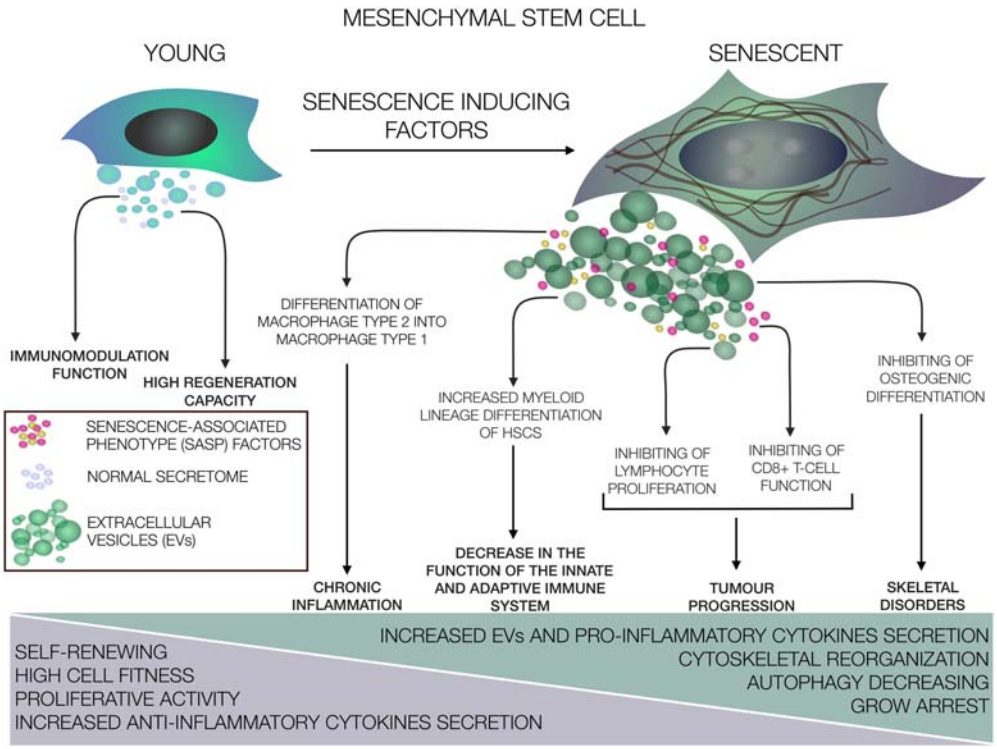

Figure 15.2 Mesenchymal stem cell senescence. The influence of senescence-associated secretory phenotype factors of the old mesenchymal stem cells on the microenvironment, and its consequences.

by miR-17, the expression of which decreases with age. Increased Gdf6 expression restored the osteogenic capacity of old MSCs in vitro and had beneficial effects in vivo on aging-related pathologies such as decreased lymphopoiesis, insufficient muscle recovery, decreased neural precursors in the brain, and chronic inflammation.[86] Probably one of the key pathways influencing the SASP phenotype formation is the JAK1/STAT3 pathway (Janus kinase 1/signal transducer and activator of transcription 3), which was exhibited in irradiated isolated BM-MSCs. Following treatment with a JAK1 inhibitor, a decrease in SASP secretion was observed.[87] Similar results were obtained on BMSCs ovariectomized (OVX) mice using a JAK inhibitor.[88,89] Gnani and colleagues showed that secretion levels of SASP-specific cytokines (IL-1, IL-6, IL-8, and monocyte chemoattractant protein 1 (MCP-1)) were significantly higher in older MSCs, while secretion of these pro-inflammatory factors may adversely affect clonogenic differentiation of HSCs.[34] SASP factors secreted by senescent SCs also affect surrounding healthy cells through paracrine activity via various mechanisms, including recruitment of inflammatory cells, remodeling of the microenvironment, induction of fibrosis, and inhibition of stem cell function.[90] Hematopoietic stem cells and progenitor cells are found in bone marrow

microenvironments, together with MSCs. Thus, the aging of MSCs, and the secretion of inflammatory factors, affect hematopoietic progenitor cells. It was previously shown that the induction of apoptosis by TNF-α in progenitor cells promotes the survival of HSCs and their differentiation into myeloid cells through the p65-nuclear factor κB (NF-κB), which prevents necroptosis, induces immunomodulatory functions, and directs the differentiation of HSCs along the myeloid pathway.[91] The relationship between TNF-α and the induction of senescence through the ERK-ETS1 (extracellular signal-regulated kinases 1 and 2/ETS proto-oncogene 1) signaling pathway and the IL27Ra receptor has been shown. Increased levels of IL27Ra expression also lead to a shift in the differentiation profile of HSCs toward myeloid cells and the development of immune senescence. At the same time, a deletion within the murine IL27Ra gene eliminates these effects, and also cancels the inhibitory effect of TNF-α on HSCs.[92] Experiments showed that MSC-derived exosomes from young donor cells had lower expression levels of miR-127−3p and miR-125b-5p, compared to MSC-derived exosomes in cells from elderly donors, and more pronounced therapeutic effects in the murine model of LPS-induced acute lung injury to reduce inflammation by polarizing macrophages for the M2 phenotype.[88] BM-MSCs of young mice, when co-cultured with RAW264.7 cells (macrophage cell line), showed greater immunomodulatory effects, increasing the polarization of macrophages toward the M2 phenotype and inducing secretion of antiinflammatory and immunomodulatory cytokines. However, in the Transwell system, BM-MSCs from aged mice increased macrophage migration stronger.[93] Ruhland et al. showed that IL-6 secreted by senescent MSCs induced myeloid-dependent immunosuppression, whereby CD8 + T cells were inhibited, leading to unrestricted tumor growth.[94] Senescent cells also facilitate the neoplastic transformation of adjacent epithelial cells.[95] After aging, these cells begin to secrete inflammatory factors, cytokines and matrix metalloproteinases, which disrupt tissue architecture and function, leading to the formation of a microenvironment that promotes the progression of mutant epithelial cells.[96] Of the many SASP factors associated with inflammation and malignant neoplasms, it is IL-6, secreted by senescent MSCs, that promotes tumor progression. Indeed IL-6 secretion increases by more that 40-fold in senescent MSCs. In addition, conditioned media from senescent MSCs can activate STAT3, the main downstream transcription factor for IL-6, in breast cancer cells. Moreover, xenografts consisting of MDA231 co-culture and MSCs aged with peroxide turned out to be larger than xenografts consisting of MDA231 co-cultures with young MSCs or only MDA231.[97]

15.8 Therapeutic strategies to rejuvenate and increase fitness

One of the goals of therapeutic approaches aimed at rejuvenating the body using MSCs, is to maintain the functionality of stem cells (their ability for self-renewal and multiple differentiation), both after expansion in the laboratory, and directly in the

body. Gaur et al., proposed a compendium of protocols which may be useful for culturing and subsequent characterization of AD-MSCs and the stromal vascular fraction.[98] Reducing the oxidative stress that cells experience during culture may improve the therapeutic potential of MSCs. The milk protein lactoferrin is able to inhibit production of intracellular ROS, and activation of caspase-3 and Akt induced by hydrogen peroxide, whilst also suppressing MSCs senescence and apoptosis.[99] Treatment of culture plastic with amphiphilic block copolymer (redox copolymer), described in the article by Ikeda et al., also helps to reduce ROS, and maintain the SOX2 gene expression levels which are responsible for stem formation.[100] In contrast, melatonin treatment of MSCs protects them from oxidative stress and its associated aging, again highlighting the correlation between oxidative stress and aging.[101,102] Regulation of miR-17 miRNA and E2F1 transcription factor expression can also slow aging and prevent the MSC growth arrest caused by oxidative stress.[62] It is also worth noting the results obtained using autologous extracellular matrix - its use improves proliferation, reduces the signs of aging which includes decreased multipotency, and is associated with increased expression of β-gal aging and accumulation of ROS.[103] EVs isolated from iPSCs after addition to the culture of senescent MSCs with an increased ROS content, showed decreased cellular ROS levels and the aging phenotypes of senescent MSCs attenuated, probably due to the delivery of the intracellular antioxidant enzyme peroxiredoxin.[104] It is known that stem cells in mesenchymal niches are present in relatively low oxygen concentrations (1%–8%).[105] Thus, 21% of the ambient oxygen will lead to the formation of oxidized biological macromolecules and result in the formation of excess ROS.[106] When UC-MSCs are maintained under hypoxic conditions (5% oxygen) they exhibit increased capacities for cell growth and expression of the Bmi1 protein, and decreases in the induction of the p53-p21 signaling cascade and MCP-1 protein secretion, when compared to cells in normoxic (21% oxygen) conditions. These results suggest that culture conditions consistent with the cellular level of ROS may prove to be a practical strategy for slowing the progression of cellular senescence during ex vivo expansion of UC-MSCs.[107]

One of the promising approaches in relation to aging cells is the use of senolytics. Dasatinib is one agent that can be used to reduce the senile phenotype and remove aged cells from tissues. Using the example of ADSCs isolated from women with normal pregnancies (NP-ADSCs) and women with preeclampsia (PE-ADSCs), it was shown that dasatinib treatment significantly increased the number of apoptotic PE-ADSCs compared to NP-ADSCs. In addition, treatment decreased the expression of the gene encoding the p16 protein and six SASP components, and also increased the angiogenic potential of some NP-ADSCs.[108] Rapamycin is one of the most popular and promising potential antiaging drugs.[48] A number of experiments carried out in rats with D-galactose-induced osteoporosis showed that rapamycin reduced cellular senescence and promoted the organization of heterochromatin BM-MSCs.[109]

Experiments performed on MSCs isolated from rat bone marrow have shown the effectiveness of rapamycin in increasing both autophagic activity and the production of lysosomes by cells. In addition, hypoxia-exposed rapamycin-treated cells increased the secretion of hepatocyte growth factor (HGF), insulin-like growth factor 1 (IGF-1), stem cell factor (SCF), stromal cell-derived factor-1 (SDF-1) and vascular endothelial growth factor, whilst expression levels of both IL-1β and TNF-α were inhibited.[110] And although research on senolytics seems to be very promising, the effects of some of the drugs can negatively affect the function of stem cells, such as the drug Navitoclax (ABT-263).[111] Other treatments, including resveratrol and noncoding RNA modulation, can also reverse the altered phenotype of aging MSCs.[112] Another possible strategy may be to replenish the lactobacilli population. It was observed that there is a link between premature aging of BM-MSCs and intestinal microbial communities, in particular, a decrease in the number of lactobacilli in the intestine led to an abnormal accumulation of D-galactose, which induced premature aging of cells.[113] The link between obesity and the initiation of the early aging program of AD-MSCs has been confirmed, this was expressed as a decrease in cell proliferative capacity, increased expression of the p16, p53, IL-6, and MCP-1 genes, and the same results were reported in experiments using BM-MSCs.[114,115] The use of the "DNA clock" method, which is based on measuring the level of histone methylation, is a promising marker for predicting MSC aging.[75] This can be used in the context of new approaches in regenerative medicine, for example, the use of MSCs derived from iPSCs.[116,117]

15.9 Conclusion

Stem cells provide the body's regenerative pool, to which the function of preserving tissue throughout life is maintained. Aging is the result of an increase in the number of dysfunctional cells that is dangerous to the surrounding tissues. The most recent data show that stem cells are also susceptible to aging, and this dramatically alters the general condition of the body. This is due to the secretion of a number of factors and EVs, key miRNAs differences, chronically senescent SCs, changes to the microenvironment, reductions in the functionality of neighboring cells and induced inflammation. It is probable that these factors can also affect the cellular microenvironment, reducing the level of competition between cells as age advances. Stem programs are triggered and reduced in fitness both directly in the body, during life, and as a result of intensive use in vitro, which ultimately leads to the loss of the therapeutic potential of these cells. Stress caused by suboptimal cultivation conditions triggers signaling pathways that form with the formation of the senile MSCs phenotype. These factors change the established strategies of cell therapy. It has been shown that autologous SC or EVs show isolated negative effects on the body and aggravate the development of

pathological conditions. However, allogeneic SCs, cultivated for a short time in vitro, and injecting younger donor cells into the elderly, are safe and improve indicators of physical performance and biomarkers of inflammation. Standardization of isolation methods and transplantation can expand the applicability of SC in the field of gerontology, and reverse age-related changes, which will prolong life and improve its quality.

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CHAPTER 16

Programming of early aging

Susana P. Pereira[1,2], Luís F. Grilo[1,3], Renata S. Tavares[1,4], Rodrigo M. Gomes[5], João Ramalho-Santos[1,6], Susan E. Ozanne[7] and Paulo Matafome[8,9,10]

[1]CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[2]Laboratory of Metabolism and Exercise (LametEx), Research Centre in Physical Activity, Health and Leisure (CIAFEL), Laboratory for Integrative and Translational Research in Population Health (ITR), Faculty of Sport, University of Porto, Porto, Portugal
[3]PDBEB - PhD Programme in Experimental Biology and Biomedicine, IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal
[4]IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal
[5]Department of Physiological Sciences, Biological Sciences Institute, Federal University of Goias, Goiania, Brazil
[6]Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[7]University of Cambridge Metabolic Research Laboratories and MRC Metabolic Diseases Unit Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, United Kingdom
[8]CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[9]Coimbra Institute for Clinical and Biomedical Research (iCBR) and Institute of Physiology, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[10]Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal

16.1 Epidemiology of early life environment and adult aging—developmental origins of health, disease, and aging?

The emergence of the developmental origins of health and disease hypothesis, also known as fetal programming or the Barker hypothesis, was derived from the observed long-term effects on adult health from a low birth weight (LBW).[1] The first studies pointing at relationships between early life experience and adult health were presented by Forsdahl[2] and Wadsworth.[3] However, it was with Barker's works that the scientific community gained awareness of the contribution of the gestational environment to the long-term health of the offspring.[4] These and other more recent studies revealed a strong relationship between fetal growth restriction and adult sequelae, demonstrating that adults who were small at birth, but not premature, were at an increased risk for coronary heart disease.[4] Association of hypertension to health status at birth was also corroborated by an epidemiological study using a Finish cohort of approximately 6000 subjects.[5] During the Dutch famine, from 1944 to 1945, investigators could determine the impact of undernutrition in early, mid or late gestation on birth weight. Babies exposed to famine only during early gestation still presented a normal birth weight, but an augmented incidence of coronary heart and renal diseases in later life compared with non-exposed individuals.[5,6] Another interesting finding was the existence of a conditional adaptive response by the fetus, which confers a survival advantage when the postnatal diet remains suboptimal, but becomes harmful when postnatal nutrition

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is adequate or in excess.[7,8] In agreement with epidemiological human observations, studies in rodents, sheep, and non-human primates report accelerated F1 aging and early disease development induced by maternal nutrition during pregnancy.[9–13]

In this chapter, we present evidence and review mechanisms regarding how the uterine environment modulates synchronization of cellular clock synthesis and regulation, and aging pace, thus imprinting the "biological time." Controlling the speed of development and aging will ultimately shape their lives and how long they will live. We discuss the early life nutrition and programming of adult aging and lifespan, the mechanisms underlying early programming of aging, the early programming of aging-related diseases, and the transgenerational passage of the aging clock.

16.2 Early life nutrition and programming of adult aging and lifespan

Aging is described as a time-dependent functional deterioration and gradual loss of physiological integrity and efficacy, increasing vulnerability to disease and consequently, death. This impaired function is a major risk factor for human pathologies, including cancer, type 2 diabetes (T2D), cardiovascular disease (CVD), and neurodegenerative diseases. Genomic instability, epigenetic alterations, telomere attrition, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, inflammation, and altered intercellular communication all contribute to mammalian aging (Fig. 16.1).[14]

16.2.1 Prenatal malnutrition, longevity, and aging

Malnutrition is a condition that may vary from exposure to suboptimal conditions to maternal obesity (MO), having distinct impacts on offspring health and life expectancy (Fig. 16.1). The relationship between reduced birth weight and increased all-causes mortality in adult women and with premature death in adult men was demonstrated in a cohort of Finnish individuals.[15] Later retrospective studies demonstrated that male individuals with above average birth weights but a reduced length of the placental surface had increased mortality, suggesting that alterations in placental biology and function can have a lasting impact on long-term health that is independent of fetal growth.[16] The lifespan of humans makes it challenging to carry out prospective human studies to assess the impact of contemporary in utero exposures such as fetal overnutrition on mortality. Therefore, a number of studies have addressed effects of the early environment on molecular proxy markers of aging, such as telomere length, which shorten as a consequence of cell division and oxidative damage and thus indicate cellular aging.[17] A recent study demonstrated that young adult men who were born very preterm (less than 33 weeks of gestation) had both a higher blood pressure and shorter white blood cell telomere length than those born at term.[18] Studies in animal models

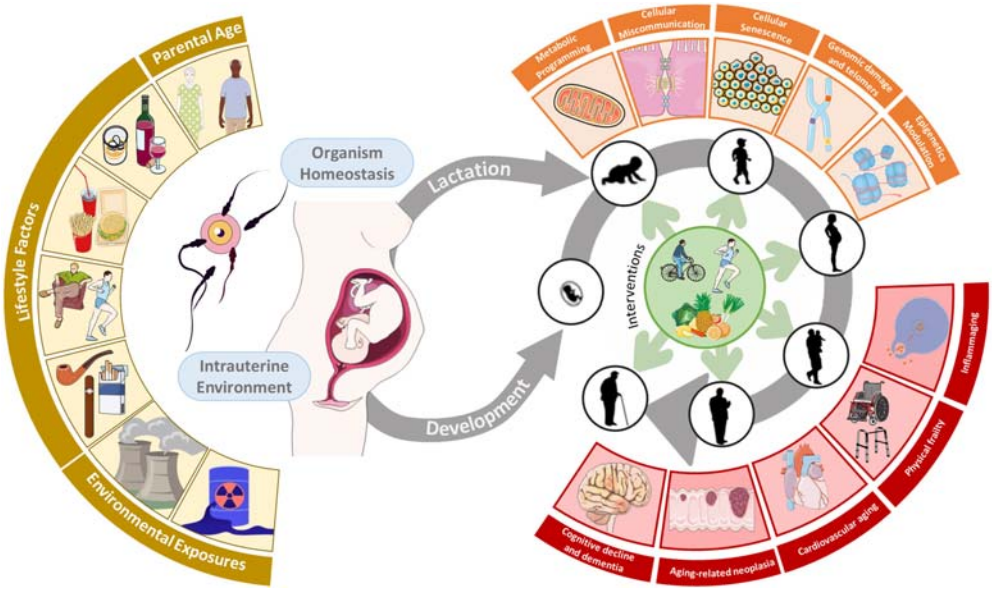

Figure 16.1 Factors and mechanisms of developmental programming in utero and in lactation period (left panel). During such developmental windows, mother exposition to insults or stressors such as under- or overnutrition, obesity, diabetes, stress, tobacco smoking, alcohol, other drugs or external pollutants may imprint changes at the molecular level that program metabolism, senescence, cellular communication, and genomic and epigenetic alterations (right upper panel). Although the descendance may adopt lifestyle changes, such molecular imprinting may increase the risk of early development of aging-associated disorders, such as inflammaging, cognitive impairment, neoplasia, CVD, and physical frailty (right lower panel).

support these findings, demonstrating that rat offspring born to dams fed a low-protein (8%) diet had a reduced birth weight but then underwent accelerated postnatal growth when suckled by a normally nourished dam.[19] When weaned onto a standard laboratory chow, these offspring had a reduced probability of survival up to 15 months of age. In addition, they displayed accelerated telomere shortening in the kidney,[19] an observation also made in a number of tissues in this model, including pancreatic islets, heart, and the aorta (reviewed in Ref. 20). In mice, offspring with nutritionally-induced LBW displayed an approximate 25% reduction in lifespan, which was reduced further if animals were weaned onto an obesogenic diet, demonstrating that the effects of maternal and offspring diet were additive.[21] This study also identified different critical time windows during early development for the impact of maternal diet on offspring longevity. Those exposed to the maternal low-protein diet during lactation alone, grew slowly during the suckling period, were protected from diet-induced obesity, and had an increased lifespan compared to control offspring.[21] These studies highlight the importance of the appropriate nutrition at the appropriate time during

development for optimal health and lifespan and the ability of alterations in early nutrition to both accelerate and decrease the aging process.

MO prevalence during pregnancy is increasing and has long-term impacts on the offspring health and life expectancy, increasing the risk of obesity, insulin resistance, hypertension, dyslipidemia, T2D, CVD, non-alcoholic fatty liver disease, and behavioral problems in adult individuals.[22] Many rodent studies have pinpointed that a maternal high-fat diet (HFD) during pregnancy induces adverse metabolic outcomes in the progeny, including pancreatic β-cell dysfunction and insulin resistance. Adult female and male offspring of obese Wistar rats showed increased β-cell mass and proportion. However, diabetic models may produce distinct results, which may be related to the different regulation of the pancreatic duodenal homeobox (PDX-1) mRNA and protein expression.[23,24] A maternal HFD diet induces molecular alterations in pathways of lipid metabolism in the liver of rat offspring.[25] Offspring of HFD mice also showed obese phenotype, hyperphagia, hypertriglyceridemia, and fatty acid synthase overexpression. In addition, changes in gluconeogenesis, hepatic insulin resistance, and increased phosphoenolpyruvate carboxykinase protein expression were observed in mouse offspring of HFD-fed females.[26]

Regarding aging markers, children whose mothers had metabolic syndrome during pregnancy had reduced salivary DNA telomere length at 10 y.o.[27] Another study has demonstrated that pre-pregnancy BMI was associated with lower cord blood and placental telomere length at the time of delivery.[28] A study using flies, which have a shorter life cycle, demonstrated decreased longevity in the flies that had progenitors fed an HFD.[29]

16.2.2 Can lactation and early life nutrition contribute to early aging?

Breast milk has the appropriate balance of nutrients and other bioactive factors, but its composition is variable according to stages of lactation, time of the day, maternal diet and maternal metabolic status.[30,31] The colostrum is produced in the first days of lactation, being richer in immunological factors. Gradual transition to mature milk raises the levels of macronutrients and micronutrients, growth factors, and a very rich microbiota.[32] Maternal diet and metabolic status, more than BMI, strongly influence milk composition, not only due to changes in lipids and glucose levels, but also to other factors, such as insulin, leptin, or inflammatory cytokines.[33] Although macronutrients, lactose, and mineral content are relatively constant, fatty acids may have significant fluctuations according to diet. Polyunsaturated fatty acids levels, for instance, seem to be regulated by the consumption of their dietary sources.[34] Protein restriction during lactation has been consistently shown to have long-term consequences on the infant's health.[30,31] On the other hand, maternal obesogenic/cafeteria diets have consistently shown to increase milk fatty acids content and to program metabolic dysfunction in

infants, including adiposity, insulin resistance, and later T2D.[35] A recent study in rats showed that MO during suckling leads to impairment of the melanocortin system, a reduction of hypothalamic IRβ, leptin signaling and POMC levels, increased NPY, and ultimately, obesity.[36] Western diets are rich in glycotoxins, such as advanced glycation end-products (AGEs), which activate membrane AGEs receptors (RAGE), triggering intracellular NF-kB signaling, inflammation, and oxidative stress. Increased maternal exposure to glycotoxins during breastfeeding leads to an impaired lipid profile, adiposity, and decreased β-cell function in rat offspring.[37]

Exclusive breastfeeding was shown to protect from telomere shortening at six weeks of age when compared to formula-feeding.[38] However, changes in milk volume or composition may also account for early programming of aging mechanisms. Mice postnatal overfeeding was shown to increase renal levels of proteins implicated in cellular senescence at weaning, such as p53, and lower levels of Sirt1, a known inhibitor of senescence.[39] Accordingly, maternal HFD feeding during gestation and lactation was associated with increased hepatic levels of senescence markers, such as the cell cycle arrest protein p16INK4a.[40] In contrast, slower postnatal growth caused by protein restriction during lactation is associated with reduced DNA damage, reduced oxidative stress, and less frequency of short telomeres.[41] Thus, altogether, changes in normal protein and fat composition of the milk, namely those resulting from overfeeding, are associated with an early increase of cellular markers of aging.

16.3 Mechanisms underlying early programming of aging

16.3.1 Metabolic programming

T2D has long been associated with birth weight and to a reduction in life expectancy. There is extensive data from animal models showing that a suboptimal early environment (including maternal protein restriction, MO, intrauterine artery ligation, and overexposure to glucocorticoids) can lead to reduced beta cell function (e.g., as a consequence of reduced beta cell mass), and peripheral insulin resistance (through changes in insulin signaling protein expression) which is often associated with increased oxidative stress (reviewed in Ref. 42) (Fig. 16.1). In addition to the role of in utero overexposure to glucocorticoids for offspring health programming (e.g., through accelerated tissue maturation), there is also evidence suggesting that long-term alterations in the hypothalamic-pituitary-adrenal-axis (HPA) can be a contributing mechanism (reviewed in Ref. 43). It is well-established that glucocorticoids act antagonistically to insulin; therefore, increased circulating levels can lead to insulin resistance.

Studies in animal models (including models of maternal undernutrition and maternal overnutrition) have identified key components of the insulin signaling pathway as being susceptible to metabolic programming as a consequence of exposure to a suboptimal in utero environment.[44] Although defects in the expression of insulin signalling

proteins have been identified in the main insulin sensitive tissues [muscle, liver, adipose tissue (AT), and the brain], AT displays some of the earliest and largest effects. Insulin receptor substrate one (IRS-1) and the p110 beta catalytic subunit of PI 3-kinase in AT appear to be common targets of developmental programming. Substantial reductions in both of these proteins were observed in AT from both rat offspring of low-protein fed dams[45] and offspring born to obese mice.[46] Consistent with oxidative stress playing a role in insulin resistance programming, postweaning diet supplementation of low-protein to offspring with Co-enzyme Q normalized the AT levels of IRS-1 and PI3-kinase p110 beta catalytic subunit. Similar effects on insulin signalling proteins have been observed in humans. AT biopsies from young adult men who either had LBW demonstrated reduced expression of IRS-1 and the p110 beta catalytic subunit of PI3-kinase.[47] As in the rodent studies, the underlying molecular mechanism was a post-transcriptional effect with no differences in the corresponding mRNA levels. A recent manuscript by Rodriguez-Gonzalez and colleagues studied rats at four time points across lifespan and demonstrated that rat offspring born to obese dams displayed accelerated metabolic aging, including the development of hyperinsulinemia.[13]

16.3.2 Early cellular miscommunication and cellular senescence

Senescence and early development of aging features have been shown to be a consequence of cell cycle defects. Senescence is defined as a state of permanent cell cycle arrest. In vivo and in vitro inhibition of the spindle assembly checkpoint protein BubR1 leads to a shorter lifespan, cachexia, and increased cell senescence. Reduced levels of BubR1 and other mitotic checkpoint genes (Bub3 and Rae1) have also been observed during natural aging and to increase senescence and p53 and p21 levels.[48,49] The *Cdkn2a* locus, encodes for $p16^{Ink4a}$ and $p19^{Arf}$, known inhibitors of the cell cycle.[50] Both are upregulated by a series of stress stimuli, such as telomere reduction, oxidative stress, DNA damage, and nutrient deprivation. Increased $p16^{Ink4a}$ levels in response to stress lead to cell cycle arrest at G1, which allows cell repair when transiently activated, but leads to senescence when chronically activated.[50] $p19^{Arf}$ is an activator of p53 in response to stress, which transiently activates p21-mediated cell cycle arrest during cell repair. p53 activation resulting from DNA damage, genomic instability, telomere shortening, or loss and oncogene activation was shown to promote a terminal cell cycle arrest, which makes cells become senescent, leading to reduced longevity, osteoporosis, organ atrophy, and lower stress tolerance.[51–53] Given that $p19^{Arf}$ was also shown to protect from senescence under certain circumstances, different levels of p53 were suggested to determine cell fate. Under severe acute stress, p53 activates p21-mediated cell cycle arrest, leading to senescence. However, under mild stress, less robust p53 activation may cause reactive oxygen species (ROS) reduction, expression of antioxidant enzymes and improved mitochondrial respiration

through regulation of enzymes involved in mitochondrial respiration. In such conditions, p53 favors cell repair, preventing senescence and allowing cell and organ functionality[50] (reviewed in Ref. 52). Importantly, in Hutchinson-Gilford Progeria no p53 overactivation was observed, showing the existence of other mechanisms involved.[54]

One important question remaining is: which stimuli lead to chronic, mild, or acute strong p53 activation? Diabetes has been suggested as an inducer of senescence in endothelial cells, causing an early-age phenotype.[55] Similarly, increased senescence markers were found in the AT of severely obese patients with markers of glucose dysmetabolism and insulin resistance.[56] Thus, metabolic dysregulation could be a trigger for early aging due to increased stress-induced cell senescence. Interestingly, changes in transcriptional activation of the *Cdkn2a* locus were associated with decreased AT plasticity and an imbalance between adipogenesis and senescence.[57] Thus, senescence is linked to an early loss of AT plasticity, contributing to the loss of metabolic dysregulation and further cell stress. In contrast, food restriction was shown to decrease cell markers of stress in the rat liver, namely HSP90 and HSP70, which was associated with lifespan-increasing effects of caloric restriction. Moreover, diabetes may also program senescence during developmental phases (Fig. 16.1). In rat, changes in DNA methylation induced by gestational diabetes were shown to decrease CDKN2A promoter methylation and to increase its expression in the pancreatic beta-cells of the offspring, which was suggested to contribute to impaired beta-cell function in adulthood.[58] A similar increase of senescence markers was observed in human umbilical cord mesenchymal stromal cells of diabetic women, showing decreased differentiation capacity.[59]

16.3.3 Programming of genomic aging and epigenetic alterations

The genomic instability designates the spontaneous tendency to create genomic alterations during the cellular life cycle, and is widely accepted as one of the hallmarks of aging.[60–62] Early embryonic genomic instability is generally a consequence of reproductive aging in human females and sperm DNA damage,[60,61,63] commonly resulting in preclinical pregnancy or fetal losses.[64] However, the role of early genomic instability in offspring programming of premature aging is unknown. Accumulation of DNA damage is well established with age, resulting from multiple stressors.[65–67] ROS are the most studied group of toxic molecules able to produce genomic instability. During pregnancy, the placenta is a supplementary source of ROS promoting a systemic state of oxidative stress in normal pregnancies that is exacerbated in gestations complicated by preeclampsia, IUGR, MO, and diabetes.[68–71] Additionally, fetal metabolic impairment and mitochondrial dysfunction represent an endogenous source of ROS. In obesogenic pregnancies, ROS generation overcomes natural antioxidant defense capacity in fetal tissues, which increases lipid peroxidation, protein carbonylation, and nuclear and mitochondrial DNA oxidation.[72–74]

Mammalian cells contain multiple mechanisms to repair DNA damage but the capacity decreases with aging.[75] While damage by DNA-alkylating agents activates the direct reversal repair mechanism and removes damage without altering the DNA backbone, other more complex repair mechanisms involve the single- or double-stranded DNA break and replacement of damaged nucleotides by undamaged bases, which potentiate mutations.[76] Defects in DNA repair-related proteins promote the premature accumulation of DNA damage and are associated with accelerated aging and the early development of age-related disorders.[77] High guanidine residues regions (such as telomeres) are particularly susceptible to oxidative stress, which potentiates telomere shortening and decreased telomerase activity.[78] In animal models, altered intrauterine environments caused by stress and malnutrition were associated with shortened telomeres and lower telomerase expression and activity in multiple tissues of the offspring[19,41,79,80] (Fig. 16.1). Several human studies have corroborated the impact of the intrauterine environmental changes on maternal stress, age, and pre-pregnancy BMI on offspring telomere biology and have related telomere shortening with hallmarks of biological aging.[78,81]

The telomere biology system is strongly regulated by epigenetic patterns due to the heterochromatin-like regions in subtelomeres.[82] In contrast to adulthood, fetal development presents elevated degrees of epigenetic plasticity that is modulated by the intrauterine environment.[83–85] Offspring epigenetic alterations due to the intrauterine environment may represent a mechanism of transgenerational disease transmission and premature aging, affecting long-term gene expression and predisposition to disease.[44] Altered histone modifications, such as acetylation and methylation, promote hepatic hypertrophy and lipid accumulation, adipogenesis, circadian dysregulation, and skeletal muscle insulin resistance, programming the offspring to obesity and diabetes.[44] It has been shown that intrauterine conditions can also regulate gene promoter methylation and impact gene expression of loci related to insulin resistance, glucocorticoid response, and cellular growth, development, and metabolism in 60-year-old humans.[86–88]

16.4 Early programming of aging-related diseases

16.4.1 Inflammaging

A noticeable aging-associated alteration in intercellular communication is "inflammaging" (inflammation + aging), a chronic, low-grade inflammation that happens in the absence of infection, is driven by endogenous signals and a disturbed immune system, and its age-association progression increases susceptibility to age-related pathologies. A major feature of inflammaging is a chronic innate immune system activation, reflected by increased serum levels of proinflammatory cytokines [e.g., interleukin 1 (IL-1), IL-1β, IL-6, IL-8], tumor necrosis factor alpha TNF-α, interferon gamma (IFN-γ), and acute-phase reactants [C reactive protein (CRP)].[89] Inflammaging can be initiated

by the buildup of proinflammatory tissue damage, a defective immune system incapable of effectively removing pathogens and defective host cells, the predisposition of senescent cells to release proinflammatory cytokines, the upregulation of the NF-κB and NLRP3 inflammasome, or dysfunctional autophagy.[90]

Suboptimal prenatal exposures have been associated with developmental deficiencies of the immune system and modulation of the inflammation process, increasing the risk of adult inflammatory/immune diseases and accelerated/premature aging.[91] Increased risk of allergy was described for infants of mothers with increased levels of proinflammatory cytokines in maternal serum due to psychosocial stress.[92] O'Connor et al. described reduced IFN-γ and increased IL-4 responder cell frequencies in 6-month old infants exposed to maternal anxiety.[93] Elevated maternal immunoglobulin E (IgE) during pregnancy was associated with increased IgE in children at the age of 1 year[94] and altered T cell subsets were found in cord blood of children from mothers with allergies.[95]

Stress-induced increases in maternal glucocorticoids can impact fetal immune cells and increase susceptibility to inflammatory diseases.[96] In lambs, prenatal betamethasone and endotoxin exposure increased the mRNA level of the proinflammatory cytokine IL-1b in the pulmonary lymphocytes of progeny, leading to lung inflammation.[97] In addition, prenatal stress increases the protein and mRNA expression of glucocorticoid receptors in lymphoid cells in offspring, which in turn modifies the immune homeostasis.[98]

Prenatal exposure to xenobiotics can also program "inflammaging." Exposure to tobacco during pregnancy had proinflammatory effects, increasing the expression of proinflammatory chemokines and bronchial epithelial cell death, affecting the development of the fetal lung and immune system, increasing the risk of asthma, allergies, and respiratory infections.[99,100] Maternal exposure to high levels of traffic particles also increased the risk of asthma and respiratory disorders in children.[101] Macrophages of newborn mice exposed in utero to ethanol presented deteriorated phagocytic activity.[102]

Prenatal malnutrition, under- or overnutrition have also been linked to a premature inflammation process and development of T2D and CVD[103,104] (Sections 16.2.2 and 16.4.4). Human populations and experimental animal studies provided a link between an adverse gestational environment (e.g. xenobiotics exposure, psychosocial stress, maternal habits, nutrition, etc.) and perturbed immune regulation in progeny that could be related to the development of inflammaging earlier in life.

16.4.2 Cognitive decline and dementia

Brain development is critical to the immediate survival being prioritized during fetal development. The most studied mechanism of psychosocial and physiological stress programming is the HPA axis.[105] Despite the clear relationship between HPA axis dysfunction and increased offspring stress, this is not the only regulatory system programmed in utero. The type, intensity, and length of maternal stressor exposure

during gestation promote different psychiatric phenotypes but consistent behaviors.[106] In animal studies, offspring exposed to prenatal stress present behavioral changes, depressive-like and anxiety-like behaviors, cognitive impairment, lower social integration, and increased aggression.[107]

Offspring sex has been suggested as a critical factor for prenatal stress programming.[108,109] In both humans and animal models, male offspring are more susceptible to neurobehavioral impairments in adulthood after early gestational stress exposure while female offspring were able to change the behavior and improve the learning performance.[110,111] Male offspring are more susceptible to attention deficit, hyperactivity disorder, and earlier-onset schizophrenia.[112,113] Female offspring exposed to prenatal stress had an increased risk of autism spectrum disorders, affective disorders, and behavioral, and intellectual disability.[114–117]

Recent human studies linked maternal overnutrition to offspring long-term decreased cognitive function and dementia. In children, MO predisposes the offspring to lower intelligence quotient and cognitive test scores, impaired neurophysiological development, and autism spectrum disorders.[118–120] Human studies on the developmental programming of dementia are challenging due to the late appearance of this spectrum of disease, lack of patient-parental medical information, and the difficult diagnosis, as in Alzheimer's Disease (AD).[119] Some evidences suggest a potential role of maternal diet in accelerating brain aging, pathological AD markers, and cognitive decline.[27,121,122] Some characteristics such as working memory and fear memory show early impairment in aged-adults exposed to MO.[122,123] Proposed programming mechanisms include decreased brain glucocorticoid and mineralocorticoid receptor binding, impaired hippocampal neurogenesis and neuronal proliferation, regulation of immunity and inflammation, alteration in metabolic and neurotrophic signalling, placental function, and epigenetic modulation.[124–130] The relationship between prenatal stress and maternal overweight with early appearance of cognitive decline, behavioural and neuropsychological disorders is well-established. However, more longitudinal studies are required to understand their association with the early appearance of age-related dementia.

16.4.3 Aging-related neoplasia

The relationship between early aging and neoplasia is complex. Early cell aging due to exposure to stress could be considered a risk factor for the development of cancer. However, mechanisms of DNA repair and tumor suppression have also been shown to cause cell senescence, leading to early aging. Nevertheless, parental history of obesity has been suggested as a risk factor for pancreatic cancer.[131] As already discussed, early development of aging features can be a consequence of cell cycle defects. Downregulation of mitotic checkpoint genes leads to increased levels of the senescent markers p53 and p21.[48,49] Also, lack of tumor suppressors *p16Ink4a* or *p19Arf*, which

can arrest the cell cycle and positively regulate p53, respectively, also leads to increased number of tumors.[50] Naturally aged mice present a natural decline of p53 function, while those with early aging display chronic p53 activation. A mice model with an overactive form of p53 has shown lower incidence of age-related neoplasia, but lower longevity, with an early-aging phenotype.[51,53]

p53 and other DNA repair mechanisms are activated in response to stressors. Increased levels of stressors, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG), glyoxal or methylglyoxal, as well as decreased levels of antioxidants, have been shown in cancers and aging-related diseases.[132] p53 activation was suggested to be related with levels of cellular stress. Basal p53 activation is involved in ROS reduction and improved mitochondrial respiration through regulation of enzymes involved in the mitochondrial respiratory chain. Thus, early exposure to stressors may lead to increased p53 expression, which in turn may be a defense mechanism against cancer. However, chronic p53 upregulation, together with unknown alterations of other DNA repair and cell cycle regulators, may induce cell senescence and early aging. It was demonstrated that the offspring of HFD dams have lower mammary gland levels of p53 and p21, but a link with cancer development was not reported.[133] Another example of the relationship between early aging, senescence, and cancer comes from the study of Ku dimer (Ku70 and Ku80), which is critical for DNA repair and telomere maintenance. Mutations in both Ku70 and Ku80 result in increased sensitivity to ionizing radiation. However, selective Ku70 mutations resulted in higher lymphoma incidence, while Ku80 mutation leads to early aging and low cancer incidence.[134] In utero protein restriction was shown to increase Ku70 and Ku80 in the thymus of the offspring.[22]

Cancer suppression and longevity seem to be related but there is not a direct causal relationship between them. Instead, activation of tumor-suppression mechanisms due to chronic stress exposure may lead to cell senescence and early aging. Recently, the increased mammary tumor incidence in the offspring born to HFD-fed dams was attributed to immune system alterations and suggests a link between neoplasia and inflammaging programming.[135]

16.4.4 Cardiovascular aging

Environmental factors including those during early life can lead to premature impairments in cardiovascular function. Perinatal stages of life are crucial to healthy aging and malnutrition; tobacco and other insults change epigenetic mechanisms, which are triggers to cardiometabolic imprinting and premature aging.[136] Cardiovascular aging leads to morphological changes, with the thickening of the vascular wall, collagen deposition, perivascular fibrosis, causing arterial stiffness and altered vascular tone, which results in an imbalance between vasoconstriction and vasorelaxation. This increases blood pressure and induces cardiac remodeling and failure.[137] CVD increase

dramatically with age in both sexes, even in individuals with no clinical evidence of CVD.[138] However, aspects of this age-dependent remodeling of the heart and blood vessels differ between the sexes. Within the general population, men have higher blood pressure than age-matched women during early adulthood. Aging reduces this sex difference and significantly increases the risk for CVD in women.[139]

Numerous experimental models of developmental insults reported increased cardiovascular risk in the offspring.[140] Human studies have demonstrated a relationship between higher maternal BMI and children's risk for higher systolic and diastolic blood pressure.[141] One important issue in the developmental programming of CVD is the sex-specific mechanisms. Female offspring are usually protected against hypertension and programmed cardiovascular risk during young adulthood.[140] Renin Angiotensin System (RAS) action through AT1R activates the mitogen-activated protein kinases and Akt pathway leading to cardiovascular dysfunctions. Estrogenic hormones crosstalk with RAS, promoting the activation of angiotensin 1−7 pathway, inducing therapeutic effects like vasodilation and anti-fibrotic effects.[142] Age-associated enhanced sensitivity to Angiotensin-2 (Ang-2) in females may be associated with the decrease of estrogen levels.[143] Prevention of redox unbalance by sex steroids may also contribute to the sex-specific risk in CVD programming.[144] RAS and NF-kB crosstalk in the development of CVD, which is also related to inflammaging and age-induced myocardial inflammation and fibrosis. In rat offspring, RAAS is developmentally regulated by maternal diet. Maternal HFD-induced obesity was observed to increase AT1R in the offspring heart and AT, predisposing for hypertension.[145,146] A better understanding of these mechanisms is important to prevent programming of CVD and increase lifespan. Understanding sexual dimorphism is an important strategy to identify potential mechanisms and reduce cardiovascular aging.

16.4.5 Physical frailty

Due to the absence of biological and clinical gold standards for frailty, different definitions emerge in scientific literature depending on the features used to describe it.[147] Frailty is often defined as a clinically recognizable state of increased vulnerability to stressors due to aging-associated impairments in reserve and function, across multiple interrelated physiologic systems causing a decline in homeostatic reserve and resiliency, compromising the ability to cope with everyday activities or acute stressors.[147] Generally applied criteria for frailty establishment comprise shrinking (weight loss, sarcopenia), weakness, and exhaustion (poor endurance), implicating a compromised muscular function.[148] The prevalence of frailty in elderly people ranges from 33% to 88% depending on the criteria used.[149,150] Sarcopenia, or age-related loss of muscle mass, is a physiological process that is responsible for 40% less muscle mass in older people (>80 years old), decreasing muscle cross-sectional area, and strength.

Sarcopenia is strongly related with features of the frailty syndrome, namely low grip strength, slowness, and low level of physical activity, low energy/exhaustion, and unintentional weight loss.[151,152] Age influences the prevalence of frailty, which rises particularly among those aged 80 years or older.[153] Nonetheless, the relationship between frailty and chronic diseases is complex and poorly understood so knowledge on how aging progression interrelates with chronic diseases, weakening biological systems function and particularly the immune system is crucial for designing protective strategies (Section 16.4.1). A Singapore longitudinal aging study, identified eight frailty-related inflammatory markers, namely sgp130, IL-2Rα, I-309, MCP-1, BCA-1, RANTES, leptin, and IL-6R and identified cellular immunity frailty-associated phenotypes, like loss of CD28 expression in $CD8^+$ T cells.[154]

Although there are known associations between early life events, stress physiology, inflammation, and telomere length, knowledge about early life influences on frailty is scarce.[155] In the Helsinki birth cohort study, individuals who were exposed to early life stress, caused by wartime separation during early life, had increased salivary cortisol and plasma ACTH.[156] Recently, this increased stress in early life was being linked to increased relative risk of frailty, specifically for men at an average age of 71 years.[157] Specific aspects of cognitive decline such as psychomotor speed have been associated with frailty,[149] so early life factors that predispose for cognitive decline may also be related with frailty (see Section 16.4.2). Several human observational epidemiological studies linked LBW with decreased adult muscle mass and strength.[158] Although there may be a common biological basis for frailty that may be programming in early life, there may be multiple trajectories for its development. So early detection of subclinical changes or deficits at the molecular, cellular, and/or physiologic level would be a key to preventing or delaying the development of frailty.

16.5 Transgenerational passage of the aging clock—reproductive cell plasticity and selection

A classical paradigm in biology is that the germline/soma separation allows the former to provide future offspring with a "blank slate," regardless of whatever took place in the latter. Changes throughout the lifetime of the individual until parenthood were therefore not considered relevant for primordial germ cells, gonadal stem cells, gametes, and resulting fetuses. However, mounting evidence suggests that what an individual experiences may shape the future health of its progeny (directly, or even in a transgenerational manner), via effects that ultimately translate to changes in gametes, potentially causing transmission of traits in a "Lamarckian" fashion. These traits are usually associated with metabolic and aging-associated disorders. Traditionally, the study of this phenomenon focused on maternal factors, but more recently spermatozoa were shown to also be involved.[159]

It is well-known that increased maternal age increases the risk for infertility, spontaneous miscarriages, and genetic defects in the offspring,[160] but several epidemiological studies also suggest that advanced paternal age may be critical in determining pregnancy outcomes and offspring health. Male aging has been associated with preimplantation loss and miscarriage. It has also been implicated in the increased incidence of neurodevelopmental psychiatric disorders (e.g., schizophrenia, autism spectrum disorders, bipolar disease, and attention deficit/hyperactivity disorder), trinucleotide repeated associated diseases (myotonic dystrophy, spinocerebellar ataxia and Hungtington's disease, among others), cardiovascular pathologies and forms of cancer in the offspring.[161] Additionally, some reports show that the offspring of older fathers present general increases in behavioral issues[162] and mildly diminished IQ when compared to children from younger fathers.[163]

Epigenetic alterations in primordial germ cells and thus spermatozoa represent the likely mechanistic explanation for this phenomenon, contrasting with the previously held dogma that sperm only delivers the paternal genome. Alterations in DNA methylation patterns, histone post-translational modifications and noncoding RNAs (ncRNAs) have been implicated in paternal programming of offspring diseases later in life.[161,164–166] Interestingly, not only spermatozoa but also seminal fluid, possibly via exosomes, can deliver molecules such as ncRNAs, with the potential to influence the fetus.[167] Similarly, the establishment of these epigenetic modifications in primordial germ cells or oocytes due to external factors/aging can also be transmitted to the offspring. It is often difficult to discriminate the effects induced by the in utero environment/maternal nutrition from the contributions of the oocyte itself, thus justifying the use of in vitro fertilization and animal models in these type of studies. For example, when fertilized oocytes of diabetic mice were transferred to non-diabetic pseudo-pregnant recipients, phenotypes such as growth, retardation and congenital malformation were still observed in the offspring,[168] thus discarding a uterine environment influence and pointing out to imprinted oocytes deficits. Furthermore, maternal diabetes as well as HFD-induced MO have been shown to alter DNA methylation patterns in oocytes.[169] Preconception factors may not only modulate offspring health, but also the health of subsequent generations. Transgenerational transmission of traits has been reported not only for extreme conditions and unhealthy lifestyle habits but also for exposure to certain environmental endocrine disrupting chemicals (EDCs). EDCs have been associated with the transmission of a transgenerational panoply of disease phenotypes which also include obesity and diabetes to the next generations, again possibly acting through epigenetic modifications in gonad stem cells, and thus gametes.[165,166,170] However, it should be noted that there is conflicting data on some of these aspects and, for example, analysis of the sperm DNA methylation patterns in humans with grandparents of varying age suggests that age-related changes may not be transmitted in a transgenerational manner.[171]

Finally, although important efforts have been made to unveil how the mitochondrial DNA (mtDNA) bottleneck and purifying selection acts to prevent silent and

unchecked dissemination of invisible mtDNA mutations across generations via the female germline, major advances are still needed to fully understand mitochondrial inheritance.[172] Even low levels of maternal mtDNA mutations transmitted by the oocyte can negatively affect he offspring's health by triggering several mitochondrial-related diseases and influencing lifespan.[172] The current evidence suggests that mtDNA mutations and deletions in oocytes become more prevalent with increasing reproductive age,[173] which is worrisome in societies where the trend is to delay motherhood.

16.6 Life interventions to "Re-set the Clock"

16.6.1 Nutrigenomics as a strategy to revert early life programming

Although adult health and disease may be programmed in early life by nutrition during pregnancy and lactation, there is a considerable lack of knowledge regarding interventions during pregnancy to prevent early-aging programming in the offspring.[174,175] Nutrigenomic studies how nutrients modulate gene expression and may permit personalized dietary interventions to promote health in a specific genotype (Fig. 16.2).[176] Nutrients can regulate epigenetic modifications through modulation of the levels of the universal methyl donor *S*-adenosylmethionine and of the methyltransferase inhibitor S-adenosylhomocysteine. Nutrients such as vitamin B6, vitamin B12, folate, riboflavin, methionine, and choline, are involved in DNA methylation. Bioactive food components such as tea polyphenols, resveratrol, retinoic acid, curcumin, and sulforaphane can affect epigenetic patterns by modulating the levels of *S*-adenosylmethionine and *S*-adenosylhomocysteine or the enzymes that catalyze DNA methylation and histone modifications.[176]

There is a scarcity of studies on ways to reverse accelerated aging programming during pregnancy. Maybe due to the methodological difficulties involved, there are few studies relating maternal interventions to delay programmed offspring aging. Several compounds such as melatonin and resveratrol exhibited beneficial effects on mitigating oxidative stress and regulating gene expression, improving fertility or embryogenesis, but long-term effects on aging are still missing.[177,178] In animal models, it was found that folic acid supplementation during pregnancy and lactation prevented the TNF-α and IL-1β upregulation and neurogenic and memory decline in 18-month-old rats of naturally aged offspring.[179] Diet can be used to neutralize inflammaging and the epigenetic changes associated with aging, promoting health.[174] However, it is important to understand how nutrients act at the molecular level during specific programming windows and recognize their signaling properties to potentiate nutrigenomic interventions for health and delay aging. For instance, human caloric restriction during adulthood is linked to increased lifespan,[180] but to increased offspring disease incidence during pregnancy (reviewed by[181]).

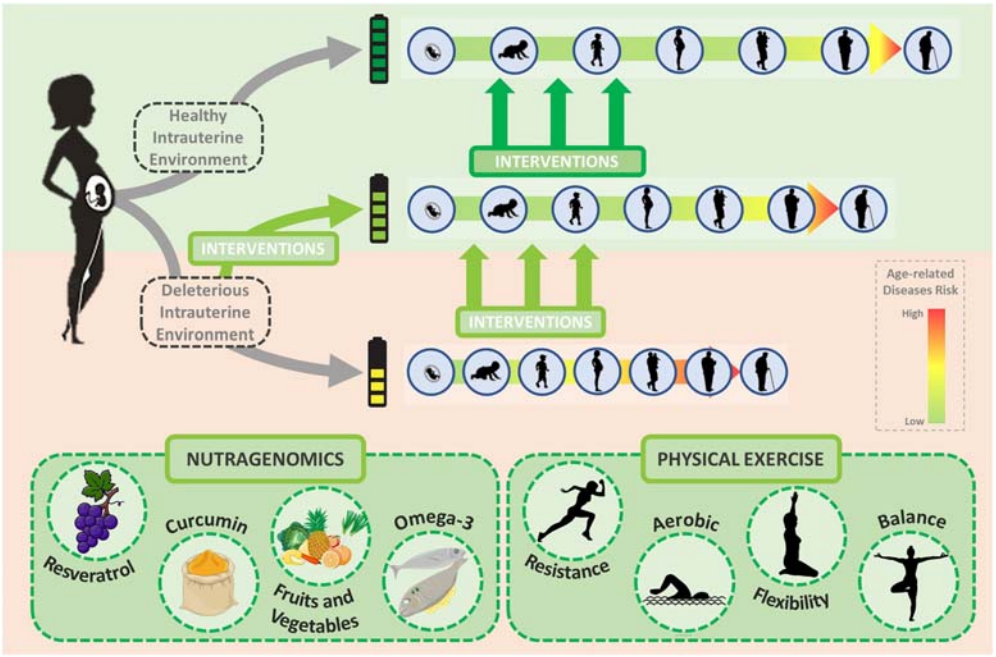

Figure 16.2 Specific developmental stages comprehending prenatal and early life can be critical periods to promote healthy aging and increase life span. Being exposed during these stages to interventions might permanently change the body's structure, metabolism, and physiology, and hence promote health in later stages of life. Some non-pharmacological interventions, such as nutrigenomics and physical exercise create beneficial effects for human health, preventing the early appearance of age-related diseases, promoting healthier aging, and increasing lifespan (upper panel). Dietary components have substantive effects on metabolic health (lower left panel); for instance, bioactive molecules capable of selectively modulating specific metabolic pathways and gene expression alter fetus programming, clinical outcomes, and disease predisposition. Different types of physical exercise (lower right panel), such as resistance, aerobic, flexibility, and balance, are also capable of regulating the inflammatory and endocrine status, and potentially improving offspring health. Minor progress in improving the early-developmental processes can potentially result in notable prevention of early age-related disease, delay aging-related morbidity and mortality, and remarkable extension of healthy lifespan.

16.6.2 Running against aging—exercise as anti-aging "medicine"

The combination of resistance, aerobic, flexibility, and balance exercises is a well-established strategy to prevent sarcopenia, consistently demonstrating good results in preventing falls in older populations with physical frailty, whether they were obese or not.[152] Such effects have been attributed to decreased muscle cell senescence and inflammation, together with increased anabolism and protein synthesis.[17] Importantly, exercise was shown to upregulate muscle IL-6 secretion and other anti-inflammatory cytokines, together with muscle hormones—myokines—such as IL-15, irisin, or FGF21, being associated with lower adiposity and better cardiovascular outcomes.[182,183]

Aerobic exercise was shown to increase VO_2 max in frail elderly people and to improve cardiorespiratory function, preventing atherosclerosis and ischemia–reperfusion injury.[152,183] Such effects were attributed to the amelioration of the inflammatory milieu, reducing inflammaging and upregulating anti-aging pathways, such as SIRT1, as well as decreasing endothelial cell senescence in elderly people, leading to preserved vascular function.[184–186] Accordingly, physical exercise was shown to delay aging-related decline of cerebral perfusion, brain aging, and dementia (AD and mild cognitive impairment), in part due to upregulating synaptic plasticity genes.[187]

The beneficial effects of exercise involve the regulation of several aging hallmarks, besides those already mentioned. At the genomic level, exercise prevents genomic instability through upregulation of DNA repair mechanisms, to reduce telomere shortening in leukocytes and to control the epigenetic regulation of SIRT1 and BDFN.[154] Exercise activates proteostasis, namely autophagy in muscle, but also in the brain and heart.[152,188] At the metabolic level, exercise prevents the age-related decline of mtDNA and mitochondrial proteins, preserving the oxidative capacity, which are features of sarcopenia. Moreover, exercise-induced activation of SIRT1 and PGC-1alpha was associated with increased mitochondrial biogenesis even in elderly people.[152]

Adherence to exercise programs in elderly people may be difficult due to several medical problems, sensory impairment, cognitive dysfunction, and depression, and should be considered in early life stages in order to prevent aging hallmarks. In the context of developmental programming, exercise during pregnancy has been widely shown to reduce the risk of preterm birth, low- and high birth weight and childhood obesity of children from mother both with normal BMI or obesity (Fig. 16.2),[189] while others found no differences.[190] Thus, the impact of exercise during critical development phases in preventing early-aging programming is currently not known. Nevertheless, in rats, maternal exercise during pregnancy was shown to program the offspring to higher liver AMPK expression and to prevent paternal-induced hepatic steatosis.[191,192] Such protective effects of maternal exercise were recently attributed to increased placental expression of Superoxide Dismutase-3.[193] With the necessary adaptations, the practice of exercise during pregnancy could be a beneficial strategy to enhance offspring health and mitigate accelerated aging.

16.7 Conclusion

The continued increase in life expectancy is a major achievement of the recent past. The population is getting older, but often not in good health and with reduced quality of life. This is confronting the population with huge challenges. The major challenge is to ensure that people not only can live longer, but also live healthy, active, and independent lives. The European Commission identified active and healthy aging as a major societal challenge. Biological aging is inevitable, but we can and must control the consequences of this aging process to make it as pleasant as possible, so we can no

longer ignore that aging begins in the same moment that a new life is created! Consequently, studying aging during early life may provide new targets to regulate this deterioration from the beginning and, prevent or delay the progress of unfavorable health outcomes throughout adulthood and elderly stages.

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SECTION 3

Aging-related physiology, disease and prevention of aging-related diseases

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CHAPTER 17

Polypharmacy and medication adherence

Marta Lavrador[1,2], Ana Cristina Cabral[1], Margarida Castel-Branco[1,2], Isabel Vitoria Figueiredo[1,2] and Fernando Fernandez-Llimos[3,4]

[1]Pharmacology and Pharmaceutical Care Laboratory, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal
[2]Coimbra Institute for Clinical and Biomedical Research (iCBR), University of Coimbra, Coimbra, Portugal
[3]Laboratory of Pharmacology, Department of Drug Sciences, Faculty of Pharmacy, University of Porto, Porto, Portugal
[4]Center for Health Technology and Services Research (CINTESIS), University of Porto, Porto, Portugal

17.1 Adherence to therapy and medication management

Nonadherence to prescribed medicines is a major public health issue intricately related to multimorbidity and polypharmacy. Increasing the number of medicines prescribed to a patient, regardless of whether polypharmacy can be classified as appropriate or inappropriate, increases the risk of medication nonadherence. Nonadherence is one of the factors more closely associated with poor control of chronic diseases, such as diabetes, hypertension and dyslipidemia but also with a rapid onset of complications and a subsequent decrease in the patient's quality of life. From a systems perspective, the impact of nonadherence in healthcare systems must also be considered because it is responsible for over 20% of hospital admissions due to preventable adverse events, leading to unnecessary costs with a major economic impact.

Aged patients are at greater risk of nonadherence behavior due to the greater number of medicines they are prescribed, their lower health literacy, and their potentially decreased cognitive capacities compared to younger populations.

17.1.1 Definition and classification

Adherence to therapy is defined as the extent to which a person's behavior (e.g., taking medication, following a diet, or executing lifestyle changes), corresponds with agreed recommendations from a healthcare provider.[1] Adherence to therapy is a multifactorial and dynamic process shaped by several interacting factors, including personal, clinical and social variables. In 2003, the WHO identified factors that influence patient adherence behaviors, highlighting five dimensions:

- Patient-related factors—encompass not only forgetfulness and misunderstanding instructions about how to take medications, but also patient knowledge, skills in managing disease symptoms and treatment, and patient beliefs about medication.

Aging
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Some patient-related factors are particularly important among aged patients, such as the preference for the use of complementary and alternative medications, lower self-care, resisting care, poor independence when taking medications, lack of interpersonal relationships, living at one's own home, poor health literacy, lack of medication knowledge, and misunderstanding of verbal instructions;

- Therapy-related factors—related to treatment itself. This dimension includes the complexity of the medication regimen, not only associated with monotherapy or polypharmacy but also the number of daily doses and the presence or absence of adverse effects of treatment.[2] In older patients, factors such as formulation, packaging, generic substitution, frequent changes to the regimen, poor or complex labeling instructions, short-term medications and lack of immediate consequences of missed doses are relevant items to consider;
- Condition-related factors—associated with the characteristics of the diseases, such as its duration, symptomatology, and changes in quality of life;
- Social/economic factors—that comprise the patient's socioeconomic status, as well as the costs of medication. In elderly individuals, the distance from the treatment setting and family support, especially if living alone or a caregiver is needed, are particularly significant;
- Health system-related factors—involve how healthcare providers interact and communicate with their patients, the quality of the relationship between patient and health professionals, physician knowledge and motivation, implementation of guidelines and therapeutic intensification. In older people who often have multimorbidities and are followed by more than one specialist, the lack of interprofessional communication or the lack of medication review and follow-up are important factors to consider.

Accordingly, adherence to therapy requires that the patient has cognitive, emotional and behavioral skills. In fact, nonadherence involves situations where the inability to follow the regime as prescribed is the main cause of inappropriate behavior but also involves situations where the patient is not willing to follow the regime, regardless of his/her ability. Therefore, nonadherence can be classified as intentional or unintentional:[3]

- Intentional nonadherence is conditioned by the patient's will, who consciously does not take medication as prescribed. It is affected by the patients' beliefs, attitudes and expectations and is influenced by the patients' motivation to begin and persist with the medication regimen. Patients' concerns about the value or effectiveness of medicines, their side effects, and the inconvenience of taking the medicines at the prescribed time and frequency are balanced against the perceived necessities and benefits of taking the medication, as explained by the necessities-concerns framework.[4]
- Unintentional nonadherence is conditioned by the patient's capacity to follow treatment recommendations. Regardless of the patient's will, adherence is prevented by practical barriers, which include forgetfulness, cognitive problems,

misunderstanding instructions, physical ability, poor organizational skills, polypharmacy, and difficulty accessing medicines. This type of nonadherence behavior is highly prevalent in aged people.

17.1.2 Assessment methodologies

Being adherent to therapy is a multifactorial process, and the assessment of medication adherence has become challenging. To accurately determine a patient's level of medication adherence, several approaches have been developed using a diversity of methods and tools for this purpose. However, different types of nonadherence behavior and the complexity of the assessment methods, together with the biases associated with any assessment of inappropriate behavior (e.g., Hawthorn effect), have prevented the development of a gold-standard method.[5]

Methods to assess medication adherence can be classified into direct and indirect measures. Direct methods, are those that assess objective parts of the medication taking process, and are more accurate, robust, and objective but are also more expensive, time and human resource consuming and, on many occasions, impractical for routine use. Indirect methods are cheaper, more practical and easier to apply and allow not only the assessment of adherence but also patient behavioral patterns. On the other hand, indirect methods are not as reliable as direct methods and are subject to bias as social desirability and response acquiescence (Table 17.1).

The nonadherence type, intentional or unintentional, may also influence the accuracy and reliability of the assessment method used. The results of self-reported questionnaires can be easily dodged by intentional nonadherence, but unintentional nonadherence may also suffer from social desirability bias and overstating their compliance. Intentional nonadherents may trick any adherence assessment method, direct or indirect, through different strategies: opening the MEMS containers or extracting the pills from the container without actually taking them, regularly acquiring the medicine in the pharmacy and discarding or storing them, and taking the medicine appropriately a few days before the medical appointment. These limitations of direct methods, together with their cost, make them useless in daily practice.

Self-reported questionnaires are the most commonly used method to assess adherence. Several validated and culturally adapted questionnaires are available in many languages. Some questionnaires assess only adherence behavior, while others identify beliefs about medicines as a proxy to differentiate intentional from unintentional nonadherence.[4] More modern questionnaires try to compile these two elements into a single questionnaire with an acceptable length for in-clinical practice.[6] However, it is important to highlight that the method of collecting patient-reported data, or mode of administration, affects the accuracy and quality of the data obtained. A questionnaire can be administered by an interviewer, usually the healthcare professional, or presented

Table 17.1 Characteristics of methods of assessment of adherence to therapy.

		Advantages	Disadvantages
Direct measures	Directly observed therapy	• Noninvasive	• Expensive • Mobilization of human resources • Requires constant return visits • Impractical for routine use
	Measurement of the level of medicine or metabolite Measurement of the biologic marker	• Accurate • Objective	• Expensive • Invasive • Depends on the pharmacokinetics of the drug • Affected by drug and food interactions • Affected by "white-coat adherence" • Not available to all drugs
Indirect measures	Rates of prescription refills	• Inexpensive • Noninvasive • Easy application	• Only valid to assess chronic medication adherence • Depends on the fidelity of the patient to the pharmacy - reliable only in hospital or pharmacy
	Pill counts	• Inexpensive • Noninvasive • Easy application	• Easily altered by the patient • Affected by social desirability
	Electronic Medication Monitors (MEMS)	• Precise • Noninvasive • Easy application • Tracks patterns of taking medication	• Expensive • Not adapted to all pharmaceutical forms • Requires return visits to download data from medication vials
	Patient questionnaires, patient self-reports	• Inexpensive • Noninvasive • Easy application • Quick data collection • Provides data on behavioral patterns of the patient, as well as their attitudes and beliefs	• Affected by phenomenon such as social desirability or response acquiescence • Influenced by health literacy level of the patient

to the patient for self-administering. Self-administration reduces costs and time and reduces the occurrence of social desirability or response acquiescence biases. However, self-administration can be a problem in aged patients because of their limited health literacy and cognitive functions, which may result in incomplete and invalid questionnaires. Conversely, professionals administering the questionnaire can ensure that patients respond to the entire questionnaire and help the patient with their limitations. However, interviewer-administered questionnaires were demonstrated to be highly influenced, underestimating the magnitude of nonadherence.[7]

17.1.3 Interventions in medication nonadherence

Considering the multifactorial characteristics and different types of nonadherence behavior, a single intervention is not sufficient to optimize medication adherence. Tailoring the adherence-enhancing intervention is a basic requirement for success (Fig. 17.1). Several simple interventions can be implemented alone or in combination.

17.1.3.1 Patient education

With the purpose of increasing the acquisition of skills and competencies and improving patients' knowledge about medications and diseases, educational strategies contribute to enhancing confidence regarding medication management and medication-taking ability, while reducing intentional nonadherence. Patient education can be performed only using oral communication, but to optimize results and systematize the information provided, education can be complemented with the use of audio-visual resources and written materials. Healthcare professionals should use patient education strategies to:

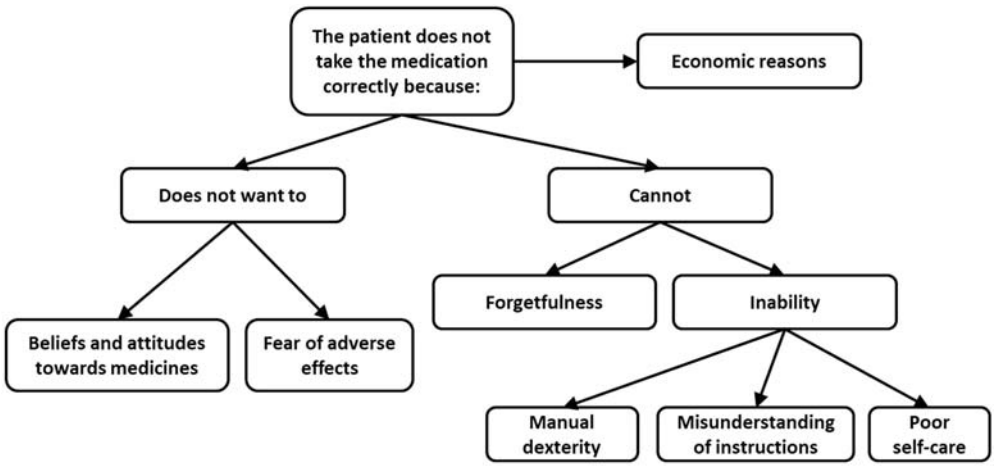

Figure 17.1 Flow chart to decide the intervention to enhance medication adherence.

- Inform patients about positive and negative consequences of adherence behavior;
- Inform patients about disease signs or symptoms and specific therapeutic goals, as well as empower patients to self-monitor symptoms/signs related to medications. This could include subjective experience of symptoms as well as information from devices, such as sphygmomanometers or blood glucose monitors;
- Teach patients to minimize or manage medication side effects (e.g., sucking on lozenges or carrying a water bottle if medications cause dry mouth);
- Understand patients' beliefs toward medicines to reduce their concerns about potential negative consequences and improve their positive attitudes toward healthcare and medication.

According to the goals of the educational intervention and the individual characteristics of the patients, strategies vary in the time spent by the health professional, the number of home visits or clinic/pharmacy visits, and the use of phone calls or video/teleconferences. The literature has demonstrated that interventions are less effective when they are delivered in patients' homes compared to other settings, such as clinics or pharmacies.[8]

Educational interventions to enhance medication adherence may involve individual or group educational programs (e.g., inpatients, family, day care centers). Group educational activities may contribute to increasing patients' social support, which is especially important in elderly individuals who live alone, creating self-help groups, adherence "buddies" or increasing social support for medication adherence from their social relationships.

The efficacy of educational interventions decreases over time, necessitating regular follow-up with patients. Complex interventions (i.e., constituted by more than one component) incorporating educational, attitudinal, and technical elements are more effective than single component interventions and demonstrate greater sustainability of adherence over time. However, complex interventions comprising too many components may overwhelm patients and produce a negative effect. Similarly, brevity is critical for maximizing the impact of educational interventions, as the patients' ability to concentrate and assimilate information is limited over time, especially in aged patients. Moreover, brief interventions are also less time consuming for healthcare professionals, which allows a greater number of patient-professional encounters to maximize the reach, scalability, and impact of interventions. Thus, brief complex interventions are preferable and have advantages over single-component or longer interventions.[9]

17.1.3.2 Supporting materials

Used alone or in combination with other types of interventions, written materials contribute to systematizing patients' education but also to preventing forgetfulness and minimizing medication-related confusion. Different supporting materials can be provided to patients according to their individual characteristics and needs:

- Information leaflets on disease, diagnosis, control and treatment;
- Individualized medication lists;

- Large print labels placed on medication packages;
- Written medication instructions, including drug cards or other formats, which could be placed on the front of the refrigerator to remind patients of the drug administration technique. This is particularly important in medications that require administration devices, such as inhalers in chronic obstructive pulmonary disease and insulin pens in diabetes;
- Calendars that chart when medications should be taken. Some of these calendars may require patients to register the use, which can be used by professionals as an adherence monitoring method.

For the appropriate use of these supporting materials, a previous assessment of patient health literacy is required. Different supporting materials can be used for different patients (e.g., replacing written instructions with icons). Testing the supporting material with each individual patient is a basic requirement to ensure comprehension. In addition to poor health literacy, cultural disparities may also limit the value of these materials.[10]

17.1.3.3 Medication regimen optimization

Medication regime complexity is a major driver of unintentional medication nonadherence, especially in an aging population, with reduced self-management abilities.[2] Aiming to optimize patient therapy, simple actions, such as medicine package modifications, medication regimen simplification, and dosage form adjustments, are important and contribute to increasing patients' treatment understanding and satisfaction. For example, changing the medication regimen to have less frequent administration of medications, by switching from multiple doses to sustained-release medications, or using fixed-dose combination pills that conglomerate more than one drug in a single pill may contribute to better health-related quality of life and reductions in adverse drug events.

These simplification measures also require patient involvement and professional monitoring after their implementation. Reducing the number of administrations may create new challenges in a patient using a given administration "beacon." For instance, weekly administration may simplify the schedule but may require creating new adherence beacons in the patient who was previously using a daily meal as a reminder. Additionally, skipping one dose in a daily administered drug may have much less impact than skipping a weekly dose. As is common, tailoring adherence-enhancing interventions is a basic requirement for success.

17.1.3.4 Medication organizing systems

Medication organizing systems are different types of gadgets that allow organization of the medication to be used by a patient according to the dosage schedule. Pill boxes, pill cassettes, blister packages, unit-of-use packaging, and special containers with the

time of the dose printed are useful aids for patients whose primary reason for nonadherence is forgetfulness and inability to manage their medication regime. Pill boxes may be prepared weekly or monthly by a healthcare professional, and they may contribute to facilitating the routine associated with medication taking.

However, two aspects must be considered before initiating medication organizing systems. As with any intervention where a new element is provided to a patient, testing the comprehension and usability of the instrument is required. However, the willingness of the patient to use these compliance aids must also be explored though a person-centered approach. Studies have demonstrated that some patients are reluctant to accept these because they considered maintaining independence and remaining in control to be important.[11]

17.1.3.5 New technologies

Although the elderly are typically more averse to new technologies, a reverse trend may exist. Electronic gadgets and smart phones are increasingly used by older people. Medication administration reminders using text messages or communication apps (e.g., WhatsApp) can be sent to each patient from a computerized system at the healthcare professional's office. These messages may be especially useful in weekly or monthly drug administrations, where a daily routine does not help. The use of medication regime schedule apps may not only help with administration reminders but also allow recording the administration time and even reporting to a monitoring system in healthcare professionals' offices if patients allow.

Home automatic pill dispensers, available at reasonable prices, can help patients living alone when they have complex medication regimens with a number of different medicines to be used at different intervals.

17.2 Concept of polypharmacy

The generalized use of medicines by the population is one of the main causes of increased life expectancy during the 20th century. However, medicines are also associated with iatrogenic harm, especially when a patient simultaneously uses too many medications. The term polypharmacy was coined to refer to situations where too much medication is being used by one patient.[12] The difference between many medicines and too many medicines is subtle. Generally, a patient is considered to be under polypharmacy when using five or more medicines concomitantly. However, the numerical definition of polypharmacy has limited value in clinical practice. For instance, according to clinical practice guidelines, a patient under secondary prevention of myocardial infarction requires the concomitant use of 5 different medicines (2 platelet aggregation inhibitors, 1 statin, 1 angiotensin-converter enzyme inhibitor, and 1 beta-blocker). If this patient suffers simultaneously from another condition, the

number of medicines would increase. Using the numerical definition of polypharmacy, this patient is under polypharmacy, although they are not using too many medicines.

However, it is important to note that the number of medicines used directly influences medication regimen complexity, which is strongly associated with two problem-prone situations: difficulty in medication self-management and a higher probability of medication nonadherence. The first produces a patient, although willing to follow the regime as it was prescribed, who fails to comply due to limited health literacy and self-efficacy, which is especially common among aged patients. The second situation is associated with negative patient behavior produced by what the patient considers an excessive number of medicines, which results in a voluntary desertion of the prescribed regime.

This paradox led to the use of polypharmacy in a nonpejorative manner and the differentiation between appropriate polypharmacy, which is evidence-based and beneficial, from inappropriate polypharmacy, which can be potentially harmful. Inappropriate polypharmacy can be defined as the prescription of multiple medicines that are either inappropriate or no longer indicated for a given patient.[13]

17.2.1 Potentially inappropriate medication in the elderly

The prevalence of multimorbidity increases with age, with more than 65% of aged patients suffering from three or more chronic conditions.[14] Single-disease guidelines frequently favor the use of several medicines to treat the condition. Aggregating the recommendations of several guidelines required for a multimorbid patient unavoidably results in polypharmacy. However, these evidence-based, single-disease guidelines usually do not take into consideration multimorbidity associated with aging and frequently ignore age-related physiological changes that occur in this population. Therefore, strict obedience to these guidelines, instead of having a holistic vision of the aged patient, often results in inappropriate polypharmacy. This situation may worsen with the prescribing cascade subsequent to the occurrence of adverse effects.

Although with some differences, inappropriate prescribing is a related concept to inappropriate polypharmacy. Potentially inappropriate medications (PIMs) are defined as medicines prescribed to a given patient for whom the risks outweigh the benefits. PIMs can also be defined as less cost-effective medicines when equally or more effective alternatives are available.[15,16] According to Spinewine, three different categories of inappropriate prescribing exist:[17]

- **Underprescribing**—omission of a medication that is needed (a clinical indication without therapy);
- **Overprescribing**—prescription of a medication that is clinically not indicated (unnecessary therapy);
- **Misprescribing**—Incorrect prescription of an indicated medication.

Misprescribing can be subdivided into the following categories associated with potential prescribing errors:

- **Medication dose**—prescribed dose is incorrect or not adapted to patients' characteristics (e.g., renal function);
- **Duration of therapy**—duration of therapy is too short or too long according to available evidence. A duration of therapy that is too long is very often associated with prescribing inertia, which can be defined as the automatic renewal of a medication even when the original indication is no longer present;
- **Therapeutic duplication**—inappropriate prescription of drugs from the same pharmacological class;
- **Drug-drug, drug-disease and drug-food interactions** have potential negative effects on therapeutic outcomes.[17]

17.2.2 Pharmacokinetic and pharmacodynamic changes in older people

Overprescribing and misprescribing are particularly challenging in older people due to age-related physiological decline and frailty. Although there is enormous variability among individuals, several physiological changes occur with a progressive reduction in organ functioning and homeostatic reserve.[18] These changes have an impact on the pharmacokinetic and pharmacodynamic characteristics of drugs and may affect older patients' response to drugs, negatively affecting patient safety.[16]

All pharmacokinetic stages, absorption, distribution, metabolism and excretion, may be affected by aging. Although several age-related changes exist in the gastrointestinal system, such as a decrease in the overall surface of the intestinal epithelium, a decrease in gut motor function, reduced blood flow, and a decrease in gastric acid secretion, absorption of the majority of drugs that permeate the gastrointestinal epithelium is not diminished in older people. However, an age-dependent reduction in absorption rate may occur for some drugs (e.g., indomethacin, prazosine, and digoxin). A reduction in the rate of transdermal, subcutaneous and intramuscular drug absorption may also occur in aged patients due to decreased tissue blood perfusion.[19]

Drug distribution is also affected by aging because important physiological changes that influence the volume of the distribution of hydrophilic and lipophilic drugs occur. In older people, skeletal muscle mass declines, body water content decreases, and fat content increases. Consequently, the volume of distribution of hydrophilic drugs decreases, and subsequent doses used in younger individuals result in higher plasma concentrations in older adults (e.g., aspirin, famotidine, lithium). Conversely, the volume of distribution of lipophilic drugs increases (e.g., amiodarone, diazepam, verapamil). A clinically relevant consequence is a prolonged half-life, which results in a longer time required to reach the steady-state concentration with a higher risk of toxicity. Nonetheless, the frailty present in very old individuals is characterized by marked weight loss, with a consequent decrease in

fat proportion. Therefore, in these patients, the volume of distribution of lipophilic drugs may be decreased.

Other age-related changes that may directly affect drug distribution are associated with plasma protein binding. Drugs can bind to plasma proteins, but only the unbound fraction is pharmacologically active. One of the most prevalent plasma proteins, albumin, exhibits reduced plasma levels with aging. Subsequently, an increase in the free fraction of albumin-bound drugs occurs in older patients (mostly with acidic compounds, such as diazepam, phenytoin, warfarin, and salicylic acid). Conversely, α1-glycoprotein, a plasma protein that predominantly binds to alkaline drugs (e.g., lignocaine, propranolol), is either increased or unchanged. These changes may affect the proportion of unbound pharmacologically active drug fractions. However, these modifications are more common in situations of acute illnesses or malnutrition.

Finally, an age-dependent increase in blood brain barrier permeability and dysfunction in the P-glycoprotein efflux pump also exists in older patients. These changes may result in a higher distribution of drugs to the central nervous system (e.g., rifampicin and cyclosporin).[20]

Drug metabolism mostly occurs in the liver. Hepatic clearance depends on hepatic blood flow, plasma protein binding, and intrinsic hepatic clearance. Important age-related changes occur, such as a decrease in liver size and a reduction in liver blood flow. The decrease in liver size leads to a reduction in CYP enzymes with a consequent reduction in intrinsic hepatic clearance. A reduction in phase I reactions may occur, while phase II or conjugation reactions remain unchanged in older people. Decreased liver blood flow mostly affects the clearance of drugs that display high hepatic extraction (e.g., lignocaine, pethidine, and propranolol), which will cause reduced hepatic metabolism. This metabolic reduction leads to a higher drug half-life, which will require dose adjustments. However, hepatic metabolism of drugs with low hepatic extraction (e.g., carbamazepine, diazepam, phenytoin, theophylline, and warfarin) is usually not affected by reduced hepatic flow, with hepatic intrinsic clearance being the limiting factor. Drugs with extensive first-pass metabolism (e.g., propranolol and verapamil) may have increased bioavailability, and prodrugs may have slower activation in the aged liver (e.g., enalapril and perindopril).[19]

Drug renal excretion is decreased with aging as well. Renal blood flow decreases, along with glomerular filtration rate and tubular secretion. As a result, an age-dependent decline in the clearance of all drugs mostly eliminated by the kidneys is expected, with a consequent increase in drug serum levels (e.g., digoxin, aminoglycoside antibiotics, and lithium). Serum creatinine may not be a reliable indicator of renal function in aged people. Reduced muscle mass, typical in this population, may affect the production of creatinine, and a normal creatinine serum value may not represent normal renal function in these patients. Several formulas to calculate creatinine clearance adapted to body weight are available to guide healthcare professionals in making dose adjustments for older patients.

Alongside these pharmacokinetic changes, aging is also associated with increased pharmacodynamic sensitivity to some drugs. These age-related changes may occur not only at the receptor and signal transduction levels but also through homeostatic mechanisms that may be attenuated, possibly compromising both drug safety and efficacy. Counter regulatory mechanisms are reduced in elderly individuals, resulting in a prolonged time required to restore the original steady state after a pharmacological perturbation. A paradigmatic example of this phenomenon is the increased susceptibility of older people to orthostatic hypotension in response to drugs that lower arterial blood pressure. Other augmented drug effects can also occur in the following common situations with aged individuals: dehydration, hypovolemia, and electrolyte disturbances in response to diuretics; bleeding risk with oral anticoagulants; or hypoglycemia with oral antihyperglycemic drugs. Changes in drug receptors and signal-transduction mechanisms also develop in older people, such as the downregulation of β-adrenergic receptors with a concomitant reduction in β-blocker effectiveness. In the central nervous system, significant modifications also exist, such as a reduction in the number of receptors, namely, muscarinic and dopaminergic receptors. As a consequence, an increased susceptibility to the antimuscarinic effects of some drugs may occur, leading to agitation, confusion, and delirium. Vulnerability to extrapyramidal effects may also be enhanced when antipsychotic drugs are used.

17.3 Inappropriate polypharmacy management

Avoiding the occurrence of polypharmacy may be an impossible mission. Increasing longevity and the development of new medicines to treat conditions more accurately diagnosed are major drivers of polypharmacy. Professional hyperspecialization, together with single-disease care, are additional challenges. To overcome the potential negative consequences of inappropriate polypharmacy, it is necessary to have polypharmacy management strategies aimed at promoting appropriate medication.[21] Polypharmacy management can be defined as a "whole system approach that optimizes the care of multimorbid patients through maximizing benefit while reducing the risks of inappropriate polypharmacy."[14] Different countries have established polypharmacy management programs, usually based on comprehensive reviews of patient medication profiles performed by family physicians or clinical pharmacists trying to identify inappropriate medications prescribed to aged patients.[13] These medication reviews are based on two different approaches: (1) implicit criteria—a systematic process that guides a subjective clinical judgment about appropriateness, (2) explicit criteria—the application of a set of previously validated sets of criteria to objectively identify PIM, and (3) a mixed method including both strategies. A systematic review identified 46 different tools to assess prescribing appropriateness.[22]

17.3.1 Implicit tools

Clinical judgment does not necessarily require the existence of an instrument to guide the assessment process. However, these systematic tools demonstrated increased efficacy with fewer mistakes when performing many clinical procedures. Implicit tools for medication review are instruments to guide clinicians in reviewing medication profiles. They consist of lists of items (or checklists) that serve as reminders of the several topics to be considered, including patient-specific characteristics and even patient preferences. This greater adaptability to patient characteristics but also to professional knowledge are their major strengths. However, implicit criteria tools are frequently criticized because they are highly subjective, time-consuming and have low interrater reliability.[16,17,22]

The medication appropriateness index (MAI) is the most relevant and internationally validated implicit tool.[23] Created in 1992 to guide medication review processes,[24] the MAI is a measure of prescribing appropriateness that assesses ten aspects of each item in a patient's medication profile: indication, effectiveness, dose, correct directions, practical directions, drug-drug interactions, drug-disease interactions, therapeutic duplication, duration of therapy, and cost.[23] The MAI provides specific instructions to standardize the assessment process and classifies each medicine in the profile as appropriate, marginally appropriate, or inappropriate. Medicines classified as inappropriate score from 1 to 3, depending on the criteria involved, using a weighted score that gives a measure of prescribing inappropriateness.[17] Although several studies defend against good intrarater and interrater reliability and the good reputation and content validity of the MAI, this instrument does not assess underprescribing and does not detect adverse drug reactions or prescribing cascades.[17]

17.3.2 Explicit tools

Explicit tools to guide medication review are instruments containing sets of specific objective criteria, ranging from simple lists of drugs to more complex conditional criteria, to identify PIM from a patient's medication profile. Their main strength is that their use implies little or no clinical judgment, which results in high interrater reliability and is amenable to use in computerized clinical decision support systems. Explicit tools are mostly derived from published reviews, expert opinions, and consensus techniques and do not always have a sufficient pharmacological basis, which is considered their major limitation. A recent systematic review identified 36 different explicit tools.[25]

17.3.2.1 Indicators for preventable drug-related morbidity

The indicators for preventable drug-related morbidity (PDRM) were an attempt to create an objective and explicit instrument to evaluate a patient's medication profile, aiming to identify medicines prescribed with an associated risk of negative outcome. Drug-related morbidity is defined as the failure of a therapeutic agent to produce the intended

therapeutic outcome, resulting from either the production of an adverse effect or the failure to obtain the desired effect within a reasonable time. Drug-related morbidity is considered preventable if four aspects exist: given an adverse clinical outcome, a preceding drug-related issue must have been recognizable; the adverse outcome or treatment failure must have been foreseeable; and the causes of the drug-related issue and the outcome must have been identifiable and controllable.[26,27]

PDRM indicators are created by collating a suboptimal process situation that involves the use of a medicine with the potential negative outcome expected if that process exists in a patient. These PDRM indicators can be created using a simple conditional criterion:

- Negative outcome expected: Fall or hip fracture or other bone fracture
- Process of care: Use of a tricyclic antidepressant (e.g., amitriptyline, imipramine, etc.)

However, these indicators can also be constituted by more complex criteria:

- Negative outcome expected: Emergency visit/hospitalization due to depression or increase in dosage of antidepressant
- Process of care: the conjunction of
  - History/diagnosis of depression
  - Use of a sympatholytic antihypertensive (e.g., reserpine, methyldopa, clonidine, etc.)

In 1999, in the USA, Mackinnon et al. created 52 PDRM indicators for older people.[28] In 2002, Robertson et al. validated Mackinnon's indicators in the older Canadian population, and in 2003, Morris et al. validated 29 PDRM indicators for use in the older UK population.[27]

To accompany scientific evidence and clinical practice evolution and to maintain their clinical relevance, PDRM indicators should be regularly updated. The updating process requires consensus panel teams and time-consuming processes that impede the normalization of these PDRM indicators.

17.3.2.2 Beers criteria

Beers criteria are probably the best known explicit criteria to identify PIMs in elderly individuals. They were created in the USA in 1991 by Mark H. Beers and have since been the subject of several updates. The first version of the Beers criteria was specifically designed for nursing home residents and included 30 medications that should be avoided in this population. In 1997, Beers updated the tool to a more comprehensive set of criteria, organized according to the following categories: (1) medications or medication categories that should generally be avoided because they pose unnecessarily high risk for older adults persons; (2) risky drugs if exceeding a maximum recommended daily dose; and (3) drugs to be avoided in patients who had specific comorbidities. This version aimed to be applied to all older people and not only to nursing

home residents. In 2003, Beers criteria were again updated to include new drugs available in the market and to integrate new scientific evidence. In 2003, the authors classified PIMs into two categories: PIMs independent of diagnosis and PIMs dependent on diagnosis. In 2012, a new version of the Beers Criteria was released with the support of the American Geriatric Society (AGS).[29] Based on a comprehensive systematic review, 53 medications or medication classes were compiled and divided into three categories: (1) PIMs and classes to avoid in older adults; (2) PIMs and classes to avoid in older adults with certain diseases and syndromes that the drugs listed can exacerbate; and (3) medications to be used with caution in older adults. With this last category, the authors aimed to include drugs that could be plausibly used in a significant number of situations but with great potential for misuse or to cause harm. After 2012, AGS updated the Beers criteria twice. The most recent version of the Beers criteria was released in 2019 and presented a more complex classification of inappropriate drugs for elderly individuals (Beers Table 1, Beers Table 2, etc.):

- Table 1—"Designations of Quality of Evidence and Strength of Recommendations";
- Table 2—"Medications to avoid for many or most older adults";
- Table 3—"Medications to avoid for older adults with specific diseases or syndromes";
- Table 4—"Medications to be used with caution";
- Table 5—"Potentially important noninfective drug-drug interactions";
- Table 6—"Drugs for which dose adjustment is required based on individual's kidney function"
- Table 7—"Drugs with strong anticholinergic properties."

Although the Beers criteria are considered explicit criteria, because their use implies little or no clinical judgment, Beers Table 4 refers to medicines that should be "used with caution," which may invalidate the explicit characteristic of the Beers criteria.

17.3.2.3 STOPP/START criteria

The Screening Tool of Older People's Prescriptions (STOPP) and Screening Tool to Alert to Right Treatment (START) criteria were created in 2008, and a second version was released in 2014.[30] The current STOPP and START criteria comprise 81 PIMs and 34 potential prescribing omissions (PPOs). STOPP criteria present a suboptimal process clinical situation and mention, between brackets the potential negative outcome, for instance: "Selective serotonin reuptake inhibitors with current or recent significant hyponatremia, that is serum Na + $<$130 mmol/l (risk of exacerbating or precipitating hyponatremia)." START criteria present the clinical situation where a given drug should have been prescribed, for instance, "Statin therapy with a documented history of coronary, cerebral or peripheral vascular disease, unless the patient's status is end-of-life or age is $>$85 years." The 2014 STOPP/START version also includes a list of references where each criterion was supported.

STOPP/START authors claimed to have created a set of criteria with more "practical clinical value" than Beers criteria because STOPP/START are evidence-based rather than consensus-based criteria.[30] STOPP/START criteria have been widely tested in interventional studies and implemented in clinical decision support systems.

17.3.2.4 EU(7)-PIM list

The EU(7)-PIM list was created in 2015 by Renom-Guiteras et al. with the aim of developing a European list of PIMs for older people that could be used to compare prescribing patterns across European countries and in clinical practice. Based on previously published explicit criteria and comprising the participation of geriatric prescribing experts from 7 different European regions, the EU(7)-PIM list includes a total of 282 chemical substances or drug classes from 34 therapeutic groups, according to ATC classification. This instrument presents monitoring suggestions, dose adjustments and therapeutic alternatives.

In contrast to the Beers criteria or STOPP/START, the EU(7)-PIM list can be used even when the patients' clinical information is scarce because authors aimed to create an explicit list restricted to drugs or drug classes. Therefore, the EU(7)-PIM list is potentially suitable for use in pharmacoepidemiological studies with databases or surveys without any clinical information.[31]

17.3.2.5 EURO-FORTA list

The FORTA (Fit fOR The Aged) list was first developed in 2012 and was updated in 2015 by experts from Germany and Austria. The FORTA list adopts a different approach to assessing medication appropriateness, combining a positive and negative classification that addresses over- and underprescribing. Therefore, FORTA categorizes each drug or drug class according to the following classification:

- "Class A (Absolutely) = indispensable drug, clear-cut benefit in terms of efficacy/safety ratio proven in older people for a given indication.
- Class B (Beneficial) = drugs with proven or obvious efficacy in older people with limited extent of effect or safety concerns.
- Class C (Careful) = drugs with questionable efficacy/safety profiles in older people, to be avoided or omitted in the presence of too many drugs, lack of benefits or emerging side effects; review/find alternatives.
- Class D (Don't) = avoid in older people, omit first and review/find alternatives."

The FORTA list is organized according to clinical indications, and each drug is labeled with a FORTA class depending on its clinical indication. This means that a given drug can have different classifications depending on the diagnosis. Drugs classified with A or B are considered appropriate, while those labeled with C or D are potentially inappropriate.

The FORTA list has been validated in a randomized controlled trial (VALFORTA) that demonstrated significant improvements in important health outcomes. As a result, in 2018, country/region-specific FORTA lists for seven countries/regions, as well as a European FORTA list, were developed to enhance the international utility of the list. The EURO-FORTA list contains 264 drugs or drug classes for a total of 26 clinical conditions.[32]

17.3.2.6 Selecting an explicit tool

There is no single ideal explicit tool for identifying PIMs because each of the instruments has different strengths and weaknesses. The selection of a tool depends on its purpose of use (i.e., clinical practice or research) and the availability of patient clinical data.[31]

Access to information recorded in patients' medical records is a major driver for the selection of an explicit tool to identify PIMs. Instruments such as the Beers Criteria, STOPP/START and EURO-FORTA list cannot be correctly used without access to patient diagnoses. However, the EU(7)-PIM list and other PIM-identification tools do not require access to patient diagnoses because they are simple lists of medicines to avoid in older people. New and updated versions of the explicit tools increasingly require more data from the patients' medical records. For instance, to properly use the STOPP/START criteria, access to extensive patient clinical information is required: the complete therapeutic regimen, duration of therapy, previous medications, current medical conditions, patient medical history, laboratory data, and other physiological parameters.

Once access to patients' electronic medical records is granted and when a computerized prescription order system exists, clinical decision support systems are useful technologies to support a more efficient medication review process. Explicit tools to identify PIMs can support these alert-generating systems that will guide professionals performing medication reviews. Although all the explicit tools can support these systems, the simple lists (e.g., Euro-FORTA) will produce nonspecific alerts that will be more frequently overridden. Conversely to what happens in person-based use of explicit tools, computerized systems require more complex criteria, such as STOPP/START, to produce highly specific and relevant alerts.[33,34] The clinical information needed to correctly apply these different explicit tools is summarized in Table 17.2.

17.4 Epilogue

The elderly population is at a greater risk of adverse drug events due to the concrescence of several contributing factors: physiological decline and frailty that leads to pharmacokinetic and pharmacodynamic modifications, a higher prevalence of multimorbidity that requires appropriate polypharmacy, and a higher risk of inappropriate polypharmacy. Polypharmacy, whether appropriate or inappropriate, also contributes

Table 17.2 Clinical information requirement to apply explicit tools for potentially inappropriate medication identification.

	Current medication	Duration of therapy	Current medical conditions	Past medical conditions	Laboratory tests	Measurable parameters
Beers criteria	✓	–	✓	✓	✓	–
STOPP/START criteria	✓	✓	✓	✓	✓	✓
FORTA List	✓	–	✓	✓	–	–
EU(7)-PIM list	✓	–	–	–	–	–

to increased medication regime complexity and subsequent challenges in medication self-management and adherence, which reinforces the increased risk of adverse drug events in this population.

Healthcare professionals should be specifically trained to address medication age-related issues at the individual level. However, multiprofessional polypharmacy management programs should also be established to reduce individual and system burden of age-related medication issues.

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CHAPTER 18

How molecular imaging studies can disentangle disease mechanisms in age-related neurodegenerative disorders

Edoardo R. de Natale[1], Heather Wilson[1], Chi Udeh-Momoh[2], Jamie K. Ford[2], Marios Politis[1] and Lefkos T. Middleton[2,3]

[1]Neurodegeneration Imaging Group, University of Exeter Medical School, London, United Kingdom
[2]School of Public Health, Imperial College London, London, United Kingdom
[3]Public Health Directorate, Imperial College NHS Healthcare Trust, London, United Kingdom

18.1 Introduction

Global improvements in healthcare have led to an unprecedented increase in life expectancy[1] and for the first time in human history, the number of individuals aged 65 is projected to potentially reach 1.5 billion by 2050. These demographic changes are associated with an increasing incidence and prevalence of age-related neurodegenerative diseases (NDDs), such as Alzheimer's disease (AD) and Parkinson's disease (PD). This in turn, constitutes a major socioeconomic and healthcare challenge, with extreme financial burden to global economies.

Aging is characterized biologically by a number of alterations of cellular metabolic mechanisms that determine the progressive loss of cellular capacity to produce energy, to cope with molecular stressors, and to renew itself.[2] These include genomic instability, telomere attrition, epigenetic alterations, and loss of proteostasis which, in turn, trigger the deterioration of other cellular functions such as mitochondrial dysfunction, cellular senescence, and deregulated nutrient sensing.[2,3] The majority of brain cells are postmitotic cells, and as such are particularly prone to accumulating age-related cellular damage and degenerate with the scarce possibility of being replaced.[4]

NDDs are characterized by a progressive deterioration of cerebral structure and function, and encompass a heterogeneous group of disorders including AD, PD, as well as other common age-related neurodegenerative disorders such as Amyotrophic lateral sclerosis (ALS), Huntington disease (HD) and Dementia with Lewy bodies (DLB). Commonalities in subcellular mechanisms and shared features of most NDDs include abnormally increased selective protein aggregation, failure of protein degradation pathways, impaired axonal transport, mitochondrial and oxidative dysfunction, impaired cerebral metabolism and synaptic activity as well as programmed cell death.[5]

Aging
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Neurodegeneration is a silent and long process starting decades before the onset of clinical symptoms where aging is regarded as the most important risk factor.[6] In this view, clinical symptoms constitute the final stage of a process where brain cells progressively degenerate and other factors such as age, gender, genetic variation and others, confer the interindividual variability determining the differences in clinical evolution[7] Fig. 18.1. The 2018 NIA-AA Research Framework criteria of AD[8] defines AD as the combination of altered biomarkers (Amyloid, Tau, and Neurodegeneration or neuronal injury [AT(N)]), have shifted the emphasis of NDDs from clinical entities that are vaguely defined by their clinical symptomatology and where diagnosis was only possible postmortem, to discrete, biological entities where individual differences are defined in vivo, by the extent of changes of specific biomarkers. According to the precision medicine approach, this view is a critical step towards minimizing ambiguity for the identification, and targeting more detailed subgroups of complex diseases, based on specific biomarker-based biological profiles, thus aiming towards the discovery and development of more targeted efficacious therapeutic approaches. In this regard, it is

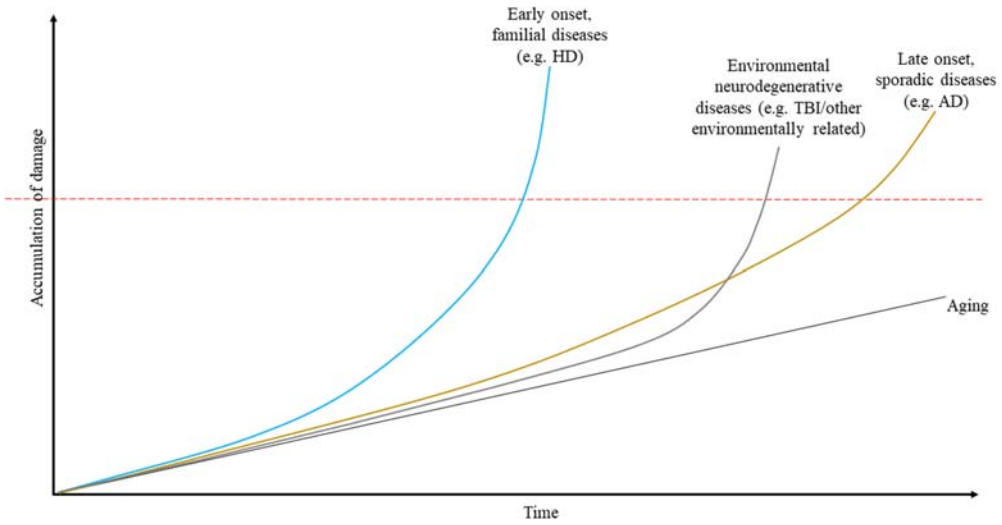

Figure 18.1 Timeline of aging and of neurodegenerative diseases. Aging (in black) can be regarded as a constant, progressive line where cellular damage accumulates but does not reach the level of clinical manifestation of symptoms (dashed red line). In familial, genetic diseases, such as Huntington's disease (blue line), the premanifest stage is characterized by a fast accumulation of cellular alterations that actually starts very early in life and gives rise to clinical symptoms at an early age. In late-onset, sporadic diseases, such as Alzheimer's disease (brown line), the deviation from the physiological line occurs many years before the onset of symptoms and cellular damage gradually accumulates over time. Environmental factors, such as traumatic brain injury (gray line) can modify abruptly the line of cellular senescence and give rise to a progressive neurodegenerative clinical picture.

essential to find methods to directly study the molecular alterations underlying aging and neurodegeneration to unravel their mutual relationship, the weight they have in each disease, and to better characterize each clinical entity according to both varying biological alterations and for differential diagnosis[9].

In vivo molecular imaging carries the advantage of studying structural and functional cellular biology in living people, with high temporal and spatial precision. PET makes use of radiotracers that bind with high affinity to specific molecules of interest gathering invaluable information that may relate to their etiopathogenesis, pathophysiology, and progression.[10–15] Over the years, several radiotracers have been developed, targeting distinct aging-related cellular mechanisms such as mitochondrial function, neuroinflammation, oxidative stress, protein accumulation, cerebral glucose metabolism and synaptic dysfunction with increasing clinical applications, giving invaluable insight about the mechanisms of dementia in vivo Fig. 18.2.[16] Recent advances in structural, microstructural, and functional MRI techniques to study molecules with paramagnetic properties, such as iron, can also contribute to the understanding of the role of each of these factors in neurodegeneration.

This chapter will illustrate the current advances in molecular imaging of the aging-related cellular processes in NDDs, across four core themes pertaining to the neuroinflammation processes, protein aggregation, brain metabolism and neuronal function, as well as iron metabolism. The chapter further describes in vivo imaging of emerging molecular mechanisms of neurodegeneration and discusses how future applications of in vivo imaging can help in the translation from basic science, to clinical trials and clinical management of these patients.

18.2 Molecular imaging of neuroinflammation

Neuroinflammation involves highly complex molecular cascades mediated by microglia and astrocytes. Microglia are ubiquitous specialized glial cells predominantly concentrated in the hippocampus, the basal ganglia, and the substantia nigra.[17] Astrocytes are essential cells for maintaining neuronal homeostasis, mediating neurotransmission, plasticity, and for communicating with the exterior through the blood brain barrier (BBB).[18] Microglia and astrocytes are particularly susceptible to misfolded proteins (such as β-amyloid (Aβ), tau, or α-synuclein), iron, reactive oxygen species (ROS), as well as other factors of the cellular microenvironment.[19–22] Under conditions of stress, trauma or exposure to neurotoxic agents, such as the soluble Aβ mono and oligomers, microglia and astrocytes initiate neuroinflammation through a dual mechanism. On one hand, they trigger the innate immunity response, by secreting a wide array of cytokines and chemokines[23,24] and by stimulating the oligomerization of the NLR family pyrin domain containing 3 (NLRP3),[25–27] hence promoting the formation of the inflammasome, a multiprotein cellular complex that triggers multiple molecular

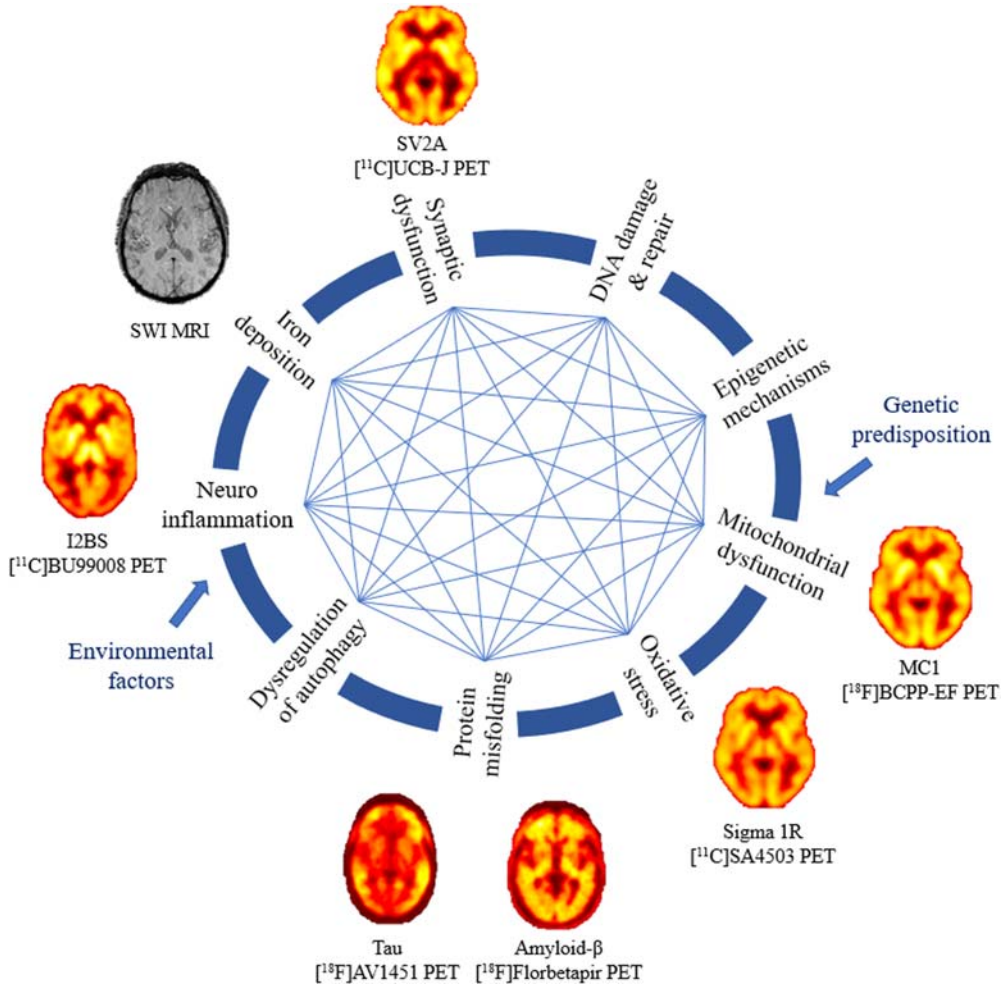

Figure 18.2 Aging is characterized by the concomitant presence of multiple molecular alterations that result in the loss of cellular homeostasis and, ultimately, in cellular death by necrosis or apoptosis. When this process is underway, it reinforces itself in a vicious circle of alterations linked one to another, which is impossible to track back to its initiation, or to isolate one element over another. In neurodegenerative diseases, external factors such as genotype and the environment intervene to reinforce the weight of some aspects over the others. This, in turn, modifies the overall balance of this process and results in a deviation from the physiological aging process, towards a detrimental, neurodegenerative one. Molecular imaging can improve our understanding of the molecular mechanisms underlying cellular alterations, disentangle the close relationship between these elements, and assess the weight played by each of them in the generation of the single neurodegenerative diseases.

cascades resulting in the activation of inflammation-related cellular death (pyroptosis).[28] On the other hand, microglia activate the adaptive immune response by acting as antigen-presenting cells for α-synuclein.[29] Overall, the inflammation cascade and the

activation of proinflammatory cytokines and chemokines, are thought to determine a microenvironment that favors cellular damage and neurodegeneration.[30,31] In addition, microglia can also diffuse toxic proteins by seeding and spreading tau and α-synuclein into the extracellular space, through exosomal release.[32,33]

The primary PET target for investigating neuroinflammation in vivo is the 18-kDa Translocator Protein (TSPO), a protein known to be highly expressed in microglia and macrophages,[34] which upregulates as a result of microglial activation[35] and is considered a non-specific marker of regional neuroinflammation.[36] The first generation TSPO-PET tracer, [^{11}C]PK1119,5 has been available since the 1990s and has been widely used across neurological conditions.[37] However, [^{11}C]PK11195 displays low signal-to-noise ratio and non-specific binding. Second-generation TSPO radioligands such as [^{11}C]ER176, [^{18}F]DPA714, [^{18}F]FEPPA, and [^{11}C]PBR28, with higher signal-to-noise ratio, are gaining applications in research. The uptake of these tracers is however dependent on the rs6971 polymorphism in the *TSPO* gene that affects the affinity of the tracer to bind to TSPO.[38]

Overall, TSPO-PET has produced a wealth of literature providing critical information into the role of neuroinflammation in neurodegeneration.[39] However, in light of recent reports raising questions over the exact biological significance of increased TSPO binding,[40–42] alternative microglial and astrocytic non-TSPO PET tracers have been developed to further elucidate the role of neuroinflammation across NDDs. Astroglial activation can be directly visualized by quantifying the imidazoline 2 binding sites (I_2BS) with the PET tracer [^{11}C]BU99008.[43,44] I_2BS are located in the outer membrane of mitochondria and regulate the expression of glial fibrillar acidic protein (GFAP) that is upregulated in activated astroglia.[45] [^{11}C]Deprenyl, a specific radiotracer to quantify monoamine oxidase (MAO)-B enzyme,[46,47] has also been utilized to study astrocytes in vivo[48]; however, MAO-B is not exclusively expressed on astrocytes and [^{11}C]Deprenyl displays off-target binding which does not solely reflect astroglia action.[49]

A common pattern across NDDs is a diffuse increase of TSPO binding in cortical and subcortical regions, which is highest in areas pathologically affected by disease-specific cellular degeneration.[50–56] In patients with early AD, elevated levels of [^{11}C]PK11195 binding were found in areas of decreased [^{18}F]FDG uptake, a marker of brain glucose metabolism.[57] Furthermore, a 14-month longitudinal study in a cohort of AD patients showed that a regional progressive increase of [^{11}C]PK11195 corresponded to a progressive decrease of [^{18}F]FDG uptake.[58] These findings suggest an association between increased microglial activation and neuronal dysfunction. Microglial activation, measured with [^{11}C]PBR28 PET, was also found to be strongly correlated with tau deposits in the temporal lobe of AD patients.[59] Moreover, sustained chronic inflammation correlating with decreased cognitive performances, has been reported in advanced stages of Parkinson's disease dementia (PDD)[60,61] and in cases of traumatic brain injury

(TBI).[62] Overall, these findings suggest a relationship between increased microglial activation, the accumulation of tau pathology, and clinical symptomatology. Increased TSPO signal has also been associated with microstructural alterations of the white matter in ALS and TBI, two conditions characterized by a vulnerability of long axonal tracts. In National Football League players with a history of TBI, as well as in patients with ALS, increased microglial activation has been reported to colocalize with areas of decreased fractional anisotropy on diffusion tension imaging (DTI) MRI, a marker of loss of white matter tissue integrity.[63–65] In these conditions, microglial activation could represent either the cause or the effect of localized white matter damage. Increased central microglial activation has also been associated with peripheral inflammation. In one study on premanifest gene carriers, increased microglial activation in the somatosensory cortex was correlated with higher plasma levels of IL-1β and TNF-α.[66] These findings suggest a concomitant role between peripheral and central immune system responses in early preclinical stages of HD, which warrants further investigations in other NDDs.

Increased TSPO uptake may represent an early feature of neurodegeneration. Patients with REM sleep behavior disorder and mild cognitive impairment (MCI), which represent prodromal and early stages for PD and AD respectively, demonstrated elevated TSPO signal.[67–69] In premanifest HD gene mutation carriers, increased TSPO signal in associative striatum and in networks associated with cognitive function predicted the probability of phenoconversion of five years.[70] Recent research also suggests that the biological relevance of neuroinflammation could vary, according to the disease stage. Early MCI patients show an initial increase of [^{11}C]PK11195 binding and a progressive decrease over time as they approach AD, with a second peak of high TSPO binding following further cognitive and functional decline leading to AD diagnosis, suggesting a bimodal peak of microglial activation.[67,71] In early disease stages, microglial activation could represent a protective countermeasure to cellular insults, as suggested by the finding of higher hippocampal and cortical volume on structural MRI and slower clinical progression, associated with high TSPO binding in cohorts of MCI and early AD patients[72,73] and by the evidence, in premanifest carriers of PSEN1 and APP mutations, of increased levels of [^{11}C]Deprenyl as early as 17 years from predicted phenoconversion.[55] Conversely, chronic inflammation in advanced disease stages may be associated with more severe cellular loss. This hypothesis is corroborated by a recent study in which early de novo PD patients displayed significantly increased cortical and subcortical uptake of [^{11}C]BU99008, followed by a sharp decline in moderate/advanced disease PD patients, that correlated with motor and cognitive deterioration.[74] These results suggest that neuroinflammation could be an early phenomenon in the neurodegenerative process. Initially, neuroinflammation can have a primary potentially neuroprotective role; however, as the disease progresses and neurotoxic effects increasingly prevail, it becomes detrimental contributing, either directly or indirectly, to further progression of neurodegeneration.

The P2X purinoceptor 7 (P2X7) has recently emerged as a novel PET target for neuroinflammation. Activation of P2X7 triggers pyroptosis, through stimulation of the NRLP3-inflammasome related cytokine cascade.[75] The PET tracer [^{11}C]JNJ717 has been recently developed for the in vivo study of P2X7 receptors.[76] However, two pilot studies in small cohorts of PD[76] and early-stage ALS[77] did not show differences in [^{11}C]JNJ717 volume of distribution, compared with healthy controls. Further studies using the more recently designed P2X7 PET tracers, such as [^{11}C]SMW139,[78] [^{18}F]JNJ-64413739,[79] or [^{11}C]GSK1482160,[80] would allow for further exploration of the potential of this PET target as a marker of neuroinflammation in NDDs.

18.3 Molecular imaging of mitochondria dysfunction and oxidative stress

Mitochondria are essential cellular organelles that oversee cellular homeostasis through several functions, such as supplying cells with energy through the mitochondrial respiratory chain, balancing cellular calcium levels, signaling cellular perturbations to microglia, and regulating apoptosis.[81] A number of these functions take place in points of close contact between the mitochondria and the endoplasmic reticulum (ER) called mitochondria associated membranes (MAM).[82] There is wealth of evidence, suggesting that mitochondrial dysfunction could represent a converging site of multiple molecular alterations in aging and NDDs.[83–92] In addition, it has been shown that MAM can be directly altered by protein aggregates of tau, α-synuclein, Aβ and TAR DNA-binding protein (TDP)-43, potentially exacerbating the functional damage to mitochondria.[93] Further supporting the central role of mitochondrial damage in the genesis of neurodegeneration, a number of pathogenic genes for familial forms of PD, ALS, and AD are physiologically located, or are active, in the mitochondria[94–96] and, as in the case of the mitophagic PINK1/Parkin pathway, intervene in several NDDs.[97–101]

An important byproduct of the mitochondrial aerobic respiratory process is the production of ROS. It is estimated that mitochondria are responsible for the formation of about 90% of the ROS present in cells.[102] In general, ROS production is counterbalanced by natural antioxidants such as superoxide dismutase (SOD) and glutathione. Excessive ROS can contribute to oxidative stress and to cellular damage of proteins, lipids, mRNA, and DNA damage.[103–105] In addition, ROS production activates NF-kB, an important mediator of the innate immune response that, in association with iron released into the cytoplasm, enhances neuronal damage.[106,107] The overproduction of ROS and the loss of antioxidant countermechanisms has been found to be an early and detrimental event preceding, and promoting, protein aggregation.[108,109]

Recently, two novel PET tracers have been developed and validated for the in vivo study of mitochondrial function in humans.[110,111] [^{18}F]BCPP-EF is a PET tracer with high affinity for the mitochondrial complex 1 (MC1), and [^{11}C]SA4503

for the sigma 1 (σ1) receptor which is highly expressed in the MAM. [^{18}F]BCPP-EF uptake was diffusely decreased in cortical areas and subcortical areas of AD patients, as evidence of regional loss of energy metabolism.[112] Strikingly, loss of [^{18}F]BCPP-EF uptake in the parahippocampal gyri was not associated with similar reduction of [^{18}F]FDG PET uptake, as reference marker of cellular glucose metabolism, suggesting that [^{18}F]BCPP-EF might be a more sensitive marker of cellular distress than [^{18}F]FDG PET.[112] A 12-month longitudinal study on de novo PD patients and controls, using both [^{11}C]SA4503 and [^{18}F]BCPP-EF showed insignificant lower levels of σ1 receptor density and of MC1 in de novo PD patients, with a trend to an annualized increase particularly in the caudate and putamen.[113] These results should be corroborated by further studies on larger cohorts to elucidate the possible pathophysiological meaning of in vivo changes of MC1, and σ1 receptor density in PD and in other NDDs.

[^{62}Cu]ATSM, a PET tracer which binds to over-reduced compounds within cells, originally devised to study hypoxia in cardiology and oncology, has been used to study oxidative stress in NDDs.[114,115] Using this tracer, two small-scale studies on PD patients demonstrated a state of diffuse oxidative stress in the striatum and a significant increase in disease severity, when corrected for the [^{123}I]FP-CIT SPECT specific binding ratio (a marker of the quantity of surviving dopaminergic neurons).[116,117] These findings indicate that oxidative stress is an ongoing process that worsens over time in vulnerable regions, in PD. In another small-sample study on twelve ALS patients, increased uptake of [^{62}Cu]ATSM in the motor cortex was negatively associated with decreases in the revised ALS functional rating scale score, a marker of disease severity.[118] The unfavorable half-life of [^{62}Cu] (about nine minutes) limits the use of this tracer to centers with on-site tracer manufacturing capabilities. Fluorinated tracers are currently in development and it is envisaged that, in future years, such tracers might permit more accurate in vivo investigation of oxidative stress.[119,120]

18.4 Molecular imaging of misfolded proteins

A great number of human proteins present one or more regions promoting self-assembly into oligomeric structures of fibrillar cross β-structures.[121] In vitro and in vivo studies indicate that several proteins implicated in NDDs, such as Aβ, α-synuclein, tau, and TDP-43, can self-aggregate into insoluble, ubiquitinated inclusions,[122–124] similarly to how prion protein (PrPSc) self-templates to induce aberrant conformation to native proteins.[125] Thus, complex and multifactorial molecular mechanisms are in place across the spectrum of NDDs which create a microenvironment that may favor protein misfolding and aggregation. Genetic variants to genes such as SNCA, MAPT, PSEN1, PSEN2, APP, SOD1, TARDBP, FUS, HTT, as well as environmental conditions, including inflammation, oxidative stress, and energy failure, increase the propensity of these proteins to self-assemble in vivo.

Similarly to classic PrPSc, which is capable of spreading throughout the CNS via neuroanatomic connections,[125] accumulating experimental evidence demonstrates that other protein aggregates like Aβ,[126,127] tau,[128,129] and α-synuclein[130,131] are transmissible by a trans-synaptic seeding mechanism through several, and still not entirely explored, cellular pathways. Human mesenchymal stem cells grafted to patients with PD showed, on post-mortem examination, aggregates of α-synuclein into Lewy-Body-like formations, suggesting host-to-graft propagation.[132,133] Moreover, cases of probable human transmission of Aβ pathology through medical procedures have been reported.[134–136] Differences in the spatial and temporal regional distribution of aggregated proteins across NDDs is hypothesized to be determined by a selective cellular vulnerability to stressors, the precise mechanisms of which are still largely unclear. Nevertheless, it has been possible to reconstruct, from post-mortem analyses, the spatio-temporal staging of protein deposition for AD and PD[137,138] which correlates with the clinical progression of these diseases but does not constitute, by itself, proof for the seeding hypothesis,[139] or explain whether protein aggregation is a cause, effect, or epiphenomenon of other pathogenic events in the neurodegeneration cascade.[140]

18.4.1 Molecular tracers of amyloid and tau in vivo PET imaging

The last decade has seen a blooming of PET tracers targeting Aβ and tau, and extensive research is underway to identify reliable radiotracers for α-synuclein and mutated Huntingtin (mHtt).[26,141] The most commonly used Aβ PET tracers are [^{11}C]PiB, [^{18}F]Flutemetamol, [^{18}F]Florbetapir, and [^{18}F]Florbetaben. These tracers bind Aβ with high affinity but show some degree of non-specific binding to white matter.[142] First-generation tau tracers ([^{18}F]FDDNP, [^{11}C]PBB3, [^{18}F]THK5117, [^{18}F]THK5351, [^{18}F]AV1451) show high affinity for AD tauopathies, but are hampered by substantial off-target binding to the basal ganglia, choroid plexus and substantia nigra, and to variable degrees of affinity for neuromelanin and MAO-B.[143–146] Second generation tau tracers ([^{18}F]RO-948, [^{18}F]MK-6240, [^{18}F]GTP1, [^{18}F]PI-2620, [^{18}F]APN-1607) show promising specificity for tau in both AD and non-AD tauopathies, have better kinetics, good brain uptake and fast washout, and do not display significant off-target binding, although this can still be present to some degree.[147,148] Because of its high prevalence and its close relationship with these biomarkers, the AD spectrum has been the main field of investigation of the dynamics of Aβ and tau deposition, using PET imaging. As with other NDDs, AD is seen as a disease continuum from the asymptomatic, preclinical stages, to MCI and clinical AD dementia,[149] all associated with different levels of brain pathology which can be used to characterize the disease in vivo according to the recent [AT(N)] classification system.[8] Abnormal levels of the classical AD biomarkers (Aβ and tau) are detectable from very early pre-symptomatic stages.[8,150,151] Aside the traditional dichotomous classification of AD biomarkers,

emerging evidence suggests the existence of a distinctive peri-threshold "gray zone" concordant with biomarker levels at the inception of the 'abnormal cut-off point'.[152] Individuals with peri-threshold biomarker levels may present similar trajectories of cognitive decline as individuals with higher-than-threshold abnormal biomarker profiles.[152] Interestingly, sub-threshold AD biomarker levels at baseline have further been associated with faster accumulation of pathology that is predictive of accelerated clinical symptom onset.[153,154]

Increased Aβ and tau deposition is not specific to NDD and can be spotted with PET imaging in cognitively healthy older adults.[155,156] Aβ is widespread in the frontal and parietal lobes, whereas tau aggregates appear preferentially in the medial temporal lobe.[157] Despite the non-overlapping distribution, gray matter areas of tau and Aβ aggregation show connectivity interactions, particularly in the inferior-lateral temporal lobe and entorhinal cortex.[157] This finding suggests a possible relationship between Aβ and tau aggregation in these areas, that may feed forward into the neurodegenerative process. This suggestion is supported by the evidence that Aβ accumulation could influence neuronal damage in the medial temporal lobe[158] and drive tau deposition. Moreover, double-tracer PET studies in the AD spectrum and in Down Syndrome have demonstrated that tau and Aβ brain retention show a significant reciprocal correlation, and cortical Aβ pathology is required for tau to deposit outside the temporal cortex.[156,159] This hypothesis is further corroborated by recent studies measuring Aβ-42 and p-tau in the cerebrospinal fluid (CSF). Aβ-42 and p-tau alterations in the CSF can represent early harbingers of cognitive impairment, as demonstrated in recent studies on PD patients.[160] P-tau-217 was found to precede increases in [^{18}F]AV-1451 uptake in the entorhinal cortex only in "amyloid positive" individuals.[161,162] Additionally, in cognitively unimpaired individuals with hippocampal tauopathy seen on tau PET, cortical retention of [^{18}F]AV-1451 was observed only in those with concomitant increased Aβ-42 in the CSF,[163] thus indicating that Aβ may trigger the spread of tauopathy outside the hippocampus, likely transforming pre-existing tauopathy to more toxic tau species, resulting in neuronal injury.

Interestingly, two PET studies in early-onset AD patients showed significantly high levels of brain tau burden, even in the absence of associated abnormal increases of cortical Aβ deposition,[164,165] suggesting that age may drive different regional levels of response to protein accumulation. In the AD spectrum, tau retention generally correlates better than Aβ with cognitive dysfunction in episodic and visual memory.[156,164,166–169] Thus, it could be posited that abnormal Aβ deposition may trigger the neurodegenerative cascade and tau accumulation, but further progression and clinical significance may be determined by the latter.

PET studies on genetically predisposed models of amyloidopathy have further confirmed the hypothesis that, in familial disease forms, abnormal brain load of Aβ deposition can represent a very early event in neurodegeneration. Premanifest carriers of the

autosomal dominant PSEN1 mutation showed widespread cortical [^{11}C]PiB deposition as early as 15 years before predicted phenoconversion.[170] Moreover, Down Syndrome patients examined before the onset of AD-dementia showed increased and widespread cortical retention of [^{11}C]PiB.[171,172]

AD dementia patients consistently show increased levels of Aβ and tau brain deposition on PET imaging.[164,173–177] In AD, abnormal levels of Aβ deposition, as seen with [^{11}C]PiB, are concentrated in the frontal, parietal, temporal and occipital cortices, posterior cingulate, and the retrosplenial cortex.[176,178] Tau PET retention, can be found in the temporal, parietal, posterior cingulate, frontal and mesial temporal cortical regions, and in the posterior cingulate.[164,174,177] A significant increase of Aβ and tau has also been detected in the parietal, temporal, occipital and cingulate cortical areas and the striatum in the majority of patients with DLB, as opposed to a small minority of PDD patients, while a general absence of Aβ and tau is reported in non-demented PD patients.[179–186] Primary tauopathies, such as progressive supranuclear palsy (PSP), corticobasal syndrome, or sporadic and MAPT-linked frontotemporal dementia (FTD), show increased levels of cortical and subcortical tau retention in studies employing first-generation tau PET radiotracers, with the degree of cerebral tau deposition correlating with clinical symptoms and severity.[187–192] Future studies employing the 3R/4R specific second-generation tau tracers may further improve the ability to interrogate the biological mechanisms of primary tauopathies.[193,194]

A striking advantage of PET imaging is the possibility to recapitulate in vivo the pathological spatiotemporal sequence of protein deposition. Recent studies in the AD continuum, have reconstructed this sequence in cognitively unimpaired, MCI and AD participants. Using [^{18}F]Florbetapir, a four-stage temporal and spatial model of Aβ deposition has been demonstrated starting from the temporobasal and frontomedial areas in cognitively unimpaired individuals, and successively affecting the associative neocortex, the primary sensorimotor areas and the medial temporal lobe and subcortical structures together with clinical progression to MCI and AD dementia.[195,196] Similarly, a pattern of tau deposition was identified, first in the transentorhinal region, and progressing to the lateral, temporal, occipital, and associative cortical regions, consistent with the pathological Braak's I-VI staging and being significantly associated with both Aβ retention and global cognition.[197,198]

The pattern of Aβ and tau brain retention has also been studied by PET imaging in relation to the *APOE* genotype. In cognitively normal older adults, *APOE ε4* genotype influences the relationship between brain Aβ and cognitive performances[199] and, in subjects with subjective cognitive decline, the *APOE ε4* allele can explain about 9%–11% of the variance of cerebral Aβ levels.[200] Cognitively unimpaired *APOE4* carriers reach abnormal levels of neocortical Aβ by age 63 compared to noncarriers that reach abnormal levels of neocortical Aβ at age 78.[201] Moreover, *APOE ε4* carriers with and without AD dementia also show a higher speed of Aβ-independent progression of tau

accumulation in the basal and lateral temporal cortical areas, compared with non-carriers.[202–204] The *APOE ε4* genotype also influences the level of tau aggregation after TBI,[205] and the level of Aβ in DLB patients.[206] In TBI, the degree of [^{18}F]AV1451 retention was higher in *APOE ε4* carriers as a function of time from the injury, being associated with low Aβ-42 CSF levels.[205] These findings suggest that the *APOE ε4* genotype may enhance the vulnerability to tau spreading.[202]

A number of longitudinal studies have assessed the degree of progression of Aβ and tau deposition. In a two-year study on AD patients, cerebral accumulation of [^{11}C]PiB did not progress significantly despite clinical progression and progressive cellular damage as evidenced by a 20% longitudinal decrease of [^{18}F]FDG.[207] By contrast, another study employing [^{18}F]AV1451 demonstrated, in both cognitively unimpaired older adults and AD patients, a progressive accumulation of tau consistent with the described Braak's progression pattern.[208] Aβ deposition, measured with [^{11}C]PiB, showed a progressive decrease of accumulation rate also in a group of DLB patients. In these patients, those who showed longitudinal change in [^{11}C]PiB also displayed rapid clinical and cognitive decline over time.[209]

18.5 In vivo imaging of brain metabolic processes and activity

18.5.1 Molecular imaging of cerebral hypometabolism

Glucose is by far the main energy source for brain metabolism,[210] to an extent that the regional reduction of glucose consumption is used as a surrogate marker of alterations of neuronal processes and cellular distress. Alterations of cerebral glucose metabolism is an early and common feature across the spectrum of NDDs.[211] Furthermore, vascular and metabolic risk factors associated with reduced energy metabolism within the brain have been linked with increased vulnerability to cognitive impairment[212] and to the severity of clinical symptoms.

[^{18}F]Fluorodeoxyglucose (FDG) is an analog of glucose that is taken up in the brain by metabolically active cells and is phosphorylated to FDG-6-phosphate. [^{18}F]FDG has long been employed to quantify local energy metabolic activity and as a sensitive downstream marker of neuronal injury.[213,214] [^{18}F]FDG has long been employed for the investigation of NDDs in both research and clinical setting[215] and has been also proposed as a marker of neurodegeneration (N) in the AT(N) diagnostic AD formula.[216] The main source of [^{18}F]FDG signal is constituted by active neurons, although astrocytes also contribute to the generation of the [^{18}F]FDG signal.[217] In AD, preclinical and AD dementia cases consistently display glucose hypometabolism in the posterior cingulate cortex and in the parahippocampus[218,219] and was shown to be correlated with clinical severity.[220]

PET imaging with [^{18}F]FDG has also been employed to assess longitudinal changes of regional brain metabolism, and its relationship with the progression of dementia in

the AD spectrum, with evidence of significant correlation between even subtle regional metabolic changes and cognitive deficits.[221,222] In this latter study, low glucose metabolism at baseline, predicted subsequent cognitive and functional decline.[221]

[^{18}F]FDG PET is also increasingly being utilized for differential diagnosis of AD versus other forms of dementia. Differential patterns of sub-regional glucose metabolism from [^{18}F]FDG PET scans have been applied for the classification of late-onset dementia forms, including AD, FTD, DLB and vascular dementia.[223,224] In an investigator-blinded study, a high concordance was found between [^{18}F]FDG PET data with clinical diagnostic information for distinct neurodegenerative conditions that included AD, FTD and DLB.[225] Overall, these findings denote the utility of [^{18}F]FDG PET for facilitating differential diagnosis of late dementia forms.

18.5.2 Molecular imaging of brain functional connectivity

Multimodal approaches combining measures of the uptake of different PET radiotracers and MRI methods of functional connectivity, offer significant insight into many biological and neurophysiological processes within the brain in vivo; one example being to measure evidence of NDDs pathology, how and where they aggregate and accumulate, and the resulting impact on brain function.[226] Due to the relatively low spatial resolution of PET imaging, MRI's comparatively superior spatial resolution, multimodal studies combining both of these approaches are highly complementary in understanding NDDs pathology across the whole brain. In the future, hybrid PET-MRI scanners may be of significant value in simultaneously collecting information on the disease mechanisms and the resulting alterations in functional connectivity, in NDDs.[227]

For AD, there is growing evidence supporting "the disconnection hypothesis," whereby those with cognitive impairment often demonstrate alterations in brain functional connectivity.[228,229] Amyloidopathy and tauopathy both affect brain functional connectivity and, consequently, act as catalysts in cognitive decline.[230] Studies conducted in preclinical AD have identified the default mode network (DMN) as a particularly vulnerable circuit to cerebral Aβ levels which may induce a "desynchronisation" effect, resulting in reduced correspondence between DMN regions and, consequently, cognitive decline.[231] Even in preclinical stages, there is a significant relationship between the accumulation of Aβ fibrils and brain function. In cognitively unimpaired older adults with sub-threshold [^{18}F]Flutemetamol Aβ cerebral retention, Aβ deposits were associated with increased functional connectivity in several resting-state networks (including, but not limited to, the DMN).[232] Early evidence of Aβ accumulation, increased dynamic functional connectivity, and cognitive performance may form a trimodal relationship. Together, these three factors may act as a compensatory mechanism, whereby increased functional connectivity may help to maintain cognitive performance among individuals with higher levels of cognitive reserve, in response to

early Aβ-induced neuronal damage, and independent of structural alterations in white and gray matter.[232] Conversely, in the presence of supra-threshold global Aβ burden, despite being cognitively unimpaired, functional connectivity was reduced between the temporal pole, orbitofrontal cortex, medial prefrontal cortex, amygdala (composing the anterior temporal system) and the anterior medial temporal lobe.[233] In these individuals, higher levels of Aβ burden may consequently have effects in reducing functional connectivity between regions associated with memory, as evidenced by lower scores of memory performance.[233] This relationship is strengthened as the disease progresses. In a group of MCI and AD patients, a dose-dependent relationship between Aβ burden, as measured with [^{18}F]AV-45 PET, and functional inter-network connectivity between the DMN and the dorsal attention network was seen.[234] In MCI, higher levels of Aβ predicted future memory decline and inter-correlational strength between the DMN and DAN, suggesting that measures of functional connectivity and global Aβ burden using PET may be used together to predict future cognitive decline.[234] To strengthen the Aβ-connectivity relationship, in a longitudinal study in preclinical AD, increased cortical Aβ and lower DMN functional connectivity was associated with gradual DMN cortical thinning.[235]

Recent studies have reported Aβ and tau acting as independent entities or interacting together to impact functional connectivity. In one study, [^{18}F]Flutemetamol for Aβ and [^{18}F]Flortaucipir for tau were found to have different spatial affinities within the brain, in that Aβ was most highly accumulated within the anterior and posterior DMN, whereas tau accumulated more within the left inferior occipital cortex, superior frontal gyri, and precuneus.[236] Partial spatial overlap between Aβ and tau was found within the superior frontal and (left) inferior occipital gyri, which suggests that some regions of the brain may be more susceptible to the combined pathological toxicity as both biomarkers accumulate; more so than other networks that do not include the aforementioned regions.[236] The higher spatial affinity of tau for functional networks could also explain why tauopathy is associated with a functional decline in more cognitive domains compared to Aβ accumulation.[236]

Within resting-state networks, Aβ and tau also appear to target different functional components. In a very recent study employing fMRI and [^{11}C]PiB and [^{18}F]AV-1451 PET imaging in atypical AD patients, it was found that Aβ deposition occurred more frequently in network epicenters (or "hubs") and tau affected region-to-region functional connectivity.[237]

Fewer studies combine molecular imaging and fMRI measures of brain connectivity in other NDDs. In one study on drug-naïve PD patients using [^{18}F]FP-CIT PET for the study of presynaptic dopaminergic dysfunction and fMRI, increased connectivity in the "motor reserve network" (basal ganglia, cerebellar vermis, insula, and inferior frontal cortex), as an index of functional motor reserve was associated with a greater motor symptom prognosis and coping capacity in the presence of developing PD

pathology as exemplified by a slower rate of longitudinal dosage increases in dopaminergic medication across a two-year period.[238] In another PD study combining metabolic PET with [^{18}F]FDG and presynaptic dopaminergic with [^{18}F]DOPA, hypometabolism in the substantia nigra was associated with reduced nigrostriatal connectivity and dopamine depletion, which in turn appeared to contribute to a disruption in striato-cortical resting-state functional connectivity. Furthermore, functional dysconnectivity from the putamen in PD patients primarily affected the sensorimotor network and subregions of the DMN and higher functional desynchronisation with the striatum was also accompanied by decreased global cognitive performance. Therefore, reduced neural input from the striatum may have led to concomitant hypometabolic changes within the cerebral cortex, in turn, triggering cognitive decline.[239] Hypometabolism caused within the salience network and DMN can also be correlated with the occurrence of specific PD-related non-motor symptoms, such as fatigue.[240]

In summary, PET imaging can be used to measure and distinguish the relative impact of discrete molecular alterations and their relative impact on brain connectivity and symptoms. Future research using multimodal approaches combining molecular imaging and MRI functional connectivity methods may provide insight into the relationship between molecular alterations, their underlying neural mechanisms, and clinical symptoms.[241,242]

18.6 Imaging of iron accumulation

Iron is an essential element for brain development and cellular communication as it participates as a cofactor for a number of enzymes, neurotransmitter receptors and protein channels.[243] Free ferric iron, however, is particularly susceptible to oxidative activity in the brain and is a major source of ROS.[244] During aging, iron levels diffusely increase in the brain, but this is not accompanied by neuroinflammatory reactions. In NDDs, by contrast, iron accumulation preferentially affects the areas affected by neurodegeneration, and is accompanied by inflammation and other alterations of cellular homeostasis.[245] In the presence of excess iron, activated microglia become less efficient[246] and, in the presence of mutant Htt, hyperactive and dystrophic.[247] Iron may also cause mitochondrial dysfunction[248] and promote conformational changes and aggregation of Aβ and α-synuclein.[249–251]

Iron can be visualized using several MRI sequences. Iron induces a magnetically inhomogeneous environment that has an effect on water protons, resulting in a faster relaxation rate (R2 and R2*), which is proportional to regional iron content.[252] More recently, variations of the magnetic susceptibility of tissues to iron have been used to quantify the regional levels of iron accumulation. Susceptibility weighted imaging (SWI) phase values,[253] and quantitative susceptibility mapping (QSM), have found

increasing applications for the in vivo quantitative determination of iron content in both aging and neurodegeneration.[254]

MRI studies have demonstrated a progressive iron deposition with increasing age in the putamen, globus pallidus, thalamus, and substantia nigra.[255–259] In these individuals, APOE ε4 genotype promotes an increase in cortical iron deposition and modification of functional connectivity. In one study, cortical iron deposition in APOE ε4 carriers was associated with an increase of connectivity along the DMN.[260] In another study, altered functional connectivity along nodes with high iron content in APOE ε4 carriers was associated with low performances in episodic memory tests.[261] Iron co-localizes with Aβ. In a study on 116 elderly subjects using QSM MRI and [^{18}F] Flutemetamol PET, clusters of co-localizations were found in the basal ganglia and the frontotemporal cortex and, in this latter region, correlated with cognitive performance.[262] This finding was consistent with what was found in patients with MCI that were Aβ and APOE ε4 positive,[263] and in a 7-Tesla QSM MRI post-mortem study on AD tissues. Both studies found a co-localization of higher susceptibility values in frontal cortex areas known to be rich in Aβ plaques.[264] Taken together, these findings highlight the potential synergistic action that iron and Aβ exert on brain cells. However, it has been hypothesized that Aβ is diamagnetic and that, in AD, part of the QSM susceptibility signal may be due to the deposition of Aβ itself.[265]

Presymptomatic carriers of genetic mutations for familial NDD forms also show increased levels of iron. Far-from-onset premanifest HD gene expansion carriers show bilateral and progressive increases in SWI field map values in the putamen and pallidum, that extend to the caudate and cortical regions.[266] Similarly, asymptomatic carriers of LRRK2 and Parkin mutations for familial PD, show increases of R2* in the substantia nigra to an intermediate level between healthy controls and idiopathic PD patients.[267]

Increased cortical and subcortical iron is a consistent finding in PD, multiple system atrophy (MSA), PSP, AD, ALS, FTD, HD, and Friedreich's Ataxia.[268–278] Increased iron correlates with the severity of motor or cognitive symptoms,[268–280] and the degree of cellular degeneration.[281,282] Excess iron has been associated with microstructural alterations in the white matter. In manifest HD, areas of higher T2 relaxation rate, such as the pallidum, were associated with a worse myelin breakdown reflected by increased fractional anisotropy on DTI.[283–285] In MSA patients, increased T2* in the putamen was associated with changes in mean diffusivity on DTI, indicating increased diffusion along damaged white matter tracts.[143]

Brain iron deposition has been studied as a progression marker. Longitudinal studies conducted in early PD patients showed fast rates of iron accumulation in the substantia nigra, whereas advanced PD patients showed significant progression also in the caudate, pallidus, and red nucleus, that correlated with disease progression.[286–289] A fast progression of putaminal iron accumulation was also seen in the Parkinsonian

variant of MSA, as opposed to the Cerebellar variant.[290] In contrast, in a small sample of ALS patients, a 6-month longitudinal observation with QSM did not find any progression of iron deposition in the motor cortex.[291] More studies are needed to clarify the role of iron deposition in disease progression across the NDD spectrum.

18.7 Emerging mechanisms of neurodegeneration

18.7.1 Glymphatic system

The glymphatic system is a clearance system of brain waste to the veins, active during non-REM sleep and composed of an intricate web of perivascular virtual spaces formed by the endfeet of astrocytes.[292] The water channel Aquaporin-4 (AQP4) is embedded in the end-feet membranes and mediates the bulk flow of fluids between the Interstitial Fluid and the CSF.[293] Loss of function and genetic variations of the AQP4 gene may affect this cerebral clearance mechanism and result in the intracerebral retention of macromolecules, such as Aβ; hence contributing to cognitive decline and AD dementia.[294,295] Two plausible mechanisms have been suggested on how dysfunction of the glymphatic system may contribute to neurodegeneration. Firstly, by facilitating the accumulation of soluble macromolecules and other neurotoxic proteins, through reduced clearance; secondly, by facilitating the seeding of toxic proteins in distant brain regions through the slow directional flow of fluids along the perivascular spaces.[296,297]

Recently, in vivo neuroimaging techniques of acquisition and analysis have been developed to study the glymphatic system under physiological and pathological conditions.[298] The flow and exchange of fluids along the glymphatic system can be visualized after intravenous or intratheacal administration of contrast, with dynamic contrast enhanced MRI (DCE-MRI), ultra-fast magnetic encephalography, near-infrared spectroscopy, ultra-fast functional MRI, and 3D phase-contrast MRI.[299] Novel analysis methods of DTI and arterial spin labeling sequences also allow for the study of the exchange of fluids along the perivascular spaces and the blood flow across the BBB.[300] DCE-MRI imaging has demonstrated an age-dependent and progressive BBB breakdown in the hippocampus which correlated with cognitive dysfunction across the AD continuum.[301] Similarly, patients with normal pressure hydrocephalus showed reduced clearance of contrast in the entorhinal cortex and adjacent white matter, suggesting impaired glymphatic circulation.[302,303] Further studies will help to better elucidate the relationship between alterations of the glymphatic system and the development of NDDs.

Attempts at studying glymphatic transport and drainage in animals have employed [^{18}F]FDG dynamic PET scans,[304] but no specific radiotracer to quantify the density and function of molecular targets of the glymphatic system have been studied yet. Recently, [^{11}C]TGN-020 has been developed as a radioligand to bind both AQP4

and Aquaporin-1 (AQP1).[305] In the first report on human healthy subjects, [^{11}C] TGN-020 bound specifically in the subpial and perivascular endfeet of astrocytes, and the choroid plexus, areas of preferential distribution of AQP4 and AQP1.[306] The in vivo evaluation of the density of AQP4 in NDDs would be extremely valuable and it is expected that novel AQP4-specific radiotracers with high sensitivity and specificity will be developed and used to increase our knowledge of this evolving field in neurodegeneration.[304,305]

18.7.2 O-GLcNAc

O-GlcNAcylation is a reversible mechanism of protein post-translational modification in which a β-N-acetylglucosamine (O-GlcNAc) residue is added to serine or threonine residues by the enzyme O-GlcNAc transferase (OGT), and removed by the enzyme O-GLcNAcase (OGA).[307,308] This process is active during periods of cellular stress, such as hypoxia, heat shock, or nutrient deprivation, so that high levels of O-GlcNAc are used as indirect markers of cellular stress.[309] Substrates of OGT and OGA include hundreds of cytoplasmic, nuclear, and mitochondrial proteins, making O-GlcNAcylation one of the most critical biochemical processes for cellular homeostasis.[310] Substrates presenting multiple sites for O-GlcNAcylation are tau, Glycogen synthase kinase 3 beta (GSK3β), α-synuclein, β-amyloid precursor protein (APP), SOD, neurofilaments and Htt, highligting the potential importance of O-GlcNAc in neurodegeneration.[311,312] It has been suggested that O-GlcNAc could play a protective role in NDDs. For example, it has been shown that decreased levels of O-GlcNAc impair mitochondrial activity in AD and are associated with disease progression.[313] In addition, O-GlcNAcylated β-amyloid, tau, and α-synuclein show, both in vitro and in vivo, decreased ability to self-aggregate.[314,315] In light of these observations, increasing the cellular levels of O-GlcNAc (by inhibiting the OGA enzyme) has become a promising target for pharmaceutical intervention in NDDs.[316,317]

Recently, [^{18}F]LSN3316612 has been developed as a high-affinity PET radiotracer for the in vivo quantification of OGA.[318] Preliminary studies, on animals and humans, have demonstrated its suitability to quantify OGA in vivo.[319,320] It is envisaged that, in the future, molecular imaging of the O-GLcNAc biochemical pathway could provide important information in the understanding the pathophysiology of O-GlcNAc in neurodegeneration.

18.8 Translational use of molecular imaging in neurodegenerative diseases

In vivo molecular imaging fulfills the dual role of allowing investigation into components of the complex etio-pathogenic puzzle of neurodegeneration, whilst also facilitating the identification of novel therapeutic targets.[321] Molecular imaging plays a

protagonistic role in revealing the complex biology underpinning neurodegeneration and characterizing biomarkers to be used as criteria to comprehend and define NDDs.[8,9] To this end, appropriate, longitudinal, multi-modal PET studies across the temporal spectrum of NDDs have the potential to track back when and how the initial cellular damage takes place, and how different possible causative pathophysiological processes interact and influence each other to give rise to clinical symptoms. Coupled with strong statistical models, and with sufficient power to predict clinical milestones, they could add value in the selection of at-risk study participants in randomized clinical trials. This approach may warrant a high likelihood of success in studies involving pre-symptomatic carriers of causative disease mutations, such as familial AD or HD.[322] On the other hand, the knowledge derived from such studies will be critical to assist research and development efforts in the design of tailored clinical trials, in which selected inclusion criteria and specific outcome measures can increase the likelihood of success of a trial. In this regard, PET imaging carries the added advantage of potentially achieving large effect sizes whilst requiring relatively small sample sizes, in the context of early development/experimental medicine studies, as opposed to other clinical or fluid outcome measures. The ability of PET imaging to directly visualize changes in brain function at a molecular level, can also be exploited in other parts of the drug development pipeline, such as in vivo studies of target engagement and receptor occupancy, or modulating effects on a metabolic or a neuro-inflammatory process; thus allowing for drug candidate or dose selection, in real-life setting.

18.9 Conclusions

Despite remarkable progress in clinical research, the nosological boundaries and heterogeneity of most NDDs still remain poorly understood and this (at least) partly explains the disappointing output of the pharmaceutical research and development efforts for effective disease-modifying medicines in the last decades. Molecular imaging carries the promise of significantly contributing to the advancement of personalized/precision medicine paradigm in age-related NDDs, by allowing a more precise phenotypic characterization of each at risk individual or patient, albeit directly related to the underpinning biology. Such development will bring the field of NDDs closer to the paradigm of personalized or precision medicine trials, targeting optimally selected study participants of disease subtypes, based on biomarker evidence, with drugs targeting key pathway(s) of the corresponding NDDs subtypes, thus carrying a higher likelihood of drug response; this paradigm of "testing the right drug, in the right patient, at the right dose," using PET imaging for diagnosis/disease staging and treatment monitoring, has proven its value in several cancer forms, resulting in significant improvements in their medical management, patients' quality of life and prognosis. Currently, infrastructures and costs for PET imaging still remain significant limiting factors for the wider

utilization of PET imaging, especially for research purposes; however, this trend is now changing in most parts of the world and the development of fluorinated PET radioligands, of significantly longer half-lives, has increased the clinical potential for PET imaging to be more widely accessible and affordable, in clinical settings. Parallel advances are under way in structural and molecular MRI imaging, with novel probes, in contrast agents and sequences.

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CHAPTER 19

Physical frailty

Yiming Pan and Lina Ma
Xuanwu Hospital Capital Medical University, Beijing, P.R. China

Abbreviations

FP	Frailty phenotype
FI	Frailty index
IC	Intrinsic capacity
SOF	Study of osteoporotic fracture
CFI	Claims-based frailty index
CAN	Care assessment need
MPI	Multidimensional prognostic index
EFS	Edmonton frailty scale
CGA	Comprehensive geriatric assessment
CFS	Clinical frailty scale
GFST	Gérontopôle frailty screening tool
FRAIL scale	Fatigue, resistance, ambulation, illness, and loss of weight scale
FSQ	Frailty screening questionnaire
FiND questionnaire	Frail nondisabled questionnaire
TFI	Tilburg frailty index
GFI	Groningen frailty indicator
SPQ	Sherbrooke postal questionnaire
IL	Interleukin
TNF-α	Tumor necrosis factor α
sICAM-1	Soluble intercellular adhesion molecule 1
CRP	C-reactive protein
MCP-1	monocyte chemoattractant protein 1
RAGE	Receptor for advanced glycation end-products
PCT	Procalcitonin
CXCL-10	CXC chemokine ligand 10
TGF-β1	Transforming growth factor-β1
IIS	Inflammatory index score
sTNFR1	Soluble tumor necrosis factor-α receptor 1
PF&S	Physical frailty and sarcopenia
MPO	Myeloperoxidase
PDGF	Platelet-derived growth factor
MIP	Macrophage inflammatory protein
HPA axis	Hypothalamic-pituitary-adrenal axis
DHEA	Dehydroepiandrosterone
DHEA-S	Dehydroepiandrosterone-sulfate
HPG axis	Hypothalamic-pituitary-gonadal axis

Aging
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SHBG	Sex hormone binding globulin
HPT axis	Hypothalamic-pituitary-thyroid axis
FT	Free thyroxine
IR	Insulin resistance
DM	Diabetes mellitus
HOMA-IR	High homeostatic model assessment for IR
CR	Caloric restrictions
CHAMP	Concord health and aging in men project
SNP	Single nucleotide polymorphism
IGF-1	Insulin-like growth factor-1
HtrA1	High-temperature serine protease A1
3-MH	3-methylhistidine
Cr	Creatinine
eGFR	Estimated glomerular filtration rate
d-ROM	Derivate of reactive oxygen metabolites
LpPLA2	Lipoprotein phospholipase A2
MDA	Malondialdehyde
PrCarb	Protein oxidation like protein carbonyl
8-OHdG	8-hydroxy-2'-deoxyguanosine
mtDNA	Mitochondrial DNA
TL	Telomere length
ApoE	Apolipoprotein E
LPC	Lysophosphatidylcholine
LPA	Lysophosphatidic acid
FI-lab	Laboratory-based murine FI tool
NMHSS	Neuromuscular health-span scoring system
IL-10$^{tm/tm}$	*IL-10* knockout model
Sod1KO	Cu/Zn superoxide dismutase knockout model
NF-B	Nuclear factor B
RSV	Resveratrol
ACEIs	Angiotensin-converting enzyme inhibitors
ARBs	Angiotensin II receptor blockers
RAAS	Renin–angiotensin–aldosterone system
RCT	Randomized controlled trial
ROS	Reactive oxygen species
BCAA	Branched-chain amino acids
BMI	Body mass index

The number of older adults is increasing globally. Those aged 60 years or older, who will require high-quality medical services, are predicted to constitute 22% of the population by 2050,[1] and thus increase the health-care cost in an aging society in. Frailty among older adults has become a subject of great interest in geriatric research. It is a common geriatric syndrome that affects 4% to 59% of older people according to their different socioeconomic conditions[2] and this continues to increase. The care and management of older adults most susceptible to risks is the core of geriatric practice, and many clinical disciplines have established guidelines to help physicians make

clinical decisions while treating older adults. We review the biology of frailty in this chapter.

19.1 The concept of frailty

Frailty refers to a geriatric syndrome that increases an individual's vulnerability due to degenerative changes and chronic diseases.[3] Frailty is a state characterized by homeostatic imbalance, increased sensitivity to stress, and being prone to adverse outcomes,[4] such as disability,[5] or even death.[6] The most common definition of frailty is an age-related biological syndrome characterized by the reduced function of several physiological systems, leading to a decrease in physiological reserves, and an increase in vulnerability to endogenous or exogenous shock. So far, there is no universal definition of frailty and currently there are two major concepts of frailty: the frailty phenotype[7] and the cumulative deficits.[8]

19.1.1 Frailty as a biological syndrome

The most common concept of frailty is the Fried frailty phenotype (FP) or frailty syndrome, which is related to but not equivalent to aging and disability.[7] Its risk factors and pathogenesis are variable, and it is accompanied by a decline in the physiological functions of multiple systems. The FP is defined by the presence of three or more of the following five components: shrinking, weakness, slowness, fatigue, and low physical activity.[7] Fried et al. proposed that chronic undernutrition, sarcopenia, low resting metabolic rate, and decreased total energy expenditure all form a vicious circle, that is, the cycle of frailty, which is the basis of FP. Once the cycle starts, it spirals downward eventually leading to death.[9]

19.1.2 Frailty as cumulative deficits

Another widely recognized concept is the Rockwood frailty index (FI).[8] The FI defines frailty as the accumulation of age-related and nonbiologically related deficits, including cognitive, medical, functional, social, and other factors, and is calculated by dividing the number of deficits by the total possible number of deficits.[8] The FI score reflects the degree of frailty, which can quantitatively evaluate the health of older adults.[8] The WHO pointed out in the "World Aging and Health Report" in 2017 that frailty was related to the decline of intrinsic capacity (IC), including locomotion, vitality, cognition, psychology, and sense.[10] Improving the IC is a way to prevent frailty.[10] The National Institute on Aging Workshop on Measures of Physiologic Resilience indicated that the concept of resilience could be applied to "psychological, behavioral, physiological, clinical, and social outcomes,"[11] implying that resilience is potentially multidimensional in nature. Therefore, both the construct of intrinsic

capacity and resilience could be multidimensional, not only regarding the biological frailty phenotype, but they also could be related to the comprehensive approach.

19.2 Frailty assessment

19.2.1 Frailty measurement tools

A large number of frailty assessment tools can predict health outcomes and disease prognosis. We categorized the tools into performed frailty tools and self-reported frailty tools.

19.2.1.1 The performed frailty tools

Performed frailty tools include FP,[7] FI,[8] the study of osteoporotic fracture scale,[12] claims-based frailty index,[13] care assessment need score,[14] the multidimensional prognostic index,[15] the Edmonton frailty scale,[16] frailty index derived from comprehensive geriatric assessment (CGA),[17] clinical frailty scale,[18] and Gérontopôle frailty screening tool.[19]

19.2.1.2 The self-reported tools

Self-reported frailty tools include the fatigue, resistance, ambulation, illness, and loss of weight scale,[20] frailty screening questionnaire,[21] The frail nondisabled questionnaire,[22] Tilburg frailty index,[23] Groningen frailty indicator,[24] Sherbrooke postal questionnaire,[25] and PRISMA-7 questionnaire.[26]

19.2.2 A two-step frailty measurement

To help with diagnosis and care planning, or to stratify the risk for worse outcomes, as well as to understand the underlying biology, frailty measures should be evaluated in line with the goal of the frailty tool. Each frailty assessment instrument should be matched to the purpose and context of its use. A two-step pathway is recommended for the management of frailty. The first step is case-finding in primary care, using quick frailty screening tools, which can easily be implemented within a short time without equipment and special training, to detect frailty. The second step (assessment) is conducted by trained professionals using complex, time-consuming equipment to perform a frailty assessment or CGA, followed by comprehensive care planning, including a personalized intervention for frailty to delay the decline in intrinsic capacity and to boost resilience.[27]

19.3 The biology of frailty

Figure 19.1 shows the different genetic and environmental factors that modulate the different hallmarks of aging (Fig. 19.1).

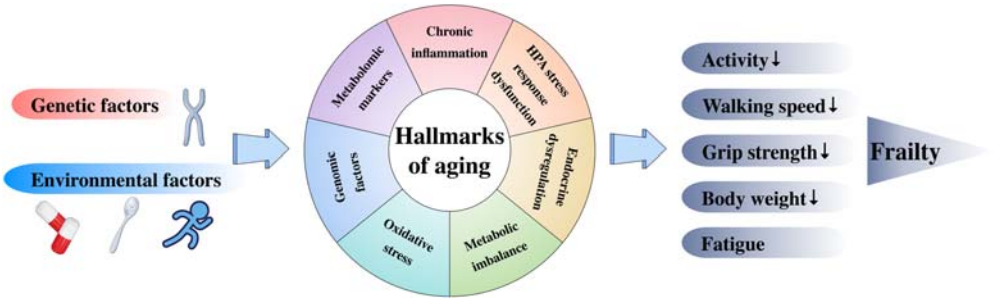

Figure 19.1 The biology of frailty.

19.3.1 Chronic inflammation

Inflammaging, an aging-associated alteration of intercellular communication,[28] refers to a chronic systemic inflammatory state,[29,30] which is considered to be significant in frail older adults.[31,32] Poor physical performance[33] and frailty[34] are associated with chronic inflammation.

Interleukin 6 (IL-6) is a key inflammatory cytokine in signaling pathways regulating aging and frailty.[35] Serum IL-6 levels increase with age,[36] independent of complications, and are negatively correlated with muscle strength,[37] gait speed,[36,38] grip strength[36] and cognitive function.[39] In a longitudinal study, IL-6 was found to be independently associated with both FP and FI,[40] indicating the significance of inflammatory cytokines in frailty. In addition, IL-6 level was found to be a potential predictor of physical and cognitive performance,[41,42] adult lifespan,[43] and the risk of mortality.[41,44]

Tumor necrosis factor α (TNF-α) is a pro-inflammatory cytokine produced by activated macrophages.[45] TNF-α is involved in muscle growth and metabolism,[46] and it triggers mitochondrial dysfunction[47] through the rapamycin-signaling pathway.[48] The muscle TNF-α level in frail older adults was found to be higher than that in healthy controls.[49] TNF-α and its soluble receptor were strongly negatively correlated with muscle mass and strength in older adults[50] and resistance exercise effectively reduces TNF-α levels.[49] Several studies have observed that TNF-α levels were associated with FP and FI.[51–53] However, in the cohort of the Toledo study for healthy aging, there was only a correlation between the TNF-α/IL-10 ratio and frailty, but no cross-sectional relationship between frailty and serum TNF-α levels.[54] Soluble intercellular adhesion molecule 1 (sICAM-1) can promote the production of pro-inflammatory cytokines, such as TNF-α, in vitro to activate inflammatory cascades.[55] sICAM-1 has been reported to be associated with frailty in community-dwelling older Taiwanese populations.[56]

High levels of circulating white blood cells, and neutrophils, as well as high monocyte counts, and low lymphocyte counts were associated with frailty.[57,58] In people

older than 65 years with recently acute coronary syndrome, a low lymphocyte percentage was associated with a high risk of frailty.[59] However, a 10-year longitudinal study has shown that higher levels of white blood cells, neutrophils, monocytes, and lymphocytes were associated with higher odds of being frail.[57] Therefore, the relationship between lymphocyte count and frailty requires verification. In addition, increased monocyte and neutrophil counts were observed to be related to frailty only in women.[60] Among community-dwelling Chinese older adults, T follicular helper cell subsets were correlated with frailty, and this correlation was stronger in females.[61] The potential sex differences in immune cells in the pathogenesis of frailty require more attention. However, some researchers have questioned whether there is any association between lymphocyte subsets and frailty, because they only observed a correlation between inflammatory mediators and frailty.[62]

Other inflammatory features are also associated with frailty. The C-reactive protein (CRP) levels were associated with frailty[63,64] and can predict frailty,[65] cognitive performance,[41] and mortality[41] in older adults. The level of circulating monocyte chemoattractant protein 1 (MCP-1) is a potential measure of frailty.[32] In wild-type mice, MCP-1 levels increased with age; in older adults with aortic stenosis, the circulating MCP-1 levels of frail participants were significantly higher than that of nonfrail participants.[66] The receptor for advanced glycation end-products (RAGE), a transmembrane glycoprotein of the immunoglobulin superfamily, can activate inflammatory responses. A prospective study in Europe showed that RAGE was an independent predictor of mortality in frail individuals and may be used in the prognosis assessment and treatment stratification of frailty.[67] Circulating procalcitonin,[68] fibrinogen,[34] CXC chemokine ligand 10 (CXCL-10),[69] and salivary α-amylase[70] are also considered to be associated with frailty.

Frailty involves changes in multiple inflammatory pathways, and the multivariate measurement of inflammation has become a promising method for reflecting frailty. Bandeen-Roche et al.[71] found that an index based on the composition of seven serum inflammatory biomediators, including TNF-α, IL-1β, IL-6, IL-18, IL-1 receptor antagonist, CRP, and transforming growth factor-β1 (TGF-β), could be used to measure functional loss in older adults. The up-regulation of scores was independently associated with morbidity function decline and frailty risk, while the down-regulation of scores was associated with frailty outcomes. Subsequently, Varadhan et al. developed an inflammatory index score including IL-6 and soluble tumor necrosis factor-α receptor 1 (sTNFR1) for detecting 15 NF-κB-mediated inflammation markers, which was a good predictor of 10-year all-cause mortality in older adults,[44] and had a stronger correlation with frailty[72] compared with age.[73] Marzetti et al.[74] identified the core inflammatory profile of older adults with physical frailty and sarcopenia (PF&S): higher levels of P-selectin, CRP, and interferon γ-induced protein 10, and lower levels of myeloperoxidase (MPO), IL-8, MCP-1, macrophage inflammatory protein 1-α, and

platelet-derived growth factor (PDGF) BB. The sex-specific inflammatory signature of PF&S in older persons included higher levels of CRP, P-selectin, and macrophage inflammatory protein-1β (MIP-1β), and lower levels of MPO, IL-8, MCP-1, PDGF-BB, MIP-1α, and eotaxin in females, with higher levels of CRP and lower levels of interferon-γ, FGF b, IL-17, TNF-α, and MIP-1β in males.[74] In summary, an imbalance in the inflammatory state that participates in physical frailty's pathophysiological mechanisms is an important feature of frailty.

19.3.2 Hypothalamic-pituitary axis stress response dysfunction

The hormones produced by the hypothalamic-pituitary axis can affect most endocrine systems in the body, especially the adrenal glands, gonads, and thyroid, which are crucial in the pathogenesis of frailty. The hormones of the hypothalamic-pituitary-adrenal (HPA) axis include cortisol, epinephrine, norepinephrine, dehydroepiandrosterone (DHEA), and its sulfated metabolites. Cortisol is a lipophilic steroid hormone that regulates the effects of environmental, physical, psychological, and social stress on the body. The frailty phenotype was found to be associated with high salivary cortisol levels[75,76] and a decreased cortisol response (lower cortisol levels in the morning and higher in the evening).[77] The diurnal cortisol ratio could thus be used to measure the degree of frailty.[77] The serum cortisol concentration was positively associated with frailty burden,[57,78] and it increased the dependence on activities of daily living and the 10-year mortality risk in older adults.[78] As a steroid hormone, DHEA is a precursor to testosterone and estrogen. A lower level of its sulfide metabolite, DHEA-sulfate (DHEA-S), was related to the higher rate of frailty in older adults.[57] The evidence above supports the hypothesis that the dysregulation of the HPA axis is related to frailty. In the hypothalamic-pituitary-gonadal (HPG) axis, low total testosterone levels were associated with decreased grip strength in men.[79] After adjusting for age, low free testosterone levels were associated with high frailty risk, weight loss, and decreased muscle strength in older men.[80] A prospective cohort study in Europe found that higher FI scores in men were associated with lower levels of testosterone and DHEA-S and higher levels of gonadotropin and sex hormone binding globulin.[81] After adjusting for the circulating testosterone levels, the increase in gonadotropin levels was still related to frailty, indicating that the changes in pituitary-testicular function may reflect the characteristics of frailty, but not its cause.[81] A longitudinal reduction in serum androgen concentration was significantly related to the progression of frailty in older men, whereas the baseline estrogen level was a better predictor of frailty than longitudinal changes in estrogen levels in older women.[82] In the hypothalamic-pituitary-thyroid axis, free thyroxine (FT) levels were found to be associated with frailty in older men and high-normal FT4 levels were an independent predictor of frailty in older men.[83]

19.3.3 Endocrine dysregulation (dysfunctional hormone regulation)

Endocrine dysregulation is associated with physical frailty. At present, most studies have focused on the effects of insulin resistance (IR) on frailty. Diabetes mellitus (DM) was found to be associated with a higher risk of physical frailty. This association could be explained by obesity, poor glucose control, and abnormal lipid distribution, with IR being the underlying mechanism.[84] In a prospective cohort, a high Homeostatic Model Assessment for IR (HOMA-IR) index was associated with reduced grip strength, weight loss, fatigue, and high frailty risk.[85]

Adiponectin is a multifunctional adipokine with the functions of insulin sensitization, antiinflammatory responses, and metabolic regulation; although high adiponectin levels were associated with a low risk of type 2 DM,[86] it was always accompanied by a decrease in muscle strength[87] and grip strength.[36] In older men, the number of frailty components increased with adiponectin levels.[88] The level of adiponectin was observed to be associated with both FP[36] and FI.[89] Adiponectin resistance related to insulin resistance may be the key mechanism. It is worth noting that adiponectin had different correlation strengths with frailty in different sexes.[88] Serum adiponectin was also a sex-specific independent negative predictor of lean mass and anxiety in women, and the Montreal Cognitive Assessment test score in men.[90]

As an adipocyte-derived hormone, leptin can maintain energy by suppressing hunger and promoting the oxidation of fatty acids in muscle to regulate body weight and metabolism. Leptin levels were found to be related to FI in a longitudinal study.[32] Older adults with higher leptin concentrations have slower gait speed[36,91] and greater frailty risk.[92] In addition, the level of leptin was positively correlated with HOMA-IR and the concentration of CRP, suggesting that IR and chronic inflammation are possible mechanisms of frailty.[92] Moreover, as a serine protease inhibitor derived from visceral adipose tissue, vaspin has insulin-sensitizing effects associated with IR. Compared with nonfrail controls, frail older adults had higher vaspin levels, that were not correlated with physical functions.[36]

19.3.4 Metabolic imbalance

Sirtuins belong to the family of NAD-dependent protein deacetylases, including seven homologous proteins, SIRT1−SIRT7, which have regulatory effects on aging through caloric restrictions (CR).[93,94] SIRT1 is a biomarker of aging and a regulator of the life cycle in animal models.[94] It prolongs the lifespan and aging process, and improve the physical activity of mice.[95,96] Some researchers have observed that after adjusting for confounding factors, the concentration of SIRT1 and SIRT3 in older adults decreased with age, especially in frail individuals.[97,98]

However, clinical studies have reported conflicting results. In the concord health and aging in men project (CHAMP), older men with low SIRT1 levels had a higher

lean mass and were less prone to frailty.[99] Their further research drew the same conclusion, and they believed that the SIRT1 single nucleotide polymorphism (SNP) and serum SIRT1 levels were more suitable parameters to reflect the nutritional status and body composition of older adults, rather than frailty or aging.[100] Similarly, data from older adults in China supported the association of high SIRT1 levels with frailty and slow gait speed.[101] These results were contrary to the expected results based on the biological function of SIRT1, and the possible explanation may be related to the functions of SIRT1 in calorie restriction. Therefore, future research in this area should refine the diet structure and nutritional status of the participants.[100]

An insulin-like growth factor-1 (IGF-1) is a circulating hormone that has an anabolic effect on the muscles. Serum IGF-1 can inhibit muscle cell apoptosis,[102] thereby increasing muscle area and density.[103] As a result, high IGF-1 levels can improve knee extensor strength[104] and physical performance,[105] and reduce adverse outcomes.[106] Frail older adults had lower levels of serum IGF-1[36,107–109] and higher levels of IGF-1 binding protein-1.[107] High-temperature serine protease A1 (HtrA1) is a multi-domain serine protease that can regulate IGF-1 levels. The increase in plasma HtrA1 levels was correlated with both FP and FI.[110]

As sarcopenia overlaps with physical frailty in both clinical symptoms (e.g., slow gait speed and low physical activity) and pathophysiological mechanisms (e.g., loss of muscle mass), sarcopenia is regarded as a biological substrate of physical frailty.[111] Certain markers of muscle protein renewal can also be used to reflect the frailty state. For example, 3-methylhistidine (3-MH), a component of actin, is a marker of myofibril proteolysis. 3-MH, 3-MH/creatinine (3-MH/Cr), and 3-MH/estimated glomerular filtration rate (3-MH/eGFR) were positively correlated with FP, indicating that the catabolism of muscle protein may increase in frailty.[112]

Vitamin D is a fat-soluble steroid, which is transformed from 7-dehydrocholesterol in the skin under ultraviolet radiation, and functions to increase the absorption of calcium, magnesium, and phosphate. A lower vitamin D level was associated with a higher prevalence and risk of frailty[113–115] and a higher risk of death.[116] The effects of vitamin D deficiency on frailty are unclear. However, vitamin D supplementation has become a pharmacological intervention for frailty.

19.3.5 Oxidative stress and mitochondrial dysfunction

The accumulation of oxidative damage during aging can impair cellular and organ function.[117] Chronic low-grade systemic inflammation and chronic oxidative stress was found to interact when the body's homeostasis, related to aging, was out of balance. The "oxi-inflamm-aging" theory was thus, proposed, in which, oxidative stress was closely related to an individual's lifespan.[118] However, life-long spontaneous exercise was found to prevent oxidation by reducing the production of free radicals and

improving the health-span, but not the lifespan of mice.[119] Based on this, it was proposed that oxidative stress was related to frailty but not to age.[120]

Studies have shown that many oxidative stress markers are closely related to frailty. After adjusting for age, markers to reflect oxidative stress levels, such as the derivate of reactive oxygen metabolites (d-ROM),[64] isoprostane and lipoprotein phospholipase A2 (LpPLA2),[121] markers to measure plasma lipid peroxidation such as malondialdehyde,[122] markers to measure protein oxidation like protein carbonyl (PrCarb),[122,123] and markers to reflect DNA oxidative damage, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG)[124] were all associated with frailty.

Mitochondria are important organelles that regulate energy metabolism and are a significant source of molecular patterns related to endogenous damage.[125,126] The accumulation of mitochondrial damage results in myocyte loss and muscle wasting,[127] leading to increased susceptibility to diseases and decreased physical function in older adults.[128–130] It was found that improving the mitochondrial function of aged *Drosophila melanogaster*, reduced the decline in metabolism, vision, movement, and cognitive abilities.[131] As a surrogate marker of mitochondrial function, mitochondrial DNA (mtDNA) can reflect mitochondrial exhaustion, energy reserves, and oxidative stress.[132] The mtDNA copy number was significantly negatively correlated with age.[129] A low mtDNA copy number has been associated with frailty, poor cognitive performance, and decreased physical strength.[128,129] The mtDNA copy number was also an independent predictor of all-cause mortality.[128,129] However, a previous study found that plasma mtDNA levels increased with age and contributed to the maintenance of chronic low-grade inflammation in older adults.[133]

19.3.6 Genomic factors

SNPs are associated with frailty. As mentioned earlier, CRP levels increase with frailty.[64] However, frail individuals with the *CRP1846G > A* polymorphism failed to respond effectively and had a higher risk of frailty,[134] indicating that SNP rs1205 (*CRP 1846G > A*) was related to frailty. Genetic association analysis showed that rs1800629 (*TNF*) and rs1566729 (*PTPRJ*) were significantly associated with frailty.[135] In the Toledo Study of Healthy Aging, researchers found through a genomics analysis that 15 SNPs were closely related to frailty, including: rs11812479 (unknown gene), rs10457204 (*FIG4*), rs737154 (*SLC12A7*), rs16889283 (*AARD*), rs3809430 (*FSCB*), rs7096031 (*OTUD1*), rs11208257 (*PGM1*), rs1929860 (*CDC42BPA*), rs1126673 (*ADH4*), rs1126671 (*ADH4*), rs1387144 (*BDNF-AS*), rs3773603 (*CACNA2D3rs*), rs4148883 (*AD613H4NF*-AS), and rs11006229 (*SGMS1*).[136] However, in the Women Health and Aging Study, 1354 SNPs were screened, and none of them were associated with frailty after multiple rounds of correction.[137]

Telomeres are tandem DNA repeats at the ends of eukaryotic chromosomes that maintain chromosome integrity. In addition to aging, telomere attrition is regarded as

a hallmark of aging.[138] However, the significance of telomere attrition in frailty remains unclear. In the Helsinki Birth Cohort Study, short telomere length (TL) in leukocytes was associated with frailty, but telomere shortening has nothing to do with it.[139] A metaanalysis showed that the association between TL and frailty might only exist in Hispanics.[140] However, some researchers have reported that there was no correlation between TL and frailty.[141,142]

Apolipoprotein E (ApoE) is associated with lifespan and cognitive function.[143] *APOE*, which encodes ApoE, is involved in various physiological functions, such as inflammation, oxidative stress, and lipoprotein metabolism. The *APOE ε4* allele is associated with many age-related diseases, such as Alzheimer's disease.[144] Carriers of *APOE ε4* alleles had lower CRP levels[145,146] and a higher vulnerability to the cognitive impairment caused by the dysregulation of the HPA axis.[147] The Hellenic Longitudinal Investigation of Aging and Diet study showed that carrying the *APOE ε4* allele increased the risk of frailty.[148]

In terms of epigenomics, since DNA methylation models are reshaped with aging,[149] the age of DNA methylation can better estimate the biological age of animals and measure the cumulative effect of epigenetics.[150] The acceleration of DNA methylation age is associated with increasing accumulated deficits.[151] Individuals with an older biological age, measured by the DNA methylation clock, were more vulnerable to frailty.[152] The epigenetic clock named DNAm GrimAge, constructed based on seven protein markers of aging, is closely related to many age-related diseases, and has great predictive ability for time-to-death.[153]

19.3.7 Metabolomic markers

Lysophosphatidylcholine (LPC) is a biologically active lipid rich in human plasma. Research on animals has shown that after knocking out *IL-6* in frail *IL-10*$^{tm/tm}$ mice, the levels of some mitochondrial-related lipid metabolites, such as LPC a C16:1, 18:1, and 20:3, were higher than those in frail mice, and had better short-term functional performance.[154] Several longitudinal studies have shown that circulating LPC content was an independent predictor of cognitive and physical function decline in older adults[155,156] and was related to the impaired oxidative capacity of skeletal muscle mitochondria.[155] LPC is the precursor of lysophosphatidic acid and participates in the synthesis of cardiolipin, which is a phospholipid specific to mitochondria.[157] The role of LPC in the cardiolipin synthesis pathway may be a potential mechanism underlying the correlation between LPC and FP.

Several studies have attempted to rely on targeted or nontargeted metabolomics platforms to characterize the metabolic profiles of frail older adults from different populations. For example, in frail breast cancer patients, the levels of hydroxyproline, 3-methylhydropyridine, cystine, and β-aminoisobutyric acid increased, and the levels

of serine, tryptophan, histidine, glycerol, and sphingosine phospholipids decreased.[158] In older adults with frailty and sarcopenia, higher levels of circulating asparagine, aspartic acid, citrulline, ethanolamine, glutamic acid, sarcosine, and taurine, and lower levels of α-aminobutyric acid and methionine were found.[159] The circulating levels of 3-methylhistidine, alanine, arginine, glutamate, sarcosine, tryptophan, and ethanolamine were higher in frail older patients with type 2 DM.[160] In frail older women, circulating uridine levels decreased, and C-glycosyltryptophan and N-acetylglycine levels increased.[161] Community-dwelling older black men had higher circulating levels of gluconic acid, N-carbamoyl-β-alanine, isocitrate, creatinine, C4-OH carnitine, cystathionine, hydroxyphenylacetic acid, and putrescine levels, and lower levels of tryptophan, methionine, tyrosine, asparagine, C14:0 sphingomyelin, and 1-methylnicotinamide.[162]

Frailty metabolic profiles show sex-specific characteristics in some studies, such as increased glutamine and isovaleryl carnitine and decreased dimethyloxazole in males, and increased threonine, and decreased dihydroxyphenylacetic acid and mannose in females.[163] In addition, with the help of metabolomics technology, researchers have explored the possible mechanisms of frailty and discovered three frailty-related metabolic pathways, including nitrogen metabolism, aminoacyl-tRNA biosynthesis, and the citric acid cycle.[162] In frailty-related metabolomics research, a variety of biological matrices have been detected, such as plasma,[161] serum,[160] blood,[164] and muscle biopsy.[165] However, most of the studies had small sample sizes, different populations, and inconsistent platforms, resulting in poor comparability, and no consensus has been reached. Research on metabolomic biomarkers for frailty is still in its infancy.

19.4 Animal models of frailty

Heterogeneity between health state and age can be observed in the aging process of humans, dogs, rodents, and even nematodes.[166–168] Some animal models have been constructed to simulate the human frailty state and the natural course of frailty.

The most widely used model is the *C57BL/6* mice, in which the FP[169,170] and FI[171,172] have already been quantified. Liu et al.[169] evaluated the FP of *C57BL/6* mice using human standard criteria, including grip strength (inverted-cling grip test), maximum walking speed (rotarod test), physical activity (voluntary wheel running), and endurance (rotating rod measurement plus grip strength test). The results showed that the prevalence of frailty in mice was 9%, which was similar to that in humans.[169] Gomez-Cabrera et al.[170] evaluated five aspects of male *C57BL/6J* mice: unintentional weight loss, running time, running speed, grip strength, and motor coordination. The results revealed that sedentariness could be a model of frailty in mice.[170] Parks et al.[171] carried out FI assessments based on accumulating deficits in naturally aging *C57BL/6J* mice on four aspects of activity levels, hemodynamic measures, body composition, and basic metabolic status, with 31 indicators. The results showed that the FI scores of old

mice were higher than that of young mice, but without differences between the sexes.[171] However, using FI to assess frailty is time-consuming, invasive, and dependent on specialized equipment, which limits its application in longitudinal research.[171] Whitehead et al.[172] further improved the FI into a clinical FI tool, based on the clinical signs of frail mice. This tool evaluated 31 indicators belonging to the integument, musculoskeletal system, vestibulocochlear/auditory systems, ocular and nasal systems, digestive system, urogenital system, body weight, and body surface temperature of *C57BL/6J* mice, including the respiratory system, discomfort, or restless state with simplified, noninvasive methods.[172] The relationship between clinical FI and age was found to be similar between mice and humans.[172] Kane et al.[173] developed a laboratory-based murine FI tool (FI-lab) for *C57BL/6* mice and included 23 indicators such as blood pressure, blood chemistry, and echocardiography. The high FI-lab score was positively correlated with pro-inflammatory factor levels, and sex differences were present.[173] The FI-lab score was higher than the clinical FI score, which may be beneficial for the identification of frailty in the early stages.[173] Graber et al.[174] developed a neuromuscular health-span scoring system (NMHSS) based on physiological and functional measurements of male *C57BL/6* mice to test the effectiveness of potential interventions on sarcopenia and frailty in aging animal models, including the performance in the rotarod, inverted-cling grip test, and in vitro muscle contraction.[174] However, this tool for muscle contractility assessment was invasive, which limited its application in longitudinal studies.[174] However, the models mentioned earlier generally had problems such as small sample size, lack of sex comparisons, and complex or invasive evaluations, thus they need to be further optimized.

Transgenic models of frailty include the *IL-10* knockout model (*IL-10*$^{tm/tm}$)[175] and Cu/Zn superoxide dismutase knockout model (*Sod1KO*).[176] As an antiinflammatory cytokine, IL-10 can inhibit the expression of inflammatory mediators induced by nuclear factor B (NF-B).[177] The *IL-10*$^{tm/tm}$ mice did not produce IL-10. As a result, *IL-10*$^{tm/tm}$ mice had chronic inflammation, leading to frailty-related phenotypes such as decreased muscle strength and increased IL-6 serum levels, compared with the *C57BL/6* wild-type controls.[175] Therefore, it can be regarded as a possible animal model of frailty. However, the *IL-10*$^{tm/tm}$ mice did not display certain aspects of FP, such as weight loss, decreased activity levels, or increased mortality.[175] Frailty was not quantified in this model. In addition, the *IL-10*$^{tm/tm}$ mice were initially designed as a model of inflammatory bowel disease, which often leads to early disease or death,[178] thereby limiting its application in longitudinal studies. *Sod1KO* mice exhibited increased oxidative stress, mitochondrial dysfunction, and cellular senescence, and were accompanied by inflammation and sarcopenia.[176] *Sod1KO* mice showed an FP similar to humans, such as weight loss, weakness, low physical activity, and fatigue, and are a promising animal model of frailty,[176] however, its frailty was not quantified. There have also been studies in which male *C57BL/6J* mice were injected intraperitoneally with lipopolysaccharide endotoxin[179] or NIH/Swiss mice were fed with

a high-fat diet[180] to induce frailty, but the stability of these models requires further evaluation.

Frailty might be a risk factor for death in dogs similarly to humans,[181] the age-related deficit accumulation measured by FI was strongly related to mortality in dogs, without evident differences of sex or age.[182] Moreover, some invertebrate models for the studies of aging, such as fruit fly *D. melanogaster*[183] and nematode *Caenorhabditis elegans*,[184] also displayed an age-related deficit on motor ability. However, the possibility and standards for frailty of these models still require further exploration.

The most promising application of frailty animal models is the testing of the effects of various interventions on frailty. Current applications of frailty models include exploring the functions of drugs (such as resveratrol,[185] rapamycin,[180] enalapril[186] and metformin[187]), polypharmacy,[188] exercise[170,189] and diet[180,185,190] on frailty. The application of these models can help promote the transformation of interventions to the clinic and our understanding of the mechanisms underlying frailty.

19.5 Interventions to attenuate frailty

Longitudinal studies have shown that frailty is reversible and preventable.[191] To maintain older adults living independently and healthy in the community, timely and effective interventions for frailty are essential.

19.5.1 Pharmacological interventions

Metformin is a widely used hypoglycemic agent. As metformin may have beneficial effects on lifespan and health span, there have been some studies on animal models to explore the effectiveness, safety, and possible mechanism of action of metformin on frailty.[192–196] Metformin treatment improved mobility,[193,194] spatial learning, and memory[193] of mice. Without CR, metformin could also increase insulin sensitivity in mice, regulate blood lipids,[194] and reduce fat content.[193] Metformin protects mitochondrial function, thereby reducing oxidative damage and chronic inflammation.[194] The application of metformin from young or middle-aged mice prolonged the lifespan of outbred Swiss-derived female SHR mice and delayed tumorigenesis.[192] Taking metformin at any stage of life improves reproductive function.[192] However, metformin has also shown harmful effects on the central nervous system[196] and sciatic nerve fibers[197] of mice, and may also increase the incidence of porcelain gallbladder.[198] Clinically, metformin can significantly increase the gait speed of patients, although it had no effect on grip strength.[199] In addition, metformin reduced the possibility of frailty-related and aging-related diseases and reduced all-cause mortality, indicating that metformin may have a protective effect on older adults.[200,201]

Rapamycin is an immunosuppressant that acts on the mTOR signaling pathway to regulate protein synthesis and redox sensing. Rapamycin was shown to improve grip strength,

stride length, mobility, endurance, and resistance to muscle fatigue in *C57BL/6Nia* mice.[202] Rapamycin reduced frailty and improved long-term memory, neuromuscular coordination, and tissue structure in male *nfκb1*$^{-/-}$ mice.[203] In addition, the effects of rapamycin on mouse frailty[180,204] and survival[205] was sex-specific. However, it should be noted that rapamycin has reproductive toxicity in male mice and can also cause cataracts.[206] Rapamycin can improve certain FPs in mouse models and may be beneficial to health, but more research is needed to explain its mechanism and sex-specific effects.

Resveratrol (RSV) is an antioxidant compound found in plants. RSV was found to improve the mobility,[207] balance,[208] motor coordination,[208] grip strength,[209,210] and antifatigue ability[209,210] in the mice model. It also improved the FI score of mice[185] and extended their lifespan.[207]

Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are antihypertensive drugs that act on the renin−angiotensin−aldosterone system. Long-term application of the ACEI, enalapril, has been shown to alleviate frailty in mice without long-term effects on blood pressure.[186] The possible mechanism may be the association with the levels of inflammatory factors such as IL-1α, IL-10, MCP-1, and MIP-1a.[186] The ARB, losartan, was shown to improve the mobility of mice and significantly reduce serum IL-6 levels.[211] In older women with hypertension but without congestive heart failure, ACEI can slow down the decline in muscle strength.[212] A randomized controlled trial (RCT) also showed that the administration of the ACEI, perindopril, for 20 weeks, can improve the exercise capacity of older adults with impaired functions without heart failure.[213]

While testosterone supplementation in frail older males can increase skeletal muscle strength and muscle capacity,[214] it can also increase the risk of heart, respiratory, and skin disease events.[215] An RCT showed that DHEA or low-dose testosterone replacement did not improve the body composition, physical function, insulin sensitivity, or quality of life in older adults.[216] Currently, there is insufficient evidence to prove that hormone supplementation is beneficial for the prevention or treatment of frailty, and there is no recommended hormone replacement therapy.

As mentioned earlier, low vitamin D levels are associated with frailty. However, vitamin D supplementation as a pharmacological intervention for frailty remains controversial. Vitamin D combined with calcium reduced the risk of falls in older women[217] and mortality in older adults,[218] while vitamin D alone was not effective. Vitamin D supplementation can effectively prevent hip fractures and nonvertebral fractures in older adults (≥800 IU per day),[219] and can be beneficial for strength and balance (800−1000 IU per day).[220] However, excessive vitamin D supplementation may increase the risk of falls and fractures, especially in older adults without vitamin D deficiency.[205]

Polypharmacy is common in frail older adults. Physical frailty affects pharmacokinetics[221−223] and pharmacodynamics[224,225]; polypharmacy, in turn, increases the incidence[226] and mortality[227] of frailty. Therefore, we must fully evaluate

the necessity, effectiveness, and safety of medication before administering pharmacological interventions.

19.5.2 Nonpharmacological interventions

Frailty can be intervened with through nonpharmacological measures such as exercise and nutrition, which has been a consensus.[228]

Moderate exercise can activate the NF-κB pathway and increase the expression of enzymes against reactive oxygen species (ROS), which has antioxidant effects.[229] Given the important role of oxidative stress in the pathogenesis of frailty, exercise as an antioxidant was thought to have antifrailty effects. Animal experiments have shown that a variety of exercise modes, including aerobic exercise and high-intensity interval training, can reverse or delay the process of frailty in mice and reduce mortality.[170,189,230,231] Physical exercise can also reduce frailty in older adults and partly change adverse outcomes.[232] Therefore, it is considered to be one of the most significant interventions for frailty. Aerobic endurance training can increase peak oxygen consumption and improve aerobic capacity.[233] Progressive resistance strength training has been shown to improve muscle strength and gait speed in older adults.[234,235] Multi-component exercise interventions, including two or more of aerobic, endurance, strength, coordination, balance, and flexibility training, can significantly improve the physical performance of frail older adults[233,236,237] and reduce the prevalence and level of frailty,[236,238] or even reverse frailty.[239] Long-term multi-component exercise interventions generally have better outcomes than other exercise programs.[240,241] Exercise is at the forefront of interventions to prevent or delay frailty. However, there are currently no recommendations for the best exercise program for individualized interventions for frailty.

Insufficient nutritional intake and selected nutrients were independently associated with FP in older adults.[242] Protein supplements rich in leucine and vitamin D can significantly increase muscle mass, especially in the muscles of the lower limbs.[243] In sarcopenic older adults undergoing low-intensity resistance training, additional branched-chain amino acids (BCAA) and vitamin D supplementation increased grip strength, calf circumference, and body mass index.[244] Supplementation of BCAA might stimulate muscle growth in older adults. BCAA supplementation in older adults could also effectively increase cognitive ability, albumin levels, ATP production and electron flux, and maintain low oxidative stress with reduced TNF-α levels.[245,246] To overcome frailty, older adults need a comprehensive, adequate, and balanced intake of nutrients. The supplementation of calories, proteins, and essential amino acids for frail older adults was also recommended in "the Asia-Pacific Clinical Practice Guidelines for the Management of Frailty" in 2017.[247] However, nutritional supplements should also be moderate. Animal research has shown that CR delayed the development of

frailty in male mice[185] and rats,[190] but not in female mice.[185] Longitudinal studies have found that in the monkey model, CR can improve FP, reduce the incidence of frailty, and prolong the health-span in nonhuman primates.[248] The balance between nutritional supplementation and CR, remains an unresolved issue in dietary interventions for frailty.

19.6 Conclusion

Because of the importance of accounting for frailty in clinical decision-making, frailty assessments should be integrated into daily clinical practice. Research investigating the biology of frailty is still in its early stages, and there is a need for rigorous and well-designed clinical trials using specific inhibitors or activators to confirm the role of the markers that have been described and further develop therapeutic targets for the management of frailty. Diagnostic and therapeutic opportunities should be taken into consideration to maintain functional mobility and independence in aging populations. However, there are still many unresolved questions in this field. Future studies exploring the mechanism of frailty will identify compounds that interfere with new targets for frailty and develop a variety of interventions and new technologies to improve the flexibility of the stress response in people with frailty.

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CHAPTER 20

The extracellular matrix in cardiovascular aging

Deolinda Santinha[1,2,3], Andreia Vilaça[1,4,5,6], Alessandro Ori[7] and Lino Ferreira[1,2]
[1]CNC - Center for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[2]Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Coimbra, Portugal
[3]Inter-University Doctoral Program in Aging and Chronic Diseases, Faculty of Medicine, University of Coimbra, Azinhaga de Santa Comba, Coimbra, Portugal
[4]CARIM School for Cardiovascular Diseases, Faculty of Health, Medicine and Life Sciences, Maastricht University, Maastricht, Netherlands
[5]Department of Molecular Genetics, Faculty of Sciences and Engineering, Maastricht University, Maastricht, Netherlands
[6]PDBEB - PhD Programme in Experimental Biology and Biomedicine, IIIUC - Institute for Interdisciplinary Research, University of Coimbra, Coimbra, Portugal
[7]Leibniz Institute on Aging, Fritz Lipmann Institute (FLI), Jena, Germany

Abbreviations

CVDs	Cardiovascular diseases
WHO	World Health Organization
ECM	Extracellular matrix
LV	Left ventricle
LA	Left atrial
CFs	Cardiofibroblasts
LOX	Lysyl oxidase
AGEs	Glycation end products
SPARC	Secreted protein acidic and rich in cysteine
TSP	Thrombospondin
TGF-β	Tumor growth factor-β
MMPs	Matrix metalloproteinases
TIMPs	Tissue inhibitor of metalloproteinases
GAGs	Glycosaminoglycans
FN	Fibronectin
MI	Myocardial infarction
HF	Heart failure
HFrEF	Heart failure with reduced ejection fraction
HFpEF	Heart failure with preserved ejection fraction

20.1 Introduction

Cardiovascular diseases (CVDs) are very connected to aging.[1] For instance, heart failure is more prevalent in people aged 75 years or older (8.4%) compared to those aged 45–54 (0.7%).[2] Thus, aging can be considered an independent risk factor for the

Aging
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development of common cardiac pathologies.[2] The prevalence of CVDs in the elderly and the increase of the aged population are indisputable concerns. According to the WHO, the percentage of the aged population 60 years or older globally will increase from 11% to 22% between 2000 and 2050, accounting for 2 billion of the world's population.[3,4]

Aging induces several structural and functional alterations in the cardiovascular system, which can occur at the cellular and non-cellular levels. For several decades, cardiac dysfunction was almost completely attributed to cardiomyocyte remodeling and no importance was attributed to dysfunctions of the extracellular matrix (ECM). However, it is currently accepted that cardiac ECM is not only a meshwork that supports cells, providing structure and strength to the tissues, but it also has several important nonstructural properties such as, intercellular communication, signal transduction to the cells and the regulation of several cellular processes such as cell migration,[5] proliferation,[6] cell communication[7] and differentiation.[8] Furthermore, it is a source of many of the signals that elicit plasticity and rapid adaptation to the environment, playing a crucial role as a mechanotransducer and sensor of the extracellular environment. Consequently, cellular organization, fate and ultimately functional properties of the tissues are defined by the ongoing cell-matrix and matrix-cell interactions.[9] Alterations in the quality, quantity and organization of the ECM proteins, can induce an altered systolic function even in the absence of cardiac myocyte contractile dysfunction.[10]

Aging has been shown to induce alterations on the cardiac ECM, however, these alterations have often been described as unspecific interstitial fibrosis, which corresponds to an increased ECM deposition.[11] However, additional levels of complexity derive from the dynamic interaction between signaling pathways, protein synthesis and degradation, as well as post-translational modifications, which collectively contribute to a healthy or dysfunctional cardiac ECM.[11] The impact of aging on cardiac ECM has been reviewed in previous studies from a remodeling point of view[12–14]; however, the impact of aging on the composition of cardiac ECM and how these alterations induce modifications in cardiac cell phenotype and activity has not been covered in previous studies. However, recent progress has been made to unravel the impact of cardiac ECM on cardiomyocyte activity, which makes this chapter timely.[15,16] It is important to note that this progress was possible due to advances in methodologies to decellularize the heart tissue, the general use of proteomic analyses to identify alterations in the composition of the ECM, and the use of induced pluripotent stem cell-derived cardiomyocytes to investigate the impact of aged ECM.

In this chapter, we cover the alterations of the heart ECM during aging. The expression and properties of collagens, elastin, matricellular proteins, glycosaminoglycans and proteoglycans, adhesive proteins and basement membrane components will be discussed and compared between young and aged hearts. The remodeling of ECM

in aged individuals, in the context of myocardial infarction and heart failure, will also be discussed as well as future directions in the research area of ECM and cardiovascular aging.

20.2 Physiological alterations of the aged heart

20.2.1 Aging induces functional and morphologic cardiac alterations

Aging is a progressive and multifactorial process. The decreased vascular compliance that occurs in aging, due to an increase in arterial stiffness, has been identified as a primary trigger to induce age-related changes in the myocardium.[15] Several cellular, morphological, and functional alterations in the myocardium have been described (Fig. 20.1). At a morphological and functional level, age-related alterations of blood pressure are consistent with the increase of vascular stiffness in the large artery.[16] It has been observed that systolic blood pressure continuously increases from ages 30 to 84 years old, whereas diastolic blood pressure falls after the sixth decade.[16] Increased

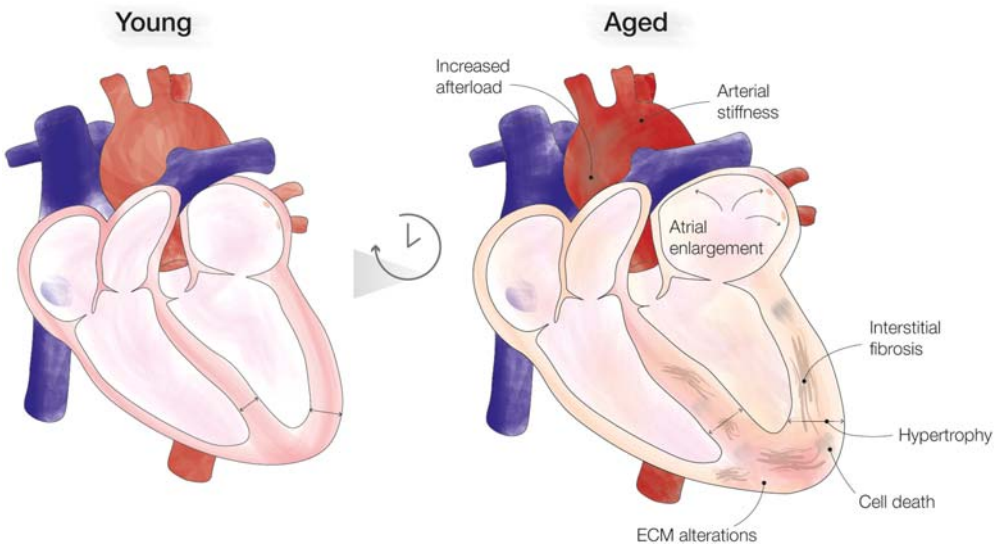

Figure 20.1 ***Aging induces morphological and functional alterations on the cardiovascular system.*** Vascular remodeling leads to arterial stiffening with aging, reducing the vascular compliance and increasing myocardial afterload. In the left ventricle, cardiomyocytes die by apoptosis or necrosis, leading to hypertrophy of the remaining cardiomyocytes, leading to a decompensated ventricular hypertrophy, demonstrated by a thicker left ventricular (LV) wall in the aged heart compared to the young heart. An increased myofibroblast proliferation promotes interstitial fibrosis and together with LV wall thickening, act as a compensatory mechanism to face the increased heart workload triggered by cardiomyocyte loss as well as vascular and ventricular stiffness. Extracellular matrix (ECM) alterations occur alongside with ventricular remodeling. In addition, aging also triggers atrial enlargement. Together, these changes induce impaired ventricular relaxation leading to diastolic dysfunction.

collagen content and/or degradation of elastin have been identified as crucial alterations occurring in the large arteries during aging, leading to a reduced vascular compliance and increased afterload.[17] These alterations increase the effort of the heart to achieve an efficient blood ejection during systole, inducing ventricular remodeling. Diastolic dysfunction in the elderly is associated with a reduced left ventricle (LV) compliance and impaired LV relaxation that are related to myocardial ECM remodeling.[18] Older individuals have also shown a substantially decreased LV compliance and stroke volume (volume of blood pumped out of the LV during each systolic cardiac contraction) compared with the young control subjects.[18]

Morphological and functional alterations in the atria have also been associated with aging. Considering that LV filling is closely linked to atrial function, atrial stiffness can also disturb diastolic function. For instance, reduced left atrial (LA) strain and increased LA stiffness have been documented in patients with diastolic heart failure.[19] Moreover, age-increased atrial contractility could result from either the LV relaxation impairment during diastole or increased atrial stiffness, leading to an impaired passive LA-mediated ventricular filling in aging.[20] Contrarily, atrial enlargement was shown to be age-independent in humans without cardiovascular disease.[21] Yet, in another study, atrial enlargement was found significantly in the eighth decade of life, perhaps as a result of long-term adaptation to LV diastolic dysfunction and reduced atrial compliance, as a consequence of interstitial fibrosis at an advanced age.[22]

At the cellular level, aging induces a progressive loss of cardiomyocytes, mainly in the LV, which has been attributed to the combination of apoptosis and necrosis.[23,24] Moreover, for the systolic function to be preserved there is increased oxygen consumption which induces a progressive decay in the cardiac myocyte metabolic capacity, leading to an energy deficit, oxidative stress and further cell death.[25–27] To compensate for this phenomena, progressive cardiomyocyte hypertrophy of the remaining myocytes takes place, resulting in the development of decompensated ventricular hypertrophy and a thicker LV wall.[15,28,29] Increased cardiac fibroblast proliferation also occurs, leading to interstitial fibrosis, increased wall stiffness and diminished myocardial compliance.[11] Although the systolic function remains moderately preserved in the elderly, myocyte hypertrophy, interstitial fibrosis and impaired ventricular relaxation cause an augmented diastolic ventricular pressure, leading to diastolic dysfunction.[30]

20.3 Young cardiac extracellular matrix

Cardiac ECM is a dynamic entity that has an essential role in several cellular mechanisms,[6–8,31,32] besides promoting tensile strength to the myocardium and supporting the force transmission.[13,14] In this section, the constituents and function of the myocardial ECM will be initially described followed by the description of its aging-related alterations.

20.3.1 Collagens

Fibrillar collagen is the most abundant protein in the cardiac ECM, comprising 5% of total protein in the adult LV.[33,34] The fibrillar collagen network is composed of different types of collagens with different α-chain combinations. Collagen types I and III are the major components of the healthy mammalian myocardial interstitium. These collagen types have been demonstrated to comprise approximately 80% and 10% of total collagen in the heart, respectively.[35] The heart is surrounded by a collagen network described as epimysium, which is located in the epicardium and in the endocardium. The myofibrillar bundles are surrounded by perimysial collagen, which is a wave-like structure that offers tensile strength.[14] The individual myocytes are attached to the endomysial collagen network through the connection of the myocyte sarcolemma (Z-band) and integrins, which sustains the myocyte alignment and prevents ventricular dilatation (Fig. 20.2).[14]

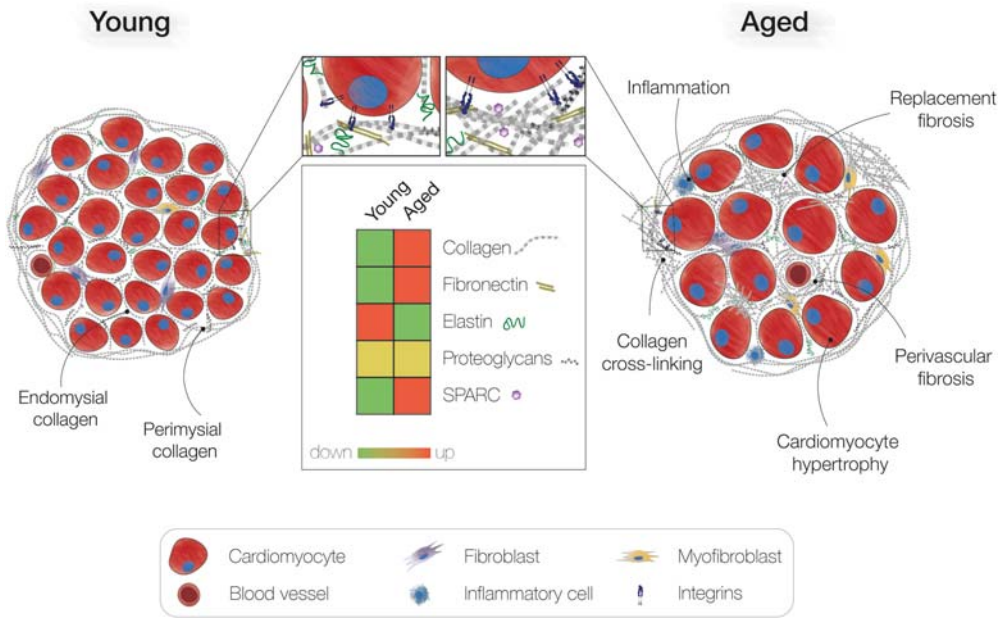

Figure 20.2 ***Schematic representation of age-associated alterations on the cardiac extracellular matrix (ECM).*** Both myocytes and non-myocytes are embedded in the meshwork composed by proteins and molecules which make up the ECM. Aging induces accumulation and cross-linking of the most abundant ECM protein, collagen, inducing interstitial and perivascular fibrosis, one of the consequences of the cardiomyocytes' death. Collagen surrounds myofibrillar bundles in a perimysial collagen network, and interconnects individual myocytes through the endomysial network. Fibronectin, another key ECM protein, also increases with aging in contrast with elastin, which is degraded during aging. ECM proteins also have important nonstructural properties by binding to integrins receptors to trigger signaling pathways involved in several cellular mechanisms. At the cellular level, aging induces cardiomyocyte loss and subsequent hypertrophy of remaining myocytes, and an increase in myofibroblasts and inflammatory cells.

Fibrillar collagen is synthesized as a triple-helical procollagen molecule by cardiac fibroblasts (CFs) and secreted into the extracellular space.[36,37] This collagen precursor has an amino (N−) and carboxy (C−) terminal propeptide, which undergoes several post-translational modifications. These include the removal of the propeptide regions by specific enzymes, the coupling with matricellular proteins and the self-assembly of collagen molecules to produce mature and insoluble collagen fibrils that are incorporated within the ECM.[14] In more detail, members of a protease family, a disintegrin and metalloproteinase with thrombospondin motifs, remove the N-terminal propeptide, whereas bone morphogenic protein-1 cleaves the C-terminal propeptide.[38,39] Subsequently, collagen fibrils are stabilized by another post-translational modification that incorporates cross-links between collagen fibrils. This cross-linking process can occur by at least two known mechanisms: (1) through the action of enzyme lysyl oxidase (LOX), which induces covalent links between either lysine or hydroxylysine residues of the adjacent collagen fibrils, and (2) by cross-linking between amino groups by reducing sugars, which induces advanced glycation end product formation. An accelerated collagen maturation and stiffening in myocardial diseases have been associated with the upregulation of LOX.[40,41] Collagen cross-linking has been associated with both aging[42] and pathological remodeling.[43]

The collagen matrix is responsible for many of the ECM properties in the heart because it: (1) provides structural support to the myocytes and their adhesion to the basement membrane; (2) offers myocardial strength, preventing tissue rupture; (3) facilitates force transmission from contracting myocytes to promote ejection of blood during systole, and (4) is responsible for the stiffness and resistance to stretching during diastole.[13] Cardiomyocyte adhesion to the myocardial basement membrane is mainly induced by non-fibrillar collagen, collagen type IV, and a fibrillar collagen, collagen type V, which are present in the pericellular space in small amounts.[36] Since slight alterations on the cardiac collagen network composition affect mechanical properties and functionality of myocardium in both a healthy and disease state, collagen synthesis, maturation and degradation are tightly controlled processes.[44]

20.3.2 Elastin

Elastin is the main component of elastic fibers, which is synthetized as tropoelastin and subsequently processed extracellularly to elastin. It consists of alternating hydrophobic and LOX cross-linked lysine-rich domains.[45] Functionally, elastin has important roles, providing stretch, recoil and resilience to dynamic tissues. By stretching more than twofold, it enables arteries to extend and retract in response to arterial pulse waves pushing blood forward in diastole. In addition, smooth muscle cell proliferation in the vessel wall can also be inhibited by elastin and polymeric collagen.[46] Elastic fibers are composed of a sheath of microfibrils formed by various components, including fibrillin 1, which surrounds an

elastin-rich core. Interestingly, Marfan syndrome shows that the dilation and dissection of large arteries is caused by fibrillin 1 mutations.[47] Similarly, fibulin 5 knockout mice exhibit tortuous and elongated aortas, indicating that fibulins and elastin microfibril components are essential for the proper formation of the elastic fibers in the vessel wall.[48] In general, studies investigating the impact of elastin expression provide limited information about myocardial elastin, because the effects on arteries dominates the phenotype.

Elastic fibers fibrillogenesis occurs largely at the early stages of development and fibers are designed to maintain elastic function for a lifetime.[49] However, loss of elasticity due to degradative modifications is the main contributing feature in the aging of numerous tissues, such as large arteries.[49,50] It has central implications for age-related myocardial remodeling, as the resulting loss of arterial compliance (arteriosclerosis) can trigger an increase in blood pressure and augment the risk of heart failure.

20.3.3 Matricellular proteins: secreted protein, acidic and rich in cysteine and thrombospondin

Matricellular proteins are nonstructural ECM components that have an essential role in the collagen maturation process, regulating collagen assembly and controlling the collagen fiber diameter. Here, the focus will be on those that are implicated in collagen remodeling in aging. These glycoproteins are minimally expressed in a healthy state, whereas they are extensively expressed throughout development and pathological conditions. An important matricellular protein, SPARC, facilitates collagen assembly and maturation as well as deposition of mature collagen in the myocardium.[51] In the myocardium, it has been demonstrated that genetic ablation of SPARC induced a decrease of the interstitial collagen amount and cross-linking leading to a diminished fibrotic response to pressure overload.[52]

TSP family is another group of matricellular proteins, which has several essential roles in collagen synthesis and assembly in the heart. TSP1 has an essential role in the regulation of angiogenesis and thrombosis, and it is a potent activator of tumor growth factor-β (TGF-β), contributing to cardiac remodeling.[53] In the heart, cardiac matrix integrity is also regulated by TSP1 and TSP2 that can interact with matrix metalloproteinases (MMPs), a vast group of proteinases responsible for ECM protein degradation.[54] TSP2 was identified as an essential nonstructural ECM component to maintain the myocardial ECM integrity, attenuating the age-related cardiomyopathies.[55] MMPs are tightly regulated at the transcriptional, post-transcriptional and inhibitory level. Tissue inhibitor of metalloproteinases (TIMPs) is an endogenous group of MMP inhibitors and alterations in its expression have also been implicated in functional cardiac changes.[56] Therefore, preservation of the cardiac ECM hemostasis is not only dependent on the balance between ECM deposition and degradation, but also on the balance between the activity of MMPs and TIMPs. The age-related modification on MMP and TIMP activities will be discussed in the following section.

20.3.4 Glycosaminoglycans and proteoglycans

Glycosaminoglycans (GAGs) and proteoglycans act as a hydrogel able to embed myocytes, non-myocytes and matrix proteins. GAGs are unbranched polysaccharides with molecular activity, found as single molecules or coupled to a core protein forming a proteoglycan. Due to their negative charge, GAGs attract water, producing a gelatinous medium that acts as a lubricant for the contractile apparatus and as a signaling medium of the ECM, allowing a quicker diffusion of the signaling molecules.[13] The main GAGs expressed in most mammalian cells include heparan/heparan sulfate, dermatan sulfate, chondroitin sulfate, keratin sulfate and hyaluronan. Heparan/heparan sulfate and hyaluronan are the most expressed GAGs in the heart.[51] Hyaluronan is predominant in the cardiac ECM and it has been involved in proinflammatory signaling.[57,58] It has also been implicated in fibroblast-myofibroblast differentiation and consequently, it is associated with disease-related cardiac remodeling.[59–61]

Proteoglycans are strongly glycosylated proteins, which comprise the main nonstructural components of ECM and present as a core protein ornamented with at least one GAG side chain. Proteoglycans can be grouped into 4 families based on their locations: (1) intracellular (serglycin), (2) cell surface (syndecans and glypicans), (3) pericellular (at basement membrane levels, e.g., perlecan and collagens XV and XVIII) and (4) extracellular proteoglycans (hyalectan lectican (e.g., versican) and small leucine-rich proteoglycans [biglycan, decorin, lumican and osteoglycin)].[51,62] Proteoglycans have been demonstrated to be crucial modulators in cardiac remodeling. They were shown to control several mechanisms such as, collagen deposition and assembly, fibroblasts infiltration and transdifferentiation, activation of hypertrophic genes, and immune cell recruitment and proinflammatory cytokines and chemokines induction, which ultimately cause cardiac fibrosis, cardiomyocyte hypertrophy and inflammation, respectively.[63]

20.3.5 Adhesive proteins: fibronectin and laminin

Adhesive proteins are responsible for the adhesion of myocytes to their basement membrane and circulating ECM, and also have an essential role in cell-ECM and/or cell-cell communication, which is ultimately essential for tissue formation, structure and integrity. One of these adhesion proteins is fibronectin (FN), a glycoprotein that is found as both an insoluble matrix adhering form, synthesized by fibroblasts and other differentiated cells, and as a soluble form produced by hepatocytes and is present in the plasma.[7] Besides its structural role, FN also has a crucial function in cell behavior via ECM-cell communication. The FN connection to its integrin receptor ($\alpha5\beta1$ integrin) can regulate several cellular mechanisms such as cell migration, cell growth, survival and proliferation.[64] It is widely expressed in regions of active morphogenesis, cell migration and inflammation.[64] In the healthy myocardium, FN is only found at low levels, mainly located at myocyte basement membranes.[65]

Laminin is another glycoprotein composed of three polypeptide chains, α, β, and γ chains, and it is also present in the cardiomyocyte basement membrane.[66] These molecules interplay with collagen type IV, integrins and dystroglycans but also with other basal membrane matrix components, contributing to the overall structure and to several mechanisms.[64] Thus, laminins are also involved in both structural and biological functions, such as cell adhesion, differentiation, migration, and resistance to apoptosis.[64] Both fibronectin and laminin have been associated with myocardial remodeling and disease.[67–69]

20.3.6 Basement membrane

The sarcolemma is surrounded by a basement membrane which promotes the attachment of each myocyte to the ECM. It is a network composed of collagen IV, adhesive proteins (fibronectin and laminin), proteoglycans (perlecan and the sulfated), linking glycoprotein and nidogen.[51,70] Collagen IV is one of the most abundant proteins of the basement membrane and its mRNA was detected in both myocyte and non-myocyte cells, demonstrating that myocytes are also able to produce their own basement membrane.[36] The significance of the basement membrane structure for myocardial functionality has been highlighted by various studies, demonstrating that changes on basement membrane composition and distribution have been associated with myocyte electrical properties, morphology alterations, and ventricular dysfunction.[71,72] Particularly, collagen IV has been shown to increase with remodeling and disease.[73,74]

20.4 Aged cardiac extracellular matrix

20.4.1 Collagen matrix—synthesis, deposition and modification with age

As the most abundant protein of the cardiac ECM, fibrillar collagen has been the most widely studied ECM protein in aging. Besides changes in abundance, collagen distribution, organization and integrity have all being implicated in cardiac ECM aging. Collagen triggers relevant functional and morphologic adaptations in the aged heart underlying age-related cardiac dysfunction.

20.4.1.1 Age-related alterations in myocardial collagen content

An increase in the amount of collagen with aging is well established and reported in the heart.[75,76] There are many studies in human and animal models which show ventricular accumulation of collagen with aging (see Table 20.1). For example, there is a correlation between an increase in insoluble collagen in the endocardium and papillary muscle and the age of the individuals.[77] Accumulation of collagen fiber content and an increase of collagen fibril diameter was also shown in LV samples acquired at autopsy from individuals aged 67–87 years compared to young adult humans aged

Table 20.1 Summary of age-related alterations on extracellular matrix components in the left ventricle.

Extracellular matrix component	Specie	Parameter studied	Aging-associated alteration	Ages studied	References
Collagen					
	Human	Soluble and insoluble collagen (Hydroxyproline content)	↑	men (25) and women (15): from 21 to 88 y	[77]
	Human	Collagen fibril content and diameter Collagen types: type I	↑	67−87 y versus 20−25 y	[78]
	Mouse	Total collagen content	↑	20 m versus 2 m	[79]
	Mouse	Collagen area (%)	↑	18−24 m versus 3 m	[80]
		Soluble and insoluble collagen	↑ insoluble		
	Mouse	Collagen content	↑	32 m versus 8 m	[81]
	Mouse	Soluble and insoluble collagen	↑ Sol./Insol. ratio	23 m versus. 3 m	[76]
	Mouse	Type IV and VI collagens	↑	18−20 m versus 4−5 m	[75]
	Rat	Total collagen content and structure	↑	1 m to 26 m	[33]
	Rat	Percentage of fibrosis	↑	4 m to 29 m	[82]
	Rat	Collagen content (SR)	↑	2 m to 19 m	[83]
		Procollagen type I and III mRNA	↓		
	Rat	Collagen area (%) Procollagen type III mRNA	↑	18 m versus 3 m	[84]
	Rat	Collagen type I/type III ratio	↑	24 m versus 4 m versus 4w	[85]
	Rat	Collagen cross-linking level	↑	20 m versus 10w	[86]
	Rat	Collagen cross-linking level Procollagen type I and III mRNA	↑ ↓	20 m versus 12 m versus 10w	[87]
	Rabbit	Collagen area (%) (SR)	↑	5−6 y versus 2−3 m	[88]
	Sheep	Collagen area (%) Procollagen type I mRNA	↑ −-	> 8 y versus 18−24 m	[89]

Matricellular proteins					
	Mouse	SPARC	↑	18–24 m versus 3 m	[80]
	Mouse	Thrombospondin 1	↑	50–60w versus 8–12w	[55]
Glycosaminoglycans (GAGs) and Proteoglycans					
GAGs	Rat	Total GAGs content	–-	7d to 10 m	[90]
	Rat	Total GAGs content	↓	25 m versus 10 m	[91]
	Rat	Total GAGs content	↑	4 m to 24 m	[92]
		Heparin Sulfate	↑		
		Capacity to bind VEGF	↑		
Proteoglycans	Mouse	Versican	↓	18–29 m versus 3–5 m	[93]
Adhesive Proteins					
Fibronectin	Rat	mRNA and protein levels	↓	40 w versus 10 w	[94]
	Mouse	Protein level	↑	20 m versus 2 m	[79]

↑, Increased; ↓, decreased; –, (no changed); *d*, days; *w*, weeks; *m*, months; *y*, years; *SR*, Sirius Red.

20–25 years.[78] Similarly, the total collagen was also increased in the LV of old mice and rats compared to young animals.[37,79,80,82] Senescent mice aged around 32 months also exhibited double interstitial fibrosis compared to adult mice (~8 months).[81]

The collagen accumulation in the heart results in increased interstitial fibrosis in the LV myocardium, which functionally induces a decreased percentage of systolic wall thickening and ejection fraction, as well as a decrease in stroke volume.[82] In contrast, the effect on the stroke volume has also been shown to be maintained despite the LV structural and functional alterations.[81] These changes were also accompanied by a decreased number of myocytes and an increased myocyte cross-section (hypertrophy).[81] Both interstitial fibrosis and cardiomyocyte hypertrophy have been considered to induce myocardial stiffness. However, it has been demonstrated that the myocardial stiffness is triggered by progressive collagen accumulation, collagen phenotype shift and higher collagen cross-linking, but not by increased LV hypertrophy.[95] Conversely, an increment of myocyte compliance with aging was observed,[96] which is maybe related to the changes in titin properties.[97] As a conclusion, the deregulation in the collagen content is critical in LV function and stiffness in aged individuals.

20.4.1.2 Age-related alterations in myocardial collagen types

Deregulation in the ratio of collagen types has also been observed in aging. Collagen type I and type III have different physical properties, and alterations in their ratio have been associated with several myocardial dysfunctions.[98–100] A decrease in collagen type III and an increase in collagen type I have been observed in rat LV during aging.[85] In addition, an increase in the number of thicker collagen fibers (collagen fibers with more collagen type I) compared to thinner fibers (fibers with more collagen type III) was also observed in the endomysium and perimysium of the aged human ventricular wall.[78] Myocardial collagen type I has been demonstrated to predominate in aged samples.[101]

Interestingly, studies that have quantified the mRNA of procollagens type I and type III in aged animals, observed a decrease in the abundance of these transcripts[83] or a non-significant alteration in procollagen I mRNA.[89] The discrepancy between protein and mRNA levels suggests age-related alterations of collagen mRNA stability and/or deregulation of collagen protein synthesis. Alternatively, the collagen accumulation in aged myocardium might derive from impaired protein degradation, thus affecting collagen turnover. Although the MMPs are the main responsible enzymes for cardiac matrix degradation, they are endogenously inhibited by the TIMPs. Therefore, activities of both must be evaluated to assess the proteolytic capacity of the aged heart. Despite both human and animal models of aging suggesting that an imbalance of the MMP-TIMP ratio and activity occurs with age, the mechanisms by which altered MMP and TIMP activity lead to cardiac remodeling with age, are still unclear. MMP-2 mRNA and protein activity of MMP-2 and proMMP-1 was shown to be downregulated in a 24-month-old rat heart, suggesting

that the reduction of ECM degradation pathway by MMP allowed the accumulation of collagen and promotion of age-associated fibrosis.[102] Protein and organelle quality control pathways, including autophagy/lysosomal and the ubiquitin-proteasome systems, decline in the heart with aging contributing to myocardial dysfunction.[103–105] The enzymatic activities of the proteasome are drastically reduced in the aged heart. As a result of compromised protein quality control, the aged heart accumulates misfolded protein aggregates (proteotoxicity), cell death proteins, and damaged mitochondria. This proteo-toxic profile is associated with augmented fibrosis, collagen deposition and cardiac hypertrophy.[106]

Non-fibrillar collagen type IV was also found in increased age-associated levels in LV tissue of old mice, suggesting that there is an increase of the basal membrane thickness with age.[75] An increase of collagen type VI content was also reported in the LV of the aged mouse.[75]

20.4.1.3 Age-related alterations in myocardial collagen cross-linking

Although changes in myocardial collagen seriously affect LV structure and stiffness, and consequently alter the systolic and diastolic heart functions, increased collagen does not always correspond to augmented myocardial stiffness.[107,108] This suggests that post-translational processes are involved in ECM left ventricular modifications. Regulation of the collagen maturation process and cross-linking have also been implicated in myocardial remodeling, stiffening and dysfunction in disease.[109,110] Indeed, increased levels of collagen cross-linking have been observed in the LV of aged rats.[86] Collagen cross-linking levels can be evaluated by the formation of the AGEs. AGE accumulation was associated with LV diastolic dysfunction in aging.[111] In addition, studies have shown that myocardial function in aged hearts has been improved by blocking the formation of AGEs. LV chamber compliance increment, due to reversal of LV stiffness, and consequently an improved cardiac function were also observed in aged dogs treated with an AGE cross-link breaker compared to untreated age-matched animals.[112] In another study, induction of diabetes in aged dogs induced LV systolic dysfunction, increased aortic stiffness, and augmented collagen types I and III content, which were reverted by AGE cross-link breaker treatment.[113] Moreover, LV collagen solubility significantly increased after treatment, suggesting that the implicated mechanism is a decrease of collagen cross-linking.[113] Interestingly, it was suggested that exercise training may reduce collagen cross-linking associated with age and can affect collagen type I and III mRNA synthesis.[86,87]

Matricellular proteins such as SPARC, which facilitate post-translational processes and deposition of mature collagen in the myocardium, play an important role in the levels of collagen in the aged heart.[51] Studies in mice have demonstrated that the SPARC absence reduced age-associated increases in ECM fibrillar collagen and diastolic stiffness.[75,80] Additionally, SPARC ablation decreased the relative amount of insoluble collagen and myocardial stiffness in aged mice.[80,93] Consequently, SPARC is likely an important mediator of collagen deposition and myocardial stiffness in aging. Another matricellular protein

that is important in myocardial collagen cross-linking is TSP-2. In this case, and in contrast to other matricellular proteins, the upregulation of TSP-2 has been found to be protective against age-related cardiomyopathy and thus cardiac aging.[55]

20.4.2 Alterations on glycosaminoglycans and proteoglycans in the aged heart

Glycosaminoglycans and proteoglycans are abundant in the myocardium and are responsible for several nonstructural properties of the cardiac ECM. These molecules and proteins have received little attention in aging compared to other structural proteins like collagen. However, glycosylation is one of the most frequent and complex post-translational modifications, which induces diversity in both structural and functional proteins.[114] Therefore, age-associated alterations on the glycosylation profile, quantity and/or distribution of these molecules strongly affects several essential mechanisms such as cell signaling and communication, and also structural properties, and consequently, cardiac function. Studies on GAGs (acid mucopolysaccharides) composition in aging of the LV are relatively scarce and not all studies are in agreement. Some studies reported that GAGs remained unchanged with age, whereas a minor increase in the most abundant GAG, hyaluronic acid, was observed along with a decrease in chondroitin sulfate.[90] Other studies showed an increase of sulfated GAGs (particularly, heparan sulfate) in the LV myocardium of old rats (24 months) compared to young rats (4 months).[92] Importantly, age-associated alterations in the sulfation pattern of heparan sulfate induced alterations in the affinity for different growth factors with impacts on several cellular mechanisms.[92] Moreover, other studies showed a decrease in total GAGs[91] and versican[93] content in myocardial tissue of old compared to young animals.

20.4.3 Alterations on matrix adhesive proteins with aging

Changes on the ECM adhesive proteins also have an impact on the structural, mechanical and functional properties of ECM, mainly in cell-to-cell communication. For example, FN expression was shown to decrease with age in normotensive rats and to remain unchanged in old hypertensive rats compared to young.[94] In contrast, a more recent study has shown that FN expression in the myocardium was higher in old (20 months) than younger mice.[79] Moreover, old mouse hearts compared to young showed higher levels of $\alpha 1$ and $\alpha 5$ integrins, which are the ligands for collagen and FN, respectively.[79]

20.5 How does the aged heart respond to disease?

Aging is considered an independent risk factor for the development of myocardial infarction (MI)[115] and heart failure (HF).[116] The following sections will shed light on ECM remodeling as a game-changer for acute myocardial infarction and heart failure survival rates and clinical outcomes.

20.5.1 Age-associated extracellular matrix remodeling in MI

Myocardial infarction is the main cause of death worldwide and its prevalence increases with aging.[117] Three different overlapping phases are involved in MI repair, namely inflammatory, proliferative, and maturation phases. In all of these phases, the ECM composition dynamically changes, taking part in the regulation of the cellular responses underlying cardiac repair.[118] During the inflammation stage, degradation of matrix proteins produces signals that trigger inflammatory and reparative signaling pathways, which precede the proliferative phase. At this next stage, myofibroblasts are abundant and deposit large amounts of structural ECM proteins, subsequently leading to the formation of a stable collagen-based scar during the maturation phase.[118]

Several studies have shown that there is an exacerbated response to the ischemic injury in the aged heart.[119–122] Strikingly, increased in-hospital and post-discharge mortality rates have been associated with patient aging regardless of the infarction area.[115] Likewise, it has been demonstrated that old mice have an increased mortality rate during occlusion and reperfusion and worse LV remodeling outcomes irrespective of the size of the infarct area.[119] For example, LV free-wall rupture, one of the most fatal complications of acute MI, has a significantly higher incidence in old mice post-MI.[122] Following ischemia-reperfusion injury, old mice show a suppressed post-infarction inflammatory response, impaired phagocytosis of dead cardiomyocytes as well as fewer myofibroblasts and reduced collagenous scar formation.[119] Age-dependent outcomes after MI have been associated with altered profibrotic and proinflammatory profiles, including increased TGF-β,[120] iNOS[120] and proinflammatory cytokines such as IL-1b,[119,122] IL-6,[119,120,122] and TNFα[119,120,122] and decreased nNOS,[120] eNOS,[120] anti-inflammatory IL-10[120] and MCP-1 chemokine.[119] Additionally, MI-driven ECM remodeling in aged individuals is altered, contributing to deleterious effects. Collagen types I and III[122] as well as MMP-9/TIMP-3[120] and MMP-2/TIMP-1[120] ratios were noticeably increased after MI in old animals. An increase in collagen content accounts for increased myocardial stiffness while the imbalance in MMP/TIMP ratios promotes exacerbated ECM degradation eventually underscoring cardiac dilation. Moreover, levels of healing-specific matricellular proteins, including a secretory leukocyte protease inhibitor, SPARC, and osteopontin were also found at significantly increased levels after myocardial injury in aged LV.[120] Interestingly, MMP-9 deletion following MI in aged animals led to an improved post-MI repair profile with increased survival rates and reduction of LV dysfunction (LV dilation),[123] further highlighting the role of ECM in the age-altered repair process post-MI.

20.5.2 Age-associated cardiac extracellular matrix remodeling in heart failure

Heart failure (HF) is a complex clinical syndrome, entailing high morbidity and mortality, which dramatically increases with aging. In young patients, HF usually develops from

ischemic heart disease or other cardiomyopathies into ventricle dilation and remodeling with reduced ejection fraction.[124] However, in the elderly, HF usually presents in the form of a hypertrophied ventricle with preserved ejection fraction (HFpEF)[124,125] together with a high prevalence of other comorbidities (hypertension, diabetes, peripheral vascular disease, coronary artery disease, valvular disease, kidney failure, among others) and multiple medications.[126,127] Furthermore, the incidence of age-related comorbidities, including but not limited to dementia,[128] cognitive decline[129] and frailty,[130,131] renders the elderly less receptive to therapy and more susceptible to medication errors as well as more likely to experience side effects.

HF in the aged heart is mainly triggered by prolonged pressure overload stimulus that is usually sustained by factors such as hypertension, arterial stiffness and aortic stenosis, whose incidence and prevalence increase as a function of age. Cardiomyocyte growth and ECM expansion initially attempt to keep up with the higher functional demands, however, over time concentric LV hypertrophy becomes deleterious and compromises LV diastolic performance. It has been shown that HFpEF patients usually present an expanded interstitial ECM network including increased fibrillar collagen accumulation and increased fibroblast/ myofibroblast proliferation within the ECM.[14] Mechanical stress has been associated with integrin-dependent activation of TGF-β,[132] which mediates the conversion of activated fibroblasts into myofibroblasts that synthesize large amounts of ECM proteins.[133] Continued or accelerated collagen accumulation together with changes in other ECM proteins increase myocardial stiffness and reduce compliance, thereby hindering LV filling during diastole.[14,33,83] Besides the total collagen amount, the degree of collagen cross-linking in HF patients may also contribute to fibrosis in aged hearts by protecting the collagen network from protease-mediated degradation. Collagen cross-linking has been associated with increased filling pressure and higher incidence of hospitalizations.[134,135] Chronic myocardial expression of MMP-1 in mice led to a loss of interstitial collagen and showed that the balance between ECM synthesis and degradation can play a critical role in the cardiac remodeling process and heart failure pathophysiology.[136] ECM deposition or other age-induced matrix network alterations further impact the myocardial excitation-contraction coupling,[10] reduce cardiomyocyte perfusion[137] and activate proinflammatory pathways.

20.6 Conclusions and perspectives

The cardiac ECM is a meshwork that plays important structural and nonstructural roles to support cardiac function. Aging induces alterations in the cardiac ECM composition and structure that have been shown to contribute to cardiac disease onset and progression in the elderly. For a long time, reductionist research focused on characterizing age-driven ECM component alterations that underlie the development of cardiac disease, individualizing the effects of specific ECM components in the aging process

mainly through alteration of the ECM strength and structure. However, emerging evidence has been pointing towards a dynamic state of the ECM composition that mediates intercellular communication and responses to the cellular microenvironment.

Recent studies have highlighted the importance of ECM in cardiac aging. For example, the recellularization of aged decellularized hearts with young iPSC-derived cardiomyocytes induced an accelerated aging phenotype, impaired cardiac function and compromised stress defense machinery.[138] Further studies are necessary to investigate the dynamics of the cardiac ECM network throughout aging and its impact on cardiac cell aging. Furthermore, it would be worthwhile to revisit the ECM impact on mechanosensing and transduction by the cells during the aging process and how this process might alter the presentation or recognition of certain components of the ECM by the cells to induce a response. Ultimately, understanding the ECM dynamics and how to manipulate the cardiac ECM during aging is expected to open avenues for cardiac disease therapies.

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CHAPTER 21

Aging-related neoplasia

Ana Bela Sarmento Ribeiro[1,2,3,4,5], Maria Filomena Botelho[2,3,4,6], Ana Cristina Gonçalves[1,2,3,4], Joana Barbosa Melo[2,3,4,7], Mafalda Laranjo[2,3,4,6], Raquel Alves[1,2,3,4], Joana Jorge[1,2,3,4], Ilda Patrícia Ribeiro[2,3,4,7], Salomé Pires[2,3,4,6], Margarida Abrantes[2,3,4,6], Vera Constâncio[8,9], Carmen Jerónimo[8,9], Isabel Marques Carreira[2,3,4,6], Manuel Santos Rosa[10] and Rafael Solana[11]

[1]Laboratory of Oncobiology and Hematology and University Clinic of Hematology, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal
[2]Coimbra Institute for Clinical and Biomedical Research (iCBR) Area of Environment, Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[3]CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal
[4]Clinical Academic Center of Coimbra (CACC), Coimbra, Portugal
[5]Clinical Hematology Department, Centro Hospitalar e Universitário de Coimbra (CHUC), Coimbra, Portugal
[6]Institute of Biophysics, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[7]Laboratory of Cytogenetics and Genomics, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal
[8]Cancer Biology and Epigenetics Group, Research Center of IPO Porto (CI-IPOP)/RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Centre (Porto.CCC), Porto, Portugal
[9]Department of Pathology and Molecular Immunology, School of Medicine and Biomedical Sciences, University of Porto (ICBAS-UP), Porto, Portugal
[10]Institute of Immunology, Faculty of Medicine (FMUC), University of Coimbra, Coimbra, Portugal
[11]Immunology and Allergy Group, Maimonides Institute for Biomedical Research of Cordoba (IMIBIC), Córdoba, Spain; Department of Cell Biology, Physiology and Immunology, University of Córdoba, Córdoba, Spain; Immunology and Allergy Service, Reina Sofia University Hospital, Córdoba, Spain

21.1 Introduction

Cancer is considered a disease of aging as the incidence of most cancers increases dramatically with age. In fact, more than half of cancers occur in individuals older than 70. The US National Cancer Institute's surveillance epidemiology and end results database refer that 43% of men and 38% of women will develop invasive cancer throughout life, and about 50% of these (in both sexes) will die from cancer.[1] In addition, according to the WHO, life expectancy is now over 80 years in most developed countries. As the population ages, cancer becomes an increasingly important health problem worldwide.[2]

The mechanisms of cancer and aging involve a time-dependent accumulation of cellular damage, and several studies demonstrate that many of the hallmarks of cancer are common with aging characteristics.[2] These include genomic instability, epigenetic changes, telomere attrition, altered intracellular communication, changes in proteostasis, mitochondrial dysfunction/redox alterations, deregulation of nutrient sensing, immune dysfunction, and cellular senescence.[3,4] Besides the changes inside cells, aging

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and cancer are also associated with alterations in the cellular microenvironment, particularly in fibroblasts, immune cells, and stem cell pools. Further, the immune system and the native microbiota could, directly and indirectly, contribute to cancer through aberrant changes in various immune responses, chronic inflammation, damage to DNA, and changes in the effectiveness of anti-cancer therapies.[3,4]

The most accepted explanation for the association between aging and cancer is that the accumulation of oncogenic mutations with age should facilitate cancer evolution (predominant model). In addition, an adaptive oncogenesis hypothesis has been proposed.[5]

This review will focus on the underlying cellular and molecular mechanisms between cancer and aging that could improve the early detection and treatment of aging-associated cancer.

21.2 Aging and the risk of cancer

Age is considered the most significant risk factor for many chronic diseases, including the majority of common cancers.[4] In people aged 65 and over, more than 60% of all cancers occur. The most common explanation is the multi-hit Knudson hypothesis. This hypothesis proposes that it takes time for cells to accumulate enough genetic mutations to reach the mutagenic threshold for carcinogenesis. Nordling suggested that probably about seven successive mutations in dividing cells would be necessary for cancer development.[6] However, this hypothesis does not explain the effect of caloric restriction in the decrease of cancer.[7] The reduction in metabolic homeostasis and gene regulation that normally occurs during aging could justify this occurrence and is consistent with the strong association between cancer prevalence and type 2 diabetes and obesity, as well as with the beneficial effect of the modulation of energy utilization by resveratrol and metformin.[8]

However, besides the numerous biological changes associated with age, the accumulation of mutations is one of the major aging mechanisms related to the higher incidence of cancer development and progression in older people. Additionally, lifestyle, environmental and psychosocial factors could also affect physiological reserve and vulnerability of aging. The environmental factors that we are exposed to as we get older, such as exposure to ultraviolet (UV) radiation, alcohol, smoke, pollution,[4] chemical substances (food additives, air/water pollutants, etc.)[9] and other genotoxic chemicals, and endogenous factors including reactive oxygen species (ROS),[10] further contribute to the chronic accumulation of DNA damage and other events associated with cell aging,[4,10] which we will address later. However, aging is associated with a reduction in gene repair, leading to an increased number of mutations and genetic instability.[9] Other pathophysiological changes related to aging affect the function of the intestinal barrier, being accompanied by an alteration in the microbiome's composition, which could modulate several age-related disorders such as cancer.[10]

Additionally, the cellular and molecular changes in non-cancerous cells during aging suggest that aging of the microenvironment may have dramatic effects on tumor progression. This permissive microenvironment tumor includes changes in extracellular matrix biophysics, secreted factors, and the immune system.[3] Furthermore, different stromal tissue environments may be reprogrammed differently during aging, which consequently, affects the growth and progression of the tumor in relation to the tissue of origin.[3]

With increasing age, the body tends to accumulate damage and the functions of different organs begin to decline. There are two prominent theories of aging that function in synergy, one related to oxidative damage and the other with the hyper-function of several survival pathways (addressed later), that could also explain cancer development during aging.[11]

While age is an important risk factor for cancer, cancer development is a multi-stage and multifactorial process that can occur over many years. The knowledge of each cancer risk factor represents opportunities to prevent cancer and for the success of a healthy aging process.[12,13]

21.3 Cellular senescence and carcinogenesis

Aging is a risk factor for cancer development, since more than half of cancers occur in individuals older than 70,[1] which is also related to an increase of senescent cells in body tissues.[14] The state of senescence seems to have a role in the protection of cancer development and, more recently, an association with aging due to the accumulation of senescent cells in tissues and the loss of stem cell function.[15] Thus, aging and cancer share several hallmarks, being interconnected in both time and mechanisms.[2]

The association of cancer and age can be explained by: (1) older individuals are more prone to the multistep and progressive carcinogenesis process, since this process usually requires several years for the neoplastic transformation of normal tissues by a process of natural selection; (2) accumulation of age-related alterations that lead to a favorable environment for tumor initiation and progression, such as increased senescence; and (3) the cancer-prone phenotype of older humans, namely the increasing of mutational rates, epigenetic alterations, telomere dysfunction, and stromal milieu alterations.[16] Cellular senescence connected directly or indirectly to a wide range of aging pathologies, including cancer,[17] is a state of irreversible growth arrest discovered by Leonard Hayflick when observed that human diploid fibroblasts cells in culture achieve a finite number of cell divisions. The progressive accumulation of senescent cells with age could be a hypothesis that explains the increase of cancer incidence with aging.[14] There are several kinds of this senescence, namely replicative senescence, oncogene-induced senescence, and therapy-induced senescence.[18] The senescent cells stay viable but did not respond to growth and death stimuli, being molecular features

of these cells, the upregulation of cell cycle inhibitors like p21 and/or p16, the positive staining of β-galactosidase, the formation of senescence-associated heterochromatin foci, and the induction of senescence-associated DNA damage.[19–21] Cellular events like telomere shortening,[22] oncogene activation,[23] and/or chemo/radiotherapy[24] can induce cell senescence probably through the activation of p53 or pRb.[18] Furthermore, senescence can also be induced by extracellular or intracellular stresses even in cancer cells that harbor mutant p53 and/or pRb, highlighting the involvement of other mechanisms independent of p53 and pRb.[25] Some triggers like cytokines, ROS, DNA damage, and nucleotide depletion could induce senescence.[26] Considering its antiproliferative effects, the senescence phenomenon seems to have antitumor functions that are explored in cancer therapy.[27] However, it can also promote cancer development through cellular microenvironment changes by a senescence-associated secretory phenotype that seems to facilitate invasion, metastasis, and resistance to therapies.[18] It is important to stress that senescence is often induced by tumor therapies to block apoptosis-resistant cancer cells, but at the same time could induce senescence in other cells and promote cancer recurrence.[14]

21.4 Oxidative stress and carcinogenesis in aging

The study of free radical mechanisms offered a basis for the free radical theory of aging that hypothesizes that functional losses associated with aging are due to the accumulation of oxidative damage to macromolecules (lipids, DNA, and proteins) induced by ROS.[28,29] Free radicals are usually produced as a result of external factors, such as pollutants, radiation, and cigarette smoke, or internal factors, like products of cellular mechanisms.[30,31] However, to maintain cellular equilibrium, cells scavenge ROS through a complex antioxidant defense system that eliminates ROS and controls their production through changes in metabolic and signaling pathways.[31,32] Since humans are exposed to ROS right from birth, if the antioxidant defenses do not eliminate ROS, they increase the susceptibility to age-related pathologies, including cancer.[33,34] During carcinogenesis and aging, the redox imbalance occurs mainly through mitochondrial dysfunction and elevated NADPH oxidase activity, a family of enzymes that specifically generates ROS.[30,35] In fact, over time the cumulative macromolecular damage can contribute to many physiologic mechanisms of aging and cancer.[29] For example, the oxidative DNA damage occurring in genomic and mitochondrial DNA leads to a transcriptional arrest, induction/replication errors, genomic instability, activation of growth factor-mediated signaling, modified metabolism, a compromised immune system, and an altering microbiome.[28,33,36] These mechanisms are all associated with aging and/or tumorigenesis.[28] During aging, ROS mediates several signaling pathways, such as NRF2, AMPK, NF-κB, and cellular mechanisms as uncoupling proteins, proteostasis, and mitochondrial dysfunction.[29] In fact, abnormalities in these

signaling pathways and mechanisms are commonly detected in elderly people and cancer patients.[29,37] When NRF2 decreases, cells are more sensitive to oxidative stress, endoplasmic reticulum stress, and protein aggregation, promoting the aging phenotype[34]. This transcription factor plays a crucial role in maintaining proper mitochondrial function and protecting cells by directly neutralizing ROS, preventing oxidative damage, and increasing metabolism of pro-tumorigenic xenobiotics.[34,37] As a consequence of mitochondrial metabolism, ROS are formed in the mitochondria respiratory chain, coupled to ATP production through oxidative phosphorylation, especially under pathological conditions.[37] With aging, inevitably mitochondrial dysfunction and/or a decrease in antioxidant defenses occur, which lead to the increase of oxidative stress/damage in elderly people and contribute to the risk of cancer development.[36,38] On the other hand, cancer cells have higher basal levels of ROS[39] that increase proliferation and survival, modify the metabolism, allow cell adaptation to nutritional and hypoxic stresses, and prevent cell death through the regulation of several transcription factors and signaling pathways, including MAPK, PI3K/AKT/mTOR, NF-kB, STAT3, HIF-1α, and ferroptosis.[29,33] The damaging and cell signaling roles of ROS contribute to aging features and are strongly connected to carcinogenesis, metastasis, and to bystander effects in the tumor microenvironment.[29] In this sense, a common oxidative stress component is observed in the mechanisms involved in aging and cancer, but if ROS is a cause or a consequence of aging and cancer remains unclear.

21.5 The hallmarks of aging and neoplasia

Aging is associated with several events at cellular and molecular levels that influence carcinogenesis and cancer development and progression. These include altered intracellular signaling pathways, telomere/telomerase deregulation, genomic instability, epigenetic changes, mitochondrial dysfunction/redox alterations, changes in proteostasis, deregulation of metabolism, immune dysfunction, and cellular senescence (Fig. 21.1).

21.5.1 Cell signaling

Aging and cancer are highly affected by different cellular processes that are orchestrated by a complex network of signaling pathways. Pathways involved in growth promotion, cell-extracellular matrix interactions, and stress responses are among the most important for both conditions, aging and cancer.[40] One of the signaling pathways that plays a crucial role in both aging and cancer is the insulin/IGF-1 signaling pathway, one of the most conserved aging-controlling pathways in evolution. Genetic abnormalities in this signaling have been linked to increased longevity and to the beneficial effects of caloric restriction. On the other hand, this pathway inhibits apoptosis and promotes cell proliferation and division, leading to the extended life span of

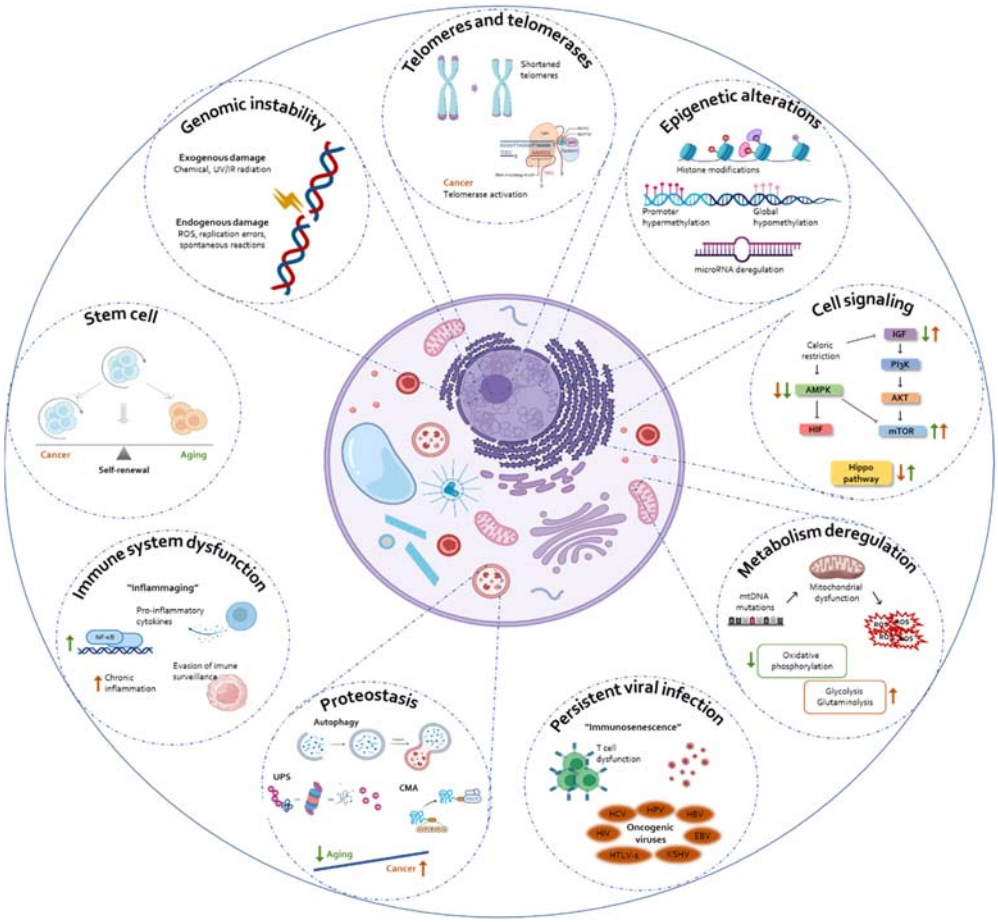

Figure 21.1 ***Cellular and molecular interplay between aging and cancer.*** Aging is associated with cellular and molecular events that influence carcinogenesis. Epigenetic alterations are common to both aging and cancer. However, global hypomethylation is frequently observed in aging, whereas hypermethylation of tumor suppressor genes associated with oncogenes' hypomethylation is a characteristic of cancer. Cell signaling and nutrient-sensing pathways are differently dysregulated in aging and cancer. Nonetheless, inhibition of insulin and mTOR signaling increases life span and provides a new antineoplastic approach. Energy supplies are predominantly obtained through glycolysis and glutaminolysis in cancer cells. Aging cells present a reduced oxidative phosphorylation activity. Persistent viral infection is associated with "immunosenescence" in the aging process and is responsible for establishing several virus-associated tumors. The proteostasis network plays opposite roles, showing decreased activity in aging while in cancer an upregulation is frequent. Aging cells are commonly characterized by a process called "inflammaging" that contributes significantly to the pathogenesis of age-related diseases like cancer. Tumor cells also have the ability to evade immune surveillance. In cancer, stem cells are highly capable of self-renewal and provide a potential niche for tumorigenesis. Further, while in aging, the main driver of malignancy is the drop in stem cells numbers and, above all, in function. Accumulation of genomic errors contributes to both aging and cancer, but they oppositely respond to genomic instability. Shortened telomeres are a consequence of aging that, combined with telomerase activation, contributes to tumor development. *AKT*, protein kinase B; *AMPK*, AMP-activated protein kinase; *CMA*, chaperone-mediated autophagy; *EBV*, Epstein—Barr virus; *HBV*, hepatitis B virus; *HCV*, hepatitis C virus; *HIF*, hypoxia-inducible factor; *HIV*, human immunodeficiency virus; *HPV*, human papillomavirus; *HTLV-1*, human T-cell leukemia virus type 1; *IGF*, insulin-like growth factor; *KSHV*, Kaposi sarcoma-associated herpesvirus; *mTOR*, mammalian target of rapamycin; *NF-κB*, nuclear factor-κB; *PI3K*, phosphoinositide 3-kinase; *ROS*, reactive oxygen species; *UPS*, ubiquitin-proteasome system. ↑ (*green arrows*): aging-associated alterations; ↑ (*orange arrows*): cancer-associated alterations.

genetically altered cells favoring carcinogenesis.[2] Similarly, the mammalian target of rapamycin (mTOR), an evolutionarily conserved nutrient-sensing protein kinase, member of the PI3K/AKT/mTOR pathway, and a target of IGF-1, is also related to both aging and cancer. This kinase is part of two protein complexes termed mTOR complex 1 (mTORC1) and 2 (mTORC2), which have a fundamental role in coordinating anabolic and catabolic processes. mTORC1 is highly regulated and responds to different stimuli from growth factors, nutrients, energy, and oxygen status controlling several cellular processes necessary for cell growth and proliferation.

mTOR activity is a major driver of aging through the deregulation of mechanosensing and altered intracellular communication. Its genetic deregulation or inhibition has been found to extend life span in different organisms from yeast to humans.[41] Hyperactivation of mTOR signaling is also a common event in neoplasia, primarily related to alterations in its upstream regulators, including PI3K-AKT and RAS-driven MAPK pathways, which are highly contributed to aging and cancer.[41] Other signaling pathways, such as AMP-activated protein kinase (AMPK), a sensor of energy deficiency, and hypoxia-inducible factor (HIF), a responder to low oxygen levels, also have fundamental roles in longevity and cancer.[42] The major regulators are several transcription factors, for example, FOXO, NF-kB, NRF2, P53 and HIF, and protein kinases, like the above mentioned mTOR and AMPK, which are able to extend life span and also favor tumor cell survival according to specific contexts.[42] More recently, the Hippo pathway, a conserved signal transduction pathway essential for development, cell proliferation, cell shape, and growth, was associated with aging due to its link with the anti-aging pathways, AMPK, and Silent information regulator 1 (sirtuin1), autophagy, and redox status.[43] The role of Hippo in cancer has been known for almost a decade; once it controls cellular functions essential to drive tumorigenesis, it induces hyperproliferation, cellular invasion, and metastasis. The abnormalities observed in this pathway are primarily due to molecular events rather than somatic mutations, which are rare in its components.[44]

Understanding the signaling mechanisms involved in both aging and cancer and their interconnections might provide new strategies that enhance not only life span but also prevent cancer.

21.5.2 Telomeres and telomerase

Telomeres are evolutionary conserved DNA–protein structures related to cellular aging and cancer processes since they preserve genomic integrity in normal cells, and their shortening during successive cell divisions leads to chromosomal instability and the loss of viability.[45] Telomeres are nucleoprotein structures located at both ends of a chromosome and composed of a non-coding, repetitive DNA sequence in association with proteins that form the shelterin complex.[46] They play a primary role not only in

the protection of chromosomal ends, avoiding unwanted recombination and degradation, but also in the prevention of coding DNA loss during DNA replication.[46] Due to telomere shortening, somatic cells can experience only a limited number of divisions before undergoing senescence or apoptosis.[15] Therefore, telomere shortening limits the life span of cells and protects against tumor development.[47] Telomere function is hampered in both aging and cancer. Short telomere length has been related to age-related diseases like poor immune function[48], diabetes[49], cardiovascular disease[50], osteoarthritis[51], atrial fibrillation[52], and Alzheimer's disease.[53] In several tumors, telomeres are maintained by telomerase, a reverse transcriptase enzyme essential to reconstitute telomere function, restore proliferative capacity, and ultimately to cancer cells' survival.[54] Most human tumor samples (85%–90%) show telomerase activity, being that telomerase expression is absent in the majority of the normal adjacent tissues.[55] Besides telomere length maintenance, telomerase has also been related to the oncogenic process since it seems to be involved in gene expression regulation, cell proliferation, apoptosis, WNT/β-catenin, and NF-kB signaling, MYC-driven oncogenesis, DNA damage response, cell adhesion and migration, and epithelial-mesenchymal transition.[56–59] Thus, telomerase appears to be able to regulate different hallmarks of cancer directly.[60] In the last years, several pathways and genes that regulate telomere length have been identified,[54] and the inhibition of telomerase being a promising target for cancer therapy.[61] Additionally, about 10%–15% of human cancers lack detectable telomerase activity, the telomere length and integrity is maintained through a mechanism called the alternative telomere lengthening (ALT), a homologous recombination (HR)-based process, that includes copying of telomeric DNA templates.[62] Both telomere maintenance mechanisms, telomerase activity and the ALT mechanism, play a role in tumorigenesis by providing unlimited proliferative capacity to cancer cells.[60,62] However, the association of ALT with the promotion of carcinogenesis and with the patient's outcomes still needs to be fully elucidated. Understanding the role of telomeres and telomerase has improved, guiding the development of novel diagnostic tools and the design of therapeutic strategies.[54] Nevertheless, nowadays, opposed to telomerase, no ALT targeting therapies have been developed, due to the scarcely available information of specific molecular players involved in the ALT maintenance of telomere length and integrity.[62] More research is warranted to decipher the genetic and epigenetic mechanisms behind telomere length regulation in aging and cancer.

21.5.3 Genomic instability

Mutations are a crucial piece in the evolution process. However, most mutations occur randomly and are deleterious, having expected adverse effects in cellular phenotypes by changing both protein-coding sequences and gene regulatory profiles.[63,64] Since DNA is chemically unstable under physiological conditions and multiple endogenous

and exogenous molecules can chemically modify the DNA, the maintenance of genomic stability is a continuous process.[65,66] The genomic integrity is constantly threatened by these stressors, and it has been estimated that 10^4 to 10^5 genomic alterations occur per cell per day in our genome.[67,68] Fortunately, cells have over 150 proteins directly responsible for safeguarding genome integrity and repairing virtually all DNA alterations.[66,68] These proteins compose several DNA repair pathways that cope with DNA lesions and are collectively recognized as DNA damage repair (DDR).[66] DNA damage activates DDR that facilitates repair and induces cell cycle arrest until the repair is complete. If DNA damage is extensive or not reparable, DDR effectors trigger cell apoptosis or cell senescence.[68] However, genome instability is the natural tendency of genomes and is known to arise from several pathways, including telomere damage, epigenetic alterations, centrosome amplification, aneuploidy, DNA damage, and unrepair or misrepair DNA.[64,69] The fact that DNA damage increases with age is well known and one possible explanation is that, with age, DNA repair capacity declines, leading to the accumulation of unresolved or misrepaired DNA damage.[65,67]

The accumulation of genomic errors over time contributes to both aging and cancer, and the cell fate is, at least in part, determined by the type of DNA damage they accumulate.[70,71] The accumulation of deleterious mutations may lead to senescence, apoptosis, depletion of stem cells, or neoplastic transformation.[2] Bulky DNA lesions can block transcription and replication, inducing cell cycle arrest and consequently cell senescence or apoptosis. These mechanisms prevent carcinogenesis but ultimately contribute to aging. On the other hand, unrepaired and misrepaired damage will persist as nucleotide substitutions, contributing to the accumulation of permanent mutations and chromosomal abnormalities that increase cancer risk.[70] Although cancer and aging may seem like opposite processes, they are in a way rivaling processes sharing common origins, such as time-dependent accumulation of DNA damage and genomic instability.[2,64] Cancer results from beneficial mutations confer advantageous features to tumor cells allowing for their establishment. If the surveillance systems that normally monitor genomic integrity and force genetically-damaged cells into either senescence or apoptosis are compromised, these initiated cancer cells will progress and metastasize.[2,72] Genomic instability plays a causal role in aging by triggering senescence and apoptosis when homeostasis cannot be maintained.[68,71] In this sense, aging can act as an antitumoral mechanism. Therefore, aging and cancer are opposite responses to the same molecular features.[71] However, the accumulation of DNA damage during aging contributes to carcinogenesis by increasing the probability of oncogenic mutations occurring.[73] Another evidence of the link between genomic instability, cancer, and aging, comes from premature aging diseases and genome instability disorders. Progeroid syndromes (as Werner and Bloom and Xeroderma Pigmentosum) have defects in DDR genes (namely in RecQ helicases and in nucleotide excision repair system, respectively) and display hypersensitivity to genotoxins, while genome instability disorders

show accelerated aging of multiple organs and may have higher cancer predisposition.[2,37,68] Additionally, some cancer survivors age more rapidly. Further, several cancer treatments exert their cytotoxic effects through the induction of DNA damage, and patients treated with these genotoxic agents also age faster.[68] In this sense, aging and cancer are opposite responses to genomic instability.[71] In fact, while cancer cells often benefit from mutations, other cells decline and age.

21.5.4 Epigenetic aging and neoplasia

During cell aging, the DNA methylation and histone landscapes change. These epigenetic alterations (regulatory mechanisms that change gene expression without altering the DNA sequence[74]) gradually perturb the epigenetic state of cells, increasing cellular entropy, which in turn impairs cell homeostasis. Because several changes observed in aging cells, such as demethylation of retrotransposons and repetitive sequences, are also observed in cancer, the link between epigenetic aging and neoplasia is not surprising. Also, the fact that cancer incidence is directly related to advanced chronological age, and that genome-wide epigenetic changes occur early in tumorigenesis and accumulate throughout its progression, magnifies this assumption.[75]

The DNA methylation age-related changes result from the effects caused by a number of extrinsic factors (e.g., microbiome, lifestyle, and environmental factors) or through the epigenetic drift associated with the accumulation of errors in maintaining DNA methylation patterns from parental strands onto newly synthesized ones during replication as well as from the defective DNA damage repair system.[76] Likewise, a loss of histone biosynthesis increasing global transcription levels is observed in aged cells.[75] These changes lead to a selective loss and reorganization of heterochromatin and an upregulation of transcripts from repeat elements, in particular retrotransposable elements associated with the formation of DNA double-strand breaks.[77] Also, age-associated hypermethylation occurs at cytosine-phosphate-guanine (CpG) islands of Polycomb target genes and promoters of tumor suppressor genes.[77] The sum of these events induces the accumulation of aberrant epigenetic changes in normal cells that can, by chance, modify the gene expression of cancer driver genes, in parallel with random mutations, supporting cancer initiation and progression.

Interestingly, a number of studies have shown that genome-wide methylation data can estimate the individual's biological age more accurately than the conventional chronological age, giving rise to the concept of "epigenetic clocks,"[78–81] which may also be applied to track cancer risk. Indeed, the epiToc (Epigenetic timer of cancer) clock focused on a set of CpGs located in Polycomb group target genes' promoters, showed an increase of the DNA methylation levels of fetal tissues with the advance of their chronological age being accelerated in cancer, including in pre-malignant lesions.[82] Complementing the concept of "epigenetic clocks," the study of epigenetic

drift can track the biological tissue aging, potentially predicting the number of overall cell divisions or turnover rate in a tissue, and tracing the age of tissue abnormalities.[76] As an example, a study of the epigenetic drift in colorectal tissues showed that the age-related drift is a genome-wide phenomenon, progressing 3–4 times faster in neoplasia (consistent with the increased cell proliferation).[83] Evidence of this study suggests that cancer precursors may sojourn for decades before turning into cancer,[83] indicating that the study of epigenetic drift in different cancer types might help to adjust the optimal age for cancer prevention.

Understanding the processes behind the differential epigenetic aging processes between individuals and associated molecular alterations holds the key to improve the understanding of age-related risk for cancer. Likewise, considering that likely only a small portion of the epigenetic drift genes are of functional significance, uncovering the key age-related epigenetic changes that may cause cancer initiation and progression is of utmost importance.

21.5.5 Proteostasis

The maintenance of the proteome is an essential process to ensure cell homeostasis and survival, and it is called proteostasis.[84] In an integrative way, the proteostasis network includes the proteotoxic stress-related cellular response, as chaperones and unfolded protein response (UPR), proteolytic systems as the ubiquitin-proteasome system (UPS), autophagy-lysosome (ALP) pathways,[84] and the transcription factors, P53, HSF1, FoxO, NRF2, as sensors.

Molecular chaperones, also known as heat shock proteins (HSP), protect cells from proteotoxicity and protein aggregation. In response to stress, the heat shock transcription factor 1 (HSF1) regulates the expression levels of HSPs to restore protein homeostasis[85]. However, during aging, the expression of HSF1 and multiple chaperones is declined, compromising protein quality control.[86] The accumulation of damaged proteins and protein aggregates made the cellular environment prone to age-related diseases, such as cancer.[85] During the tumorigenic process, the chaperones network is remodeled, giving an advantage to malignant cells. Neoplasia is associated with a chronic, stressful environment that leads to constitutive activation of HSF1 and upregulation of HSPs, which is associated with poor prognosis. One example is HSP90, overexpressed in cancer, which contributes to tumorigenesis by stabilizing aberrant proteins as BCR-ABL1 and mutated P53 and in the assembly of active telomerase, essential to senescence escape.[86,87]

The UPR system is activated in response to unfolded or misfolded protein in the endoplasmic reticulum (ER) lumen, promoting proteostasis. UPR acts by reducing de novo protein synthesis or activating chaperones for correct folding, but apoptosis is induced when the repair is impossible. While in aging, the UPR system is declined due to failure of chaperones and UPR components,[88] in cancer, this system is exacerbated to overcome

excessive protein synthesis necessary for malignant transformation. Transient UPR activation at early stages of carcinogenesis often hinders tumor progression but chronic activation observed in later stages confers resistance to ER-stress induced apoptosis.[89]

UPS is the major proteolytic system that controls protein degradation and regulates the stress response. This system is constituted by E1, E2, and E3 enzymes that modify protein substrates using ubiquitin and proteasome responsible for proteolysis. This last component is a multi-catalytic and multimeric protease formed by a catalytic core (20S) and a regulatory unit (19S).[90] UPS deficiency is at the same time, the cause and a consequence of aging.[91] This dysfunction can be due to lower expression or alterations in subunits, disassembly, or inactivation by protein aggregates of proteasomes.[91] With an increased need for protein degradation and maintenance of cell metabolism, in cancer, UPS's expression and activity increase. Mutations and overexpression of ubiquitin-enzymes or proteasome subunits are described in neoplasia and associated with poor prognosis.[90] Another degradation system in the cell is ALP, responsible for removing misfolded proteins and damaged organelles through lysosomes.[92] ALP includes three mechanisms: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy (MA). In the aging process, LAMP2A expression (essential in CMA) is decreased and lysosome activity is impaired. Additionally, problems in autophagosome fusion, a critical step in MA, and mTORC1 enhanced activity have been described as associated with aging.[92] In cancer, ALP may have a dual role, promoting or suppressing carcinogenesis, depending in part on the cancer phase evolution. Once the disease is installed, ALP is activated to increase the recycling of nutrients and sustain tumor viability.[93]

21.5.6 Metabolism deregulation

All biological functions of living organisms require energy consumption. Considering the regulation of longevity and neoplasia, and despite aging and cancer being characterized by different hallmarks,[72,84,94] the biological processes enrolled in aging and cancer are connected and can be related to metabolism alterations.[3] Although the two conditions share the same metabolism alterations, the metabolic rate does not always decline with age, and there are existing controversies about it.[95]

The relationship between metabolism and cancer was first described by Otto Warburg in the 1950s.[96] However, this theory attracted little attention in relation to the oncogenic theory that emerged in the meantime and gained ground. Only in the last decade, the Warburg's metabolic shift gained a new impetus.[97] According to this theory, cancer cells show an adaptive metabolic shift to maintain high proliferation and growth rates. In this context, cancer cells use two main metabolic pathways to obtain energy, glycolysis, even in aerobiosis, and the catabolism of glutamine. One explanation for the vital role of glutaminolysis in cancer, is to provide important fuel for the TCA cycle

when the supply of glucose to tumor cells becomes scarce.[98] Furthermore, nicotinamide adenine dinucleotide (NAD +) has emerged as an important aging metabolite that may be a common link between age-related genome instability, metabolic decline, and associated comorbidities such as neurodegeneration and cancer.[99] However, not all cancer cells types are dependent on the Warburg effect.

In the cell, mitochondria regulate different metabolic and signaling pathways. During aging, it has been considered that a mitochondrial function decline occurs including morphological alterations. The molecular mechanisms underlying this physiological decline are not well understood. The mitochondrial free radical theory of aging is one of the most studied theories related to aging metabolism, advocating that ROS, products of aerobic metabolism, are considered toxic due to their high chemical reactivity, inducing oxidative damage to different cellular macromolecules.[100]

According to Hanahan and Weinberg, ROS production and the tumor-promoting inflammation hallmarks have an essential role in cancer, considering that ROS is actively mutagenic, it can accelerate the genetic evolution towards a state of malignancy.[72] Not only that, but cancer cells can be affected by direct signals from mitochondria through ROS or other intermediates that can affect cellular physiology via genetic and epigenetic mechanisms and consequently contribute to the carcinogenesis process. Considering aging as a chronic process, the alteration of cell energy supply from the TCA cycle to glycolysis is a time-dependent process that occurs with aging and, at the same time, is a hallmark of cancer. Gradually, the cell tries to compensate through substrate-level phosphorylation considering that over, time there are damages accumulated. These damages are translated as mtDNA mutations that affect ATP production and alter the way through which cells obtain energy, according to the Warburg effect.[96,97] When cells undergo the Warburg effect transition, they predominantly will obtain energy from glycolysis and glutaminolysis, increasing the production of different substrates such as lactate, succinate, alanine, and aspartate. These substrates can regulate gene expression through epigenetic alterations contributing to cancer development.[96,97]

21.5.7 Immune system dysfunction

The function of the adaptive immune system is to protect the host from pathogens by generating a broad repertoire of pathogen-specific lymphocytes that clonally expand after specific recognition and activation. After this clonal expansion, most effector cells generated are eliminated. However, a small percentage of activated cells develop into long-lived memory cells that efficiently eliminate the same pathogen after future encounters. Lymphocyte populations are preserved by complex regulatory processes necessary not only to maintain the repertoire of newly generated naïve lymphocytes to respond to new antigens but also to proliferate, differentiate into different functional lineages, and sustain immune memory.

Self-renewal of naive and memory lymphocytes is essential to maintain an adequate balance of immune cell numbers and to keep their capacity to develop an efficient immune response against pathogens throughout life (for review see Zhang et al., 2021[101]). Endogenous age-associated disturbances and repeated exogenous infections substantially affect this highly regulated process resulting in changes of the components of the immune system in older individuals.

Immunosenescence refers to the altered immune response associated with aging, and it affects immune cells involved both in innate and adaptive immunity. It results in a decreased response to new viruses and vaccine effectiveness observed in older individuals. The clinical consequences of immunosenescence in the aging population include higher morbidity and mortality due to infections, in particular influenza and pneumococcal disease. The recent evidence from the COVID-19 pandemic by SARS-CoV-2 infection has clearly shown the high case fatality rate after the age of 50 years that further increased in elderly compared to middle-age adults.[102] Responses to vaccines, such as the flu vaccination, are also reduced with age, although vaccination against SARS-CoV-2 confers adequate protection rates.[103] The major alteration observed in immunosenescence is the decreased percentage of naive T (Tn) cells. It is clearly established that the percentage and absolute numbers of CD^{4+} and CD^{8+} Tn cells, defined by the combined expression of several markers including CD45RA, CCR7, CD28, and CD27, decreases markedly with age. Naive T cells are generated in the thymus, but thymus involution, which initiates by the time of puberty, results in a reduction in the output of new Tn cells. The number of naive T cells is maintained for decades, likely by the process of cytokine-induced homeostatic proliferation. T-cell decline is particularly relevant in naive CD^{8+} T cells rather than in naive CD^{4+} cells, representing one of the hallmarks of T-cell aging.[104] Associated with the decreased number of naive T cells, a limited shrinkage of the T-cell repertoire is found although, at least in healthy older individuals, the diversity of T-cell receptors is enough to respond to the universe of foreign peptides. The decreased proportion of naive T cells with aging is associated with increased percentages of effector-memory T cells. In addition to aging, infection by latent viruses such as cytomegalovirus (CMV), significantly affects the distribution of T-cell subsets, promoting the expansion of effector-memory T cells.[105]

21.5.8 Persistent viral infection

In addition to alterations in the naive T-cell compartment, age-associated alterations in the immune memory have also been extensively demonstrated.[104,106] After antigen stimulation in secondary lymphoid organs, naive T cells differentiate to effector-memory T cells, characterized by the lack of expression of CCR7. These effector-memory T cells are heterogeneous, and the differential expression of CD45RA, CD28, or CD27 defines several effector-memory T-cell subsets. In particular, CD45RA expression on CCR7null T cells defines a specific subset of effector cells termed TemRA cells.

Examples of the clinical consequences of a decline in the functional capacity of memory T cells are shingles, a reactivation of varicella zoster virus that is strongly associated with aging or the increased incidence of pneumococcus or respiratory syncytial virus infections that are common childhood infections. On the other hand, other viruses such as latent EBV and CMV induce expansion of memory T lymphocytes that are likely protective of symptomatic infections.[107] These virus-specific expansions of CMV and EBV-specific memory T cells also represent a hallmark of immunosenescence.

It has been challenging to separate the differential effects of CMV infection and aging on T-cell subsets because CMV seropositivity increases with age in all human populations studied, independently of other health, ethnic or socioeconomic considerations.[108] Recent advances support this, whereas the effect of aging on the decrease of naive T cells is independent of CMV serostatus, it has been shown that accumulation of effector-memory T cells is associated with CMV seropositivity but it is not age-dependent. Reports from several research groups support that increased percentages of effector-memory and TemRA CD^{8+} and CD4 T cells found in older donors are mainly observed in CMV seropositive individuals but not in CMV seronegative donors. CD^{8+} TemRA cells exhibit several signs of senescence such as telomere shortening, low proliferation capacity, high resistance to apoptosis, or high levels of cytokine production and cytotoxicity. Thus, it has been hypothesized that the accumulation of CD^{8+} TemRA cells in older individuals may be detrimental, contributing to the increased morbidity and mortality of inflammatory diseases in the elderly.[109] To analyze the differential effects of age and CMV seropositivity on immune cell phenotype and function, it is required to better understand the process of immunosenescence and design novel interventions to reduce the morbidity and mortality of infectious diseases in older populations.

21.5.9 Stem cells

Among the heterogeneous cell populations that compose tumors, cancer stem cells (CSC) are capable of self-renewal and multi-lineage differentiation.[110] Stemness underlies drug-and radiation resistance, invasion, and metastasization; the latter being supported by the plasticity of transition between epithelial and mesenchymal states.[111,112] CSCs are identified through the formation of spherical colonies in vitro, the ability to expel DNA dyes (side-population), the formation of tumor xenografts, and through specific surface markers.[113] The transformation of tissue stem cells and the dedifferentiation of tissue cells are hypotheses for the origin of CSC.[112] In fact, key stemness factors as OCT3/4, SOX2, KLF4, and NANOG are active in certain tumors.[112]

While aging is the main driver of malignancy, stem cell number and function drops with age.[112] However, the number of cancer stem-like cells increased with aging in rat colonic crypts and in the macroscopically normal mucosa of patients with

adenomatous polyps.[114] Corroborating evidence that aging predisposes the colonic mucosa to processes of carcinogenesis was pointed out by Nautiyal et al.[115] The linear increase in adenomatous polyps and CSC in macroscopically normal mucosa with advancing age was supported by the higher expression of several CSC markers, such as CD44, CD166, and ESA, activation of EGFR, and its downstream signaling events.[115,116] *SIRT1*, one of the most conserved longevity genes, increases the number of ALDH1 + CSC, elicits partial MET, and promotes lung metastasis by upregulating KLF4 in human and mouse breast cancer cells, pointing to this gene as central in the regulation of age-related CSC.[112] Further evidence of the links between aging and cancer is telomere shortening and increased chromosome instability, which is frequent in precancerous lesions and creates a permissive microenvironment for the emergence of CSC.[117,118] In fact, certain epithelial cells become privileged in senescent microenvironments, which are more likely to occur with aging, forming tumors, and engaging in stemness.[117] Menendez et al. designated this status at the intersection of aging and cancer, where depletion of adult stem cell function and reduced health meets the acquisition of tumor-initiating and metastatic abilities in CSCs as metabostemness.[119]

With age, all cells accumulate genetic and epigenetic alterations, which affect the cellular, molecular and physiological functionality of tissues. These effects may lead to a higher risk of tumorigenesis when concomitant with deregulated cell signaling and changes in the microenvironment.[120]

At this time, further research is needed to enlighten age-associated alterations involved in CSC generation and performance. Advances in this area will certainly point to developments in cancer diagnosis and treatment.

21.6 Neoplasias and aging

21.6.1 Aging-related neoplasias

Aging is a fundamental risk factor for cancer development since the majority of cancers are diagnosed after 65 years.[3] In fact, the incidence of common malignancies, including breast, lung, prostate, colorectal, gastric, thyroid, pancreatic, and ovarian carcinomas, as well as some types of leukemia, increased exponentially with age.[4]

As a person grows older, the accumulated risk for specific cancers is combined with the tendency for progressive organ loss of function, declined tissue renewal capacity, and reduced immune function. Simultaneously, mutations accumulate in tissues throughout life, and cellular repair mechanisms become less effective.[10,121]

Estimates of the worldwide cancer incidence are available online in the GLOBOCAN 2020 database, compiled and disseminated by the International Agency for Research on Cancer. For both sexes combined incidence, the most commonly diagnosed cancer types are breast cancer (11.7% of the total cases), followed by lung cancer (11.4%), colorectal cancer (10.0%), and prostate cancer (7.3%)[122] (Fig. 21.2).

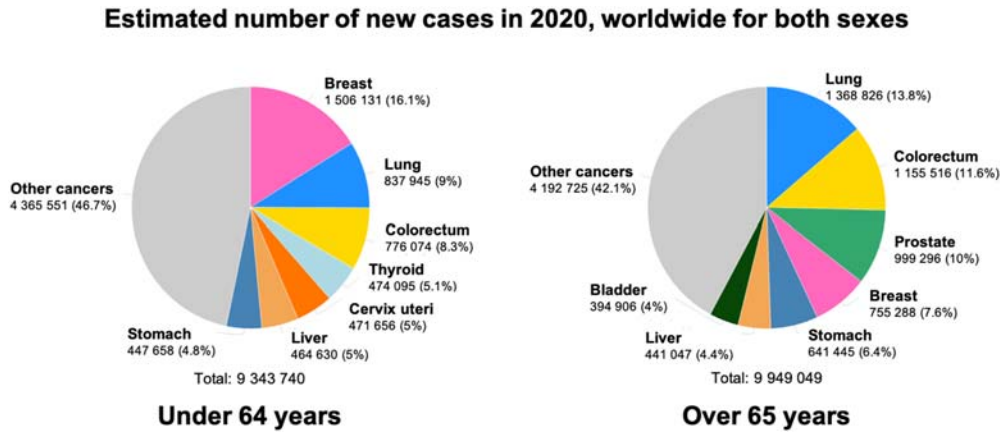

Figure 21.2 ***Age-related neoplasia's incidence.*** Estimation of the worldwide cancer incidence for both sexes in the different age segment, according GLOBOCAN 2020 database.[122]

A careful analysis of the GLOBOCAN 2020 estimates for two age groups,[122] "−65Y" (less than 64 years) and "+65Y" (more than 65 years), makes it clear that there are a group of cancer types whose incidence is most affected by age, namely lung, prostate and colorectal. Comparing the worldwide estimated number of new cases in 2020, for both sexes, between −65Y and +65Y groups, there is an increase of lung (from 9% in −65Y group to 13.8% in +65Y), prostate (from <4.8% to 10.0%) and colorectal (from 8.3% to 11.6%) cancers. The same comparative analysis, but now just considering males, reveals the same profile with an increase of the incidence of lung (from 12.7% to 15.4%), prostate (from 9.4% to 17.6%), and colorectal (from 10.1% to 10.9%) cancers. The same increasing tendency of the number of new cases, when compared −65Y and +65Y groups, is observed for females in lung (from 5.6% to 11.5%) and colorectal (from 6.7% to 12.5%) cancers.

Lung cancer is clearly an aging-related disease with less than 2% of all lung cancer cases occurring in young patients (under 45 years).[123] In parallel, elderly report high rates of respiratory symptoms, which are frequently associated with chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, and lung cancer. Although DNA damages due to smoking are the leading cause of malignant transformation of lung cells, natural biological processes also contribute and are positively associated with age. Recent results from the genome-wide association studies showed that there are risk genes that overlap between the aging lung diseases referred to previously, which include *FAM13A*, *DSP*, and *TERT*.[124] Another link of lung cancer with aging is the altered miRNA expression and biogenesis. Many age-related processes are described as playing an important role in miRNAs regulation in lung cancer. These processes include epigenetic modifications through prolonged exposure to environmental toxicants, the aberrant activity of transcription factors triggered by other aging-related

pathologies, as inflammation, and genetic alterations due to age-related mutations or deletions. miRNA changes can then influence lung cancer initiation, progression, and resistance to treatment, which in contrast also emphasizes their potential as biomarkers and therapeutic tools.[123]

The environmental and endogenous stress to which the prostate is exposed to explains the increased incidence of prostate cancer with age. There are several epigenetic molecular mechanisms, such as DNA methylation, genomic printing, and histone modifications, which are known to undergo changes with aging and which justify the increased susceptibility of the prostate to cancer development.[125] Another existing link between aging and prostate cancer is oxidative stress. Directly or indirectly, aging increases intracellular oxidative stress through the decrease of vitamin D, decrease of the efficiency of the p53 stress response, mainly due to its absence or mutation in the majority of prostate cancers, decrease in antioxidant gene expression levels, and by an increase in ROS levels mediated by androgen levels and upregulation of the androgen receptor (AR). The resulting oxidative damage can mediate the neoplastic transformation of prostate cells.[126]

Colorectal cancer is another age-related disease, with a marked and progressive incidence increase with aging. Several studies have demonstrated that specific epigenetic modifications, like miRNA expression and DNA methylation, are common in the biological aging process and might be associated with the risk of developing colorectal cancer and also with survival from colorectal cancer. Beyond epigenetic variations, some risk behaviors known to be biological aging accelerators, such as smoking, unhealthy diet, and physical inactivity, might also modulate the risk of colorectal cancer development.[127] Moreover, its carcinogenic process is influenced by an interplay of an aging-related deficient microbiota and the immune system through chronic inflammation, aberrant changes in the immune response, and DNA damage.[128] However, in colorectal cancer, tumor aggressiveness does not correlate with age. Although the incidence remains higher in older patients, younger patients diagnosed with colorectal cancer usually have a worse prognosis. This might be due to the lack of screening and the rising rates of obesity in younger patients.[3]

In summary, the incidence of cancer in older people may vary according the world region and sex, lung, prostate, colorectal, liver, and stomach cancers are the five cancers that represent over two-thirds of the total burden of cancer among people aged 65 years in all regions.[129]

21.6.2 Cancer therapeutics and aging

Cancer is a disease of aging, and for most malignancies, the mortality rate is higher in the older age portion, requiring adjustments to treatment protocols.[130] There are

complexities inherent in cancer treatment among older populations and possible undertreatment of the elderly can occur. The lack of evidence on the benefits and risks of treatment among older patients is partly due to their common exclusion from clinical trials.[129]

In elderly patients, the primary outcome of treatment may change from the potential for improved survival to be the most well tolerated, in terms of toxicity. However, in clinical trials, patients over 65 years are underrepresented, as already mentioned, comprising the information in terms of risks associated with treatment toxicity. In elderly patients, it is necessary to take into consideration multiple aspects when a treatment selection is made. The treatment approach should consider the patients' functional age, which reflects the cumulative effects of the aging process, rather than chronological age (based on time alone).[131] Additionally, the presence of comorbidities, drug interactions, and organ function reserve are some of the characteristics essential to determine eligibility for a specific cancer treatment approach. One of the tools available to facilitate treatment selection is the comprehensive geriatric assessment (CGA) that examines different age-related domains, including comorbidity, function, physical performance, cognition, nutrition, emotional status, polypharmacy, social support, and living environment.[132] This type of assessment is extremely important to identify frail patients. With CGA, it is possible to stratify patients into three distinct groups: fit, vulnerable, and frail patients. For a fit patient, the treatment approach is similar to younger patients, while the best offer for the frail group is supportive care. The vulnerable category becomes more challenging to management treatment, necessitating individualized approaches. Since CGA determination could be very time-consuming and hard to use on a daily basis in the clinical context, many other assessment tools are used for geriatric assessment, as Eastern Cooperative Oncology Group Performance Status (ECOG-PS) and Vulnerable Elders Survey (VES-13).[133]

Although advanced age does not represent a prohibitive factor to chemo- and radiotherapy, the physiological alterations due to aging may affect these approaches' safety and efficacy. Changes in body composition and reduction in organ function, like the kidney and liver, potentially alter the pharmacokinetic and pharmacodynamic properties of drugs.[134] One example is the decrease in total body water content with age and, consequently, an impairment in the distribution of water-soluble drugs occurs. These characteristics modify the peak drug concentrations and/or prolong some compounds' half-life, with possible consequences in therapeutic efficacy and toxicity profile. For instance, the vulnerability of normal tissues to chemotherapy effects increases, particularly in the bone marrow, mucous membranes, nervous system, and cardiac tissue.[135] Based on this, the chemotherapy protocol can be modified by administering suboptimal doses, omitting drugs, or prescribing monotherapies (more common in palliative care) (Table 21.1).

Table 21.1 Examples of treatment modifications to elderly patients.

Neoplasia	Treatment protocol	Modifications to elderly patients	Refs.
CRC	FU OxFU FLOFOX	Starting doses with 80% of the standard doses Discretionary escalation to full dose after 6 weeks	136
DLBCL	R-mini-CHOP	Rituximab plus dose reduction on CHOP For 5 days every 3 weeks	137
NSCLC	Doublets Singlets	Carboplatin combinations for fit patients Single agents (vinorelbine, gemcitabine or docetaxel) for unfit patients	138
Prostate Cancer	Docetaxel Cabazitaxel	Reduction to two weeks of treatment Dose reduction to 20 mg/m^2	139

CHOP, cyclophosphamide, doxorubicin, vincristine, and prednisone; *CRC*, colorectal cancer; *DLBCL*, diffuse large B-cell lymphomas; *FOLFOX*, Fluorouracil plus Leucovorin plus Oxaliplatin; *FU*, fluorouracil; *NSCLC*, non-small-cell lung cancer; *OxFU*, oxaliplatin plus fluorouracil; *Ref.* reference.

Using diffuse large B-cell lymphoma (DLBCL) as an example, the gold standard of DLBCL treatment is combination chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone) with the monoclonal antibody anti CD20 rituximab (R-CHOP). The R-CHOP protocol is used for fit patients, but for unfit patients a modified version is used, the R-mini-CHOP. With this approach, respectable response rates were observed and associated with manageable toxicity.[137] Even in cancers treated with targeted therapies, dose adjustments may be needed to avoid toxicity effects. Tyrosine kinase inhibitors are associated with cardiotoxicity, and this effect is more pronounced in elderly patients due to cardiac function impairment. Due to a higher incidence of toxic effects of cancer treatment, older patients may not be able to complete the full extension of prescribed treatment compromising results, particularly relevant in radiotherapy. To overcome this issue, one of the strategies used in this segment of patients is the hypofractionated treatment schedules. In this approach, the total dose administrated is the same but condensed in a small number of sessions.[140]

Especially for older cancer patients, it is crucial to determine if the expected benefits and outcomes of treatment are superior to the associated risks. To improve treatment selection, there are available tools that can be used to predict cancer treatment toxicity combining clinical and geriatric information, such as the cancer and aging research group (CARG) and chemotherapy risk assessment scale for high-age

patients (CRASH) models.[141,142] With the ability to predict the risk of grade 3 to 5 treatment toxicities, the CARG model includes geriatric assessment variables, laboratory test values, as well as patient, tumor, and treatment characteristics.[141] The CRASH model takes into consideration specific chemotherapy regimen use, blood pressure, LDH levels, ECOG performance status, nutritional and mental status. This model can predict the risk of grade 4 hematological or grade 3 to 4 non-hematological toxicities.[142] On the other hand, cancer treatments contribute to accelerated aging in cancer survivors by provoking aging hallmarks such as stem cell exhaustion, cellular senescence, telomere attrition, DNA damage, and epigenetic alterations.[143]

The knowledge of the underlying mechanism in both cancer and aging could also lead to the development of new selective and targeted therapeutic strategies in the elderly. The recent advances in the role of an aged tumor microenvironment in therapy response, namely in chemotherapeutic resistance, will allow a more efficient and less intensive targeting of the different types of cancer in elderly individuals, improving the quality of life of cancer survivors.

21.7 Conclusion

The underlying mechanism in both cancer and aging is the result of an accumulation of cellular damage over time. Cancer and aging may seem like opposite processes, but these conditions share many common characteristics, as represented in Table 21.2.

Cellular aging results from altered intercellular communication, genomic instability, stem cell exhaustion, cellular senescence, mitochondrial dysfunction, deregulated nutrient sensing, loss of proteostasis, epigenetic alterations, and telomere attrition.[84] On the other hand, cancer is a complex disease caused by a combination of genome instability and mutation, enabling replicative immortality, deregulating cellular energetics, tumor-promoting inflammation, evading growth suppressors, activating invasions, and metastasis, avoiding immune system destruction, sustaining proliferative signaling and resisting cell death.[72,94] Despite aging and cancer being characterized by different hallmarks, they have an undeniable and remarkable interaction.

The expected increase in cancer incidence at older ages will have substantial economic and social impacts worldwide, representing a considerable and unique challenge for health systems in all world regions. The knowledge of the underlying mechanism in both cancer and aging could contribute to the development of cancer prevention strategies and new selective and targeted therapeutic approaches in the elderly, improving clinical care and quality of life during aging.

Table 21.2 Common and divergent hallmarks in aging and cancer.

Hallmark	Aging	Cancer
Cellular senescence		
	Increased	Prevalent in pre-malignant tumors but evaded in fully malignant tumors Apoptosis evasion
Oxidative stress		
	Increased Mitochondrial dysfunction	Increased Tumor-promoting inflammation
Genomic instability		
	Increased	Increased
Telomere attrition		
	Shortened telomeres	Shortened telomeres but telomerase activation Limitless replicative potential
Epigenetic alteration		
DNA methylation Histone modification Non-coding DNA	Global hypomethylation Complex miRNA deregulation (e.g., miR17−92 downregulation)	Hyper- of tumor suppressors and hypo- of oncogenes Complex miRNA deregulation (e.g., miR17−92 upregulation)
Proteostasis		
Chaperoning Proteasome activity Autophagy-lysosome activity	Impaired Impaired Impaired	Augmented Augmented Augmented

Main metabolic pathways to obtain energy		
	Oxidative phosphorylation	Glycolysis and glutaminolysis
Nutrient sensing		
	Dysregulated nutrient sensing Inhibition of insulin and mTOR signaling increase life span	Dysregulated cellular energy Inhibition of insulin and mTOR signaling is antineoplastic
Immune system dysfunction		
	"Inflammaging"	Avoiding immune destruction
Cell signaling and communication		
	Altered intercellular communication	Tissue invasion and metastasis Self-sufficiency in growth signals Insensitivity to antigrowth signals
Stem cells		
	Exhausted	Potential niche for tumorigenesis

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CHAPTER 22

Multidimensional frailty as an outcome of biological aging: immunosenescence and inflammaging in the life course perspective

M. Cristina Polidori[1,2], Luigi Ferrucci[3] and Claudio Franceschi[4]
[1]Ageing Clinical Research, Department II of Internal Medicine and Center for Molecular Medicine Cologne, University Hospital of Cologne, Cologne, Germany
[2]Cologne Excellence Cluster on Cellular Stress-Responses in Aging-Associated Diseases (CECAD), University of Cologne, Cologne, Germany
[3]National Institute on Aging, Baltimore, MD, United States
[4]Institute of Information Technologies, Mathematics and Mechanics and Photonics Center, Department of Fundamental and Applied Research, National Research Lobachevsky State University of Nizhni Novgorod, Nizhny Novgorod, Russia

22.1 Introduction

One of the indirect effects of the ongoing severe acute respiratory syndrome-corona virus 2 (SARS-CoV-2) pandemic, as a global emergency that has claimed 3.7 million cases as of June 6th, 2021,[1] is that it unmasked vulnerabilities at the micro-, meso-, and macro-levels. From the patient interaction level, to the healthcare organization and community level, to the policy level, the current pandemic has dramatically unveiled that medical systems were not designed to cope with the emergence of a pandemic and its acute as well as chronic consequences. Due to their unpreparedness, systems reacted, often too late, with tools borrowed from previous global emergencies, largely neglecting the substantial change in the protagonist of the 21st century, the aging of the world's population. In other words, during COVID-19, the healthcare systems did not consider the profound epidemiological changes that had occurred, the new emphasis on multimorbidity the expanding gap between life span and health span and the multidimensionality of frailty.[2]

Even more so, the pandemic uncovered the complex nature of the vulnerability of a person. This spans from the level of biomolecular profiles to that of physical, psychosocial, and functional aspects and is profoundly influenced by personality, life events, lifestyle and geographical and cultural identifiers. After groundbreaking descriptions, paving the way to critical discoveries to maintain and restore robustness,[3] frailty is accepted as the very core of geriatric medicine, going far beyond multimorbidity and chronological age.[4] Frailty is based on and incorporates fundamental aging processes

Aging
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essential to its development and reversibility, but, within this complex figure, there is still a lack of understanding of single processes and age-related changes so that their modulation can lead to a sustainable, proactive management of functional loss.

Decades of research on aging and age-related diseases have shown the clear benefit of the comprehensive approach at the person-centered level to improve understanding and the management of frailty and functional loss.[4,5] Among the several age-related physiological changes described so far, two dynamically interwoven phenomena profoundly impact life trajectories and frailty outcomes: immunosenescence and inflammaging. A wealth of recent literature developed by experts has addressed these conditions in great details; here we shift the spotlight on the evidence that immunosenescence and inflammaging play an important role in the pathogenesis of frailty, perhaps stronger than often believed.

22.2 Relevance of mechanisms of aging for medicine in the 21st century

Life-course evidence indicates that there is a clinical condition of frailty as an outcome of biological aging. Physiological systems that maintain homeostasis, robustness, and resilience show altered integrated effectiveness over the life course. While these are mutually regulating, their dysregulation during aging predicts emergence of frailty.[6,7] Within this conceptual framework, energy dysregulation as the key promotor of age-related changes in the continuum from system integrity to disease, is at the same time, the causal factor prompting weakness, slow gait speed, low physical activity, exhaustion, and unintentional weight loss. These are the biologically interconnected symptoms and signs that are diagnostic elements of the frailty phenotype.[3] The greatest challenge associated with the triad of aging- physiological dysregulation-frailty (Fig. 22.1) is not only that energy balance and stress responses are highly dysregulated, but that this dysregulation is characterized by a wide intra- and interindividual heterogeneity. Such heterogeneity currently hinders the identification of unerring pathophysiological and "one size-fits all" diagnostic algorithms which are traditional pillars of medicine.

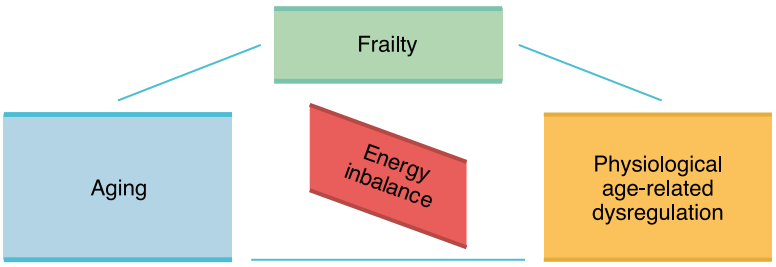

Figure 22.1 The triad, aging-physiological age-related dysregulation-frailty and the central role of energy imbalance.

The latter is strongly based on evidence highly focused on a singular sign or symptom, its diagnosis and management. This disease-centered approach often does not capture the multifactorial nature and the diversity of the person with that particular symptom or sign. However, with increasing age, a large number of factors beyond organ illness profoundly influence disease trajectories. The Comprehensive Geriatric Assessment (CGA)[8] is the accepted tool to disentangle complexity in aging medicine, but current systems still pose many barriers to its systematic use.[9] The CGA, coupled with a feasible multidimensional biomolecular assessment of the main known hallmarks of aging,[10] might solve the issue of clinical usefulness of the frailty concept and overcome the challenge of confinement to immediacy.

22.3 Two facets of immunosenescence and inflammaging

The recognition of the immune system as an important player in human health is quite a recent step in the history of medicine, well mirroring the discipline of aging medicine and geriatrics. The knowledge that inflammatory components of the immune system are often chronically elevated in older persons and associated with an increased incidence of degenerative diseases including cancer, cardiovascular, and neurodegenerative diseases is even newer. This has enabled important research pathways on inflammation as a fundamental trigger of physiological aging regulation. Interestingly, the so far known hallmarks of aging, genomic instability, shortening telomere length, epigenetic modifications, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion and altered intracellular communication,[10] have all been shown to be associated to different extents with the so-called sustained systemic inflammation. While the acute response is typically triggered by infection, chronic and systemic inflammation have been postulated to have a physical, chemical or a metabolic stimulus as the initiating factor. For this reason, the latter are called "sterile agents" and the inflammation associated with is is called "sterile inflammation".[11–13]

A common misbelief in medicine is that immunosenescence and inflammaging are signs of waste, detrimental for life and thus should be counteracted, treated and deleted. However, as for every other accepted age-related condition from biolomolecular to clinical changes, a universally accepted understanding and definition of inflammaging and immunosenescence is still lacking. "Immunosenescence" is still often understood as a "declining function of the immune system leading to a higher incidence of infection, cancer, and autoimmune disease related mortalities in the elderly population," and it is often considered independent of the differences between younger and older individuals. In general, immunosenescence is a concept related to the changes sometimes shown, sometimes assumed, in the output of immune cells from the bone marrow, the distribution of immune cells in the periphery and their

functionality. As opposed to the prevailing current opinion that the most marked changes that occur with aging in the adaptive immune system determine the state of immunosenescence, since the 1980s, it has been recognized that the innate system is influenced by aging but perhaps not always in the same negative direction.[11] Furthermore, with the turn of the century, the new concept that aging is associated with a chronic, sterile, low-grade inflammation called inflammaging[13] has arisen. The recent scientific advances leading to the knowledge that both immunosenescence and inflammaging are not always signs of ongoing pathology and end-of-life indicators has prompted a large body of research aimed at differentiating age-related changes based upon their effective association to degeneration, energy failure, and frailty.

Parallel to the mounting knowledge about the molecular mechanisms of immunosenescence and inflammaging, a number of important questions have emerged on what are the characteristic aging-associated changes in the various compartments of the immune system and which of them are robust indicators of senescence and adaptation (Table 22.1). In other words, immunosenescence and inflammaging are exposed, as other age-related conditions like oxidative stress, to the dangerous interpretation that, if they are associated to aging, their neutralization by any means including the pharmaceutical "solutions" will reverse the aging process. Similar to the case of recent

Table 22.1 Summary of some immune changes associated with aging in innate and adaptive immune systems.

Features	Change
Innate immunity	
Phagocytosis	↔
Free radical production	↑↓
Chemotaxis	↓
Cytokine production	↑
Myeloid cell number	↑
Adaptive immunity	
Naïve cell number	↓
Memory cell number	√
T regulatory cell number	√
T regulatory cell function	↓
Proliferation	↓
IL-2 production	↓
B regulatory cell number/function	↓
B cell immunoglobulin production	↓
B cell autoantibody production	↑

Source: Adapted from (Front Immunol. 2017; 8: 1960).

discoveries in the field of oxidative eu/distress, pharmacologically intervening on physiological immunosenescence overall and sterile inflammaging might be rather detrimental. Indicators of immunosenescence, in fact, may indicate beneficial effects under particular circumstances, consistent with the notion of antagonistic pleiotropy.

22.4 Pathophysiological relevance of immunosenescence and inflammaging in the context of frailty: relevance for COVID-19

The pathophysiological and clinical relevance of immunosenescence and inflammaging in the context of frailty has been conceptualized in the recently described multilayer model of frailty,[14] which was just revisited to explain the challenges linked to the diagnosis and management of COVID-19[15] (Fig. 22.2). The complexity of frailty goes beyond organ medicine, multimorbidity, and chronological age and is based upon three interwoven layers, each of them strongly affected by advanced biological aging. The inner layer

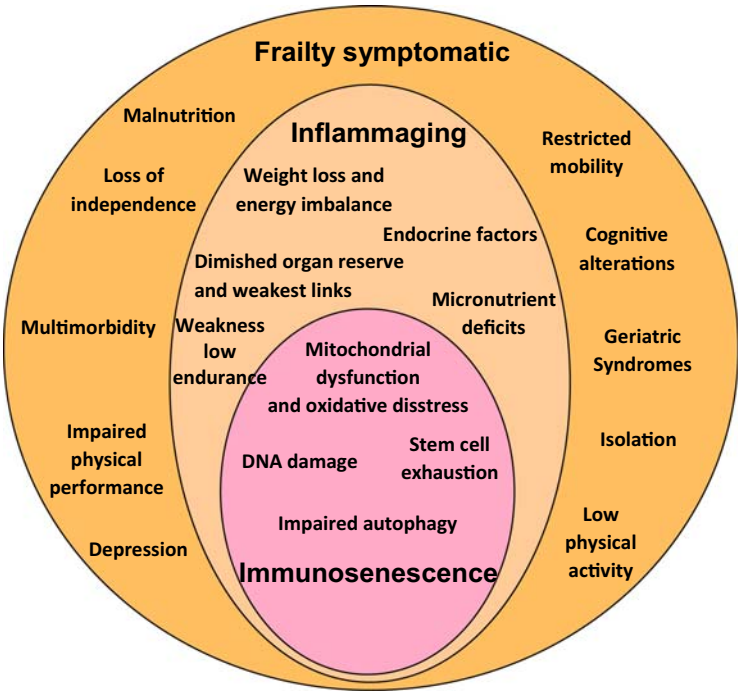

Figure 22.2 Unique and precise positioning of immunosenescence and inflammaging in the multilayer model of frailty. The inner layer includes biomolecular mechanisms and immunosenescence. The intermediate layer includes inflammaging. The outer layer is the multidimensional presentation of frailty. *Modified from Ferrucci, L., Fabbri, E., Walston, J.D. Frailty. In Hazzard's geriatrics, McGraw-Hill; 2017, pp. 691–708*[14] *and from Polidori M.C., Sies H., Ferrucci L., Benzing T. COVID-19 mortality as a fingerprint of biological age. Ageing Res Rev. 2021;67:101308.*[15]

includes biomolecular mechanisms hypothesized to be the primary causes of frailty, seeing immunosenescence as one of the protagonists, and largely the predisposition to the SARS-CoV-2-related pathophysiological cascade. The intermediate layer includes, perhaps the most pervasive homeostatic dysregulation feature of aging, the acquisition of a proinflammatory state with chronically elevated cytokine levels. This is associated with a blunted immune response to vaccination and/or infection, in turn leading to predisposition to infection. The outer layer (Fig. 22.2) represents the dimensions of the aged person, often presenting with a symptom potentially related to a (pre)frailty condition, but considered in its DRG-based form in clinical routine. Fried et al., however, previously showed that a simple symptom could explain geriatric problems in only roughly 50% of the patients.[16] This observation has been repeatedly confirmed in recent years and is the basis of complexity in aging medicine. As displayed in the outer layer of Fig. 22.2, indeed, it is the several dimensions of the person that form his/her overall frailty and determine not only the course of the specific symptomc but, most importantly, the health trajectory altogether. This multifactorial nature and multidimensionality of frailty constitutes the basis of its complexity. It is therefore not surprising that monodimensional prognostic tools of organ illness including COVID-19, often lack good prediction ability for stratification purposes in older patients.[17,18]

Within this context, one of the main drivers of COVID-19 lethality is senescence. Research in animal models and some initial data in humans suggests the hypothesis that the typical proinflammatory state of aging is primarily sustained by the accumulation of senescent cells and the spilling in the blood of senescence-associated secretory phenotype molecules that include cytokines and chemokines. This has been suggested to be the reason why chronic inflammation has been associated with many age-related chronic conditions such as insulin resistance, CVD, osteoarthritis, chronic obstructive pulmonary disease and neurodegenerative processes, all characterized by the accumulation of senescent cells.[19–23] Systemic, sustained chronic inflammation due to persistent tissue damage, environmental stressors, unhealthy lifestyle, and social and psychological stress is associated with the risk of developing many chronic diseases. Dysfunction in many of the so-called hallmarks of aging often converge into a proinflammatory response and exogenous compounds such as bacteria, viral fragments, or endogenous chemicals have been shown to interact with pattern recognition receptors (PPRs) expressed on the cell surface and in the cytoplasm. PPRs like Toll-like receptors, NOD-like receptors and aryl hydrocarbon receptors, trigger inflammatory responses thereby inducing inflammaging.[24] Patients with different age-related chronic conditions do display high circulating levels of several inflammatory biomarkers, including CRP, IL-6, IL-18, and TNF,[25] while IL-6 serum levels have been also shown to predict disability and frailty.[26] Furthermore, high blood IL-6 values have been found to be associated with slower walking speed,[27] and the risk of developing disability over time was observed to increase linearly for IL-6 levels higher than 2.5 pg/mL.[28]

Importantly, immunobiography, the individual's history of exposure to certain microorganisms (e.g., HIV and CMV) or antigens, may condition not only the degree and characteristic of the inflammatory response to various stimuli, but also inflammaging and frailty. Immunobiography may be the mechanism by which obesity and the metabolic syndrome are characterized by chronic inflammation, abnormal production of cytokines (TNF, IL-1, and IL-6), and altered immune T cell response, which appear to increase the risk of infection as well as its severity and consequences, particularly regarding COVID-19-related outcomes.[29,30]

For this reason, the observation that prognostic factors for mortality from COVID-19 are similar to those that have been associated with a high risk of chronic inflammation, like older age, male sex, obesity, smoking, and cardiovascular diseases, has prompted the suggestion that COVID-19 might induce accelerated aging and frailty across a wide range of severity (Betkas et al., 2020).[15]

22.5 Concluding remarks and research outlook

There is strong evidence that the biology and mechanisms of aging contribute to the onset of age-related diseases and conditions.[31] Similarly, longitudinal studies have found a thread between immunosenescence and inflammaging display with the development of frailty, although unfortunately, this knowledge has failed to be implemented in clinical research and practice. Advances in this field are slowed down by the idea that every age-related change is detrimental and should be counteracted a priori. Despite the highly relevant recent advances on the association between deep-learning immunosenescence-related profiles and multimorbidity patterns,[32] further research is needed to better disclose which immunosenescence- and inflammaging-related changes are indicators of stress, adaptation, and response. Moreover, as profiles of immunosenescence- and inflammaging-related changes occur across the continuum of multidimensional frailty, longitudinal research on robust older persons is likely to uncover immunological factors associated with the gradual onset of frailty. Finally, more research is needed to identify profiles of immunosenescence- and inflammaging-related changes in the context of other known hallmarks of aging.

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CHAPTER 23

Geroscience: a unifying view on aging as a risk factor

Cristina Mas-Bargues, Aurora Román-Domínguez, Consuelo Borrás and José Viña

Freshage Research Group, Department of Physiology, Faculty of Medicine, University of Valencia, CIBERFES-ISCIII, INCLIVA, Valencia, Spain

23.1 Centenarians: a growing population

We live in a world where "100 is the new 80." Centenarians are becoming more and more common, and the list of countries with people over 100 years is rising. While a population growth of 1% is the norm, the number of centenarians is increasing at an exceptionally rapid rate of about 8% per year.[1] This means that centenarians are the fastest growing age group and research estimates that by 2050, there will be 3.5 million centenarians across the world (Fig. 23.1).

According to the Population Division of the United Nations (UNDP), the top locations to age to a century are Japan, the Caribbean (Puerto Rico, Cuba, Jamaica, and Dominican Republic), Europe (Italy, France, and Spain) and South America (Uruguay and Chile) among others.[2]

Although trends differ between countries, populations of nearly all such countries are aging as a result of low fertility, low immigration, and long lives. Life expectancy improvements over the past 165 years were not propelled by constant reductions in mortality at all ages.[3] In fact, improvements in infant and childhood survival contributed most to the increase in record life expectancies until the 1920s. After successfully overcoming infectious diseases at young ages, gains in record life expectancy were triggered by progress at older ages. This reduction in old-age mortality was unprecedented and unexpected.[4,5] Since the 1950s, and especially since the 1970s, mortality over 90 has continued to fall, in some countries even at an accelerating pace.[6,7]

23.2 Morbidity compression in centenarians

Life expectancy should no longer be the primary goal of medicine, but the extension of healthspan.[8] Morbidity compression hypothesis states that, in order to achieve extreme aging, centenarians delay or even escape the predicted diseases and comorbidities associated with aging.[4] This hypothesis also sustains the possibility that chronic

Aging
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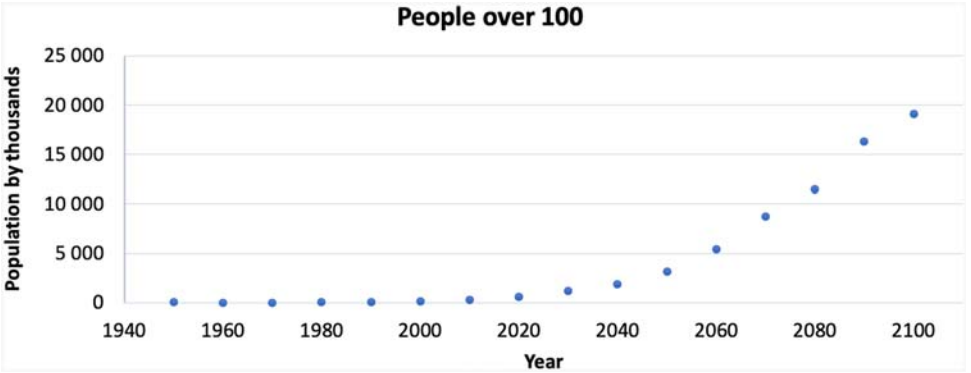

Figure 23.1 Prospect of world population over 100 years old.[2]

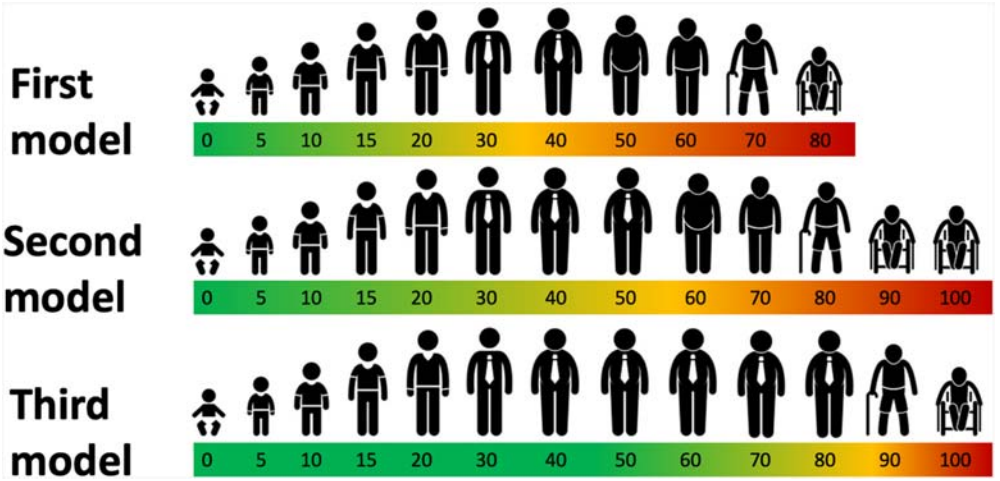

Figure 23.2 Aging models.

morbidity would begin later in life, and that the delay of the onset of morbidity would exceed the increase in life expectancy. Accordingly, there are three models of predicted morbidity that we propose on the basis of extended longevity (Fig. 23.2). In the first model of life extension, the age at initiation of morbidity remains constant and life years gained are accompanied by increased morbidity. In a second model, both the initiation of morbidity and the accumulated life years are shifted to the right, with no gain or loss of morbidity. In the third model, initiation of morbidity is delayed and accompanied by the added years accrued, resulting in the compression of morbidity. For these longer-lived individuals, the duration of chronic diseases and associated disability is lowered, resulting in a reduction in cumulative morbidity.[9]

Moreover, centenarians have also been classified into three categories: survivors, delayers, and escapers, according to the different routes taken to achieve exceptional longevity.[10] In a similar fashion, another point of view states an "aging deceleration" in older individuals, where healthy and unhealthy aging coexist, enabling the establishment of three different age-trajectories: accelerated, normal, and decelerated aging depending on the age they display age-related diseases.[11] In fact, this "aging deceleration" has been reported to be accompanied by improvements in mobility, based on indicators that are geared towards the highest level of physical functioning such as walking or climbing stairs.[3] Large improvements have been documented for Spain,[12] USA,[13] The Netherlands,[14] Finland,[15] and Japan,[16] where daily-living activity disabilities have been falling from 0.4% to 2.7% per year.[17]

Despite the well documented sex differences regarding functional status,[18] common facts in centenarians have also been revealed: resilience, intrinsic capacity, and functional integrity maintenance.[19] Resilience, in essence, refers to the capacity to respond or recover from stressors. Aging is characterized by damage accumulation and a reduced capacity to cope with it. Consequently, in a physiological context, resilience can be defined as the organism's ability to recover from homeostatic alterations.[20] Thus, resilience may differentiate individuals' ongoing, exceptional, and normal aging. Furthermore, the intrinsic capacity or biological reserve of the organism allows for a faster recovery,[21] therefore, a higher intrinsic capacity would result in a more resilient individual.[19] Taken together, we suggest that survival at these high ages mainly depends on maintaining functional integrity rather than on preventing diseases,[22] such as in supercentenarians, where, as first reported by Pearls and his colleagues, health span approximates lifespan.[23]

23.3 Limits of human longevity

How long can we live? The current record, set by Jeanne Calmet, stands at 122 years and has held for over 20 years now. It is likely that this record will be broken soon or have we reached the upper limit of human lifespan? Studying human lifespan is a tricky task, not only due to the fact that the process of aging is a multifactorial process, but because it takes several decades to study longevity in long-lived species like us and its study relies on observational data. This is the reason why there has been much debate regarding the length of human lifespan. Even now, there is still no consensus on the matter among experts.

Several authors claim that there is evidence of a longevity plateau around 115–120 years that could be explained by a natural limit human lifespan.[24,25] Dong et al. analyzed maximal reported age at death (MRAD) and found that the probability of exceeding 125 years is less than 1 in 10,000, suggesting that the duration of human life is limited.[26] However, this work has been criticized.[27–30] Similar results were shown by Modig et al. who claimed that maximum lifespan is unlikely to increase,

analyzing data from cohorts of Swedish and Danish centenarians born from 1870 to 1901.[31] Thus, the chances of breaking Calment's longevity record are far-off.[32]

The fact that we reached a plateau of lifespan in this precise moment of human history does not guarantee that this stage will remain as it is in the future. Furthermore, similar phases of no increase have been recorded, followed by an increase in lifespan.[33] Some authors are still confident that a limit to human lifespan does exist but it is not yet in sight.[34,35] In fact, there is evidence that the MRAD will increase in the long-term.[36] Other authors reject the previous hypothesis and remain unsure about the existence of an "unbreakable ceiling" of human maximal lifespan.[33,37] An analysis of different interventions performed in multiple species to increase lifespan performed by Ben-Haim et al. showed that once the main cause of death has been defeated, an increase in both median and maximum lifespan is observed.[38]

23.4 Exceptional aging "must-haves"

Centenarians exist throughout the world and they differ in education level, socioeconomic status, religion, ethnicity, and diet, that is none of these traits have been significantly correlated with the ability to survive to an extreme old age. However, centenarians still share several keys to success (see Fig. 23.3).

23.4.1 Low-grade inflammation

The immune system deals with chronic exposure to antigens, leading to an overstimulation over time. This process is called "inflammaging."[39] Indeed, the phenotypic characteristics of old and very old people support the concept that aging is a remodeling process whereby the immune system progressively adapts to decades of exposure to internal and external damaging agents. Within this perspective, healthy aging and longevity are likely the result not only of a lower propensity to initiate inflammatory

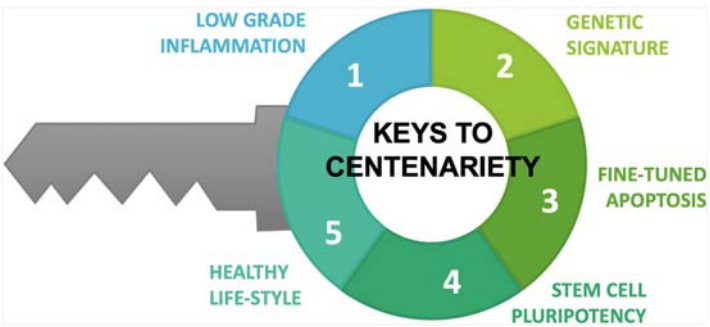

Figure 23.3 Keys to centenariety. 1. Low grade inflammation,[39–45] 2. Genetic signature,[46–49] 3. Fine-tuned apoptosis,[50–53] 4. Stem cell pluripotency,[54–57] 5. Healthy lifestyle.[58–65] The numbers refer to the publications supporting these statements.

responses, but also of efficient anti-inflammatory networks, which in normal aging, fail to fully neutralize the inflammatory processes, as a consequence of the lifelong antigenic burden and exposure to damaging agents.[40]

In centenarians, the number of circulating CD^{34+} cells in peripheral blood are about half that found in young individuals. Interestingly, when CD^{34+} cells from centenarians were put in culture and exposed to optimal concentrations of hemopoietic cytokines and growth factors, their response was indistinguishable (number and size of colonies) from that of young subjects, demonstrating that hemopoietic potential is preserved, but the hemopoietic cytokine network (milieu) undergoes a complex remodeling with age.[41] In centenarians, this chronic pro-inflammatory state of aging is counteracted by an increased expression of anti-inflammatory cytokines such as high levels of interleukin-6 (IL-6), fibrinogen, and coagulation factors.[42,43,66] While both inflammation and anti-inflammation are important for survival, centenarians have achieved a peculiar balance between the two. Centenarians are unique in that, despite high levels of pro-inflammatory markers, they also exhibit anti-inflammatory markers that may delay disease onset.[67] In fact, they are remarkably free of most age-related diseases that have an inflammatory component.[44] Thus, it is important for successful aging and longevity to decrease chronic inflammation without compromising an acute response when exposed to pathogens.[45]

23.4.2 Genetic signature

There is a substantial distinction to be made between the genetics of aging and the genetics of exceptional longevity. Lifespan experiments in lower organisms as well as in mammals, together with molecular genetic studies of centenarian sibships, suggest that genetic factors play an important role in exceptional longevity.[46]

Candidate gene variations can be determined in centenarians and controls using a hypothesis-driven approach. Since small non-coding RNAs (including microRNAs) are implicated in the regulation of gene expression, we previously hypothesized that longevity of centenarians may reflect alterations in small non-coding RNA expression.[47] Our previous analysis of miRNA microarray data revealed that miRNA expression pattern of centenarians overlapped young people's pattern but was significantly different from the septuagenarians' pattern. Results from this laboratory showed that centenarians overexpressed seven characteristic small noncoding RNAs, of which four (scaRNA-7, miR-130a, miR-21 and miR-494) are known to be involved in a wide range of lifespan-enhancing gene modulation. Among them are telomerase overexpression in Cajal bodies, neuroprotection in ischemia, cardio protection, and inhibition of mitochondrial damage and apoptosis.[68–71]

A few years later, we performed transcriptomic analysis of centenarians, septuagenarians, and young people's blood, and found 1721 mRNA differently expressed in

centenarians vs. the other two groups.[48] Further classification of these mRNAs into biological processes gave us an idea of the major processes that may be involved in exceptional aging. Some of these processes are immune response, cell signaling, damage repair, stress resistance, and chromosome structure and function. Experiments performed in yeasts, nematode worms (*Caenorhabditis elegans*), fruit flies (*Drosophila melanogaster*) and mice suggest that genes modulating the aforementioned processes can exert a powerful influence on life expectancy.[49]

23.4.3 Fine-tuned apoptosis

The following sub-network analysis let us converge on six genes: interferon (IFN)-γ (IFNG); T-cell receptor; tumor necrosis factor (TNF); SP1 transcription factor (SP1); transforming growth factor (TGF)-β1 (TGFβ1); and the cytokine IL-32. All of them are related to three apoptosis-related genes: Bcl-xL, Fas and Fas ligand (FasL).[48] The former group inhibits the intrinsic pathway to apoptosis, whereas the latter ones are mainly involved in the control of the extrinsic pathway.

The intrinsic apoptosis pathway, which involves conserved signaling proteins, is physically associated with mitochondria, and is sensitive to mitochondrial oxidative stress. The pathway is tightly regulated by the Bcl-2 family of proteins which act as pro- or anti-apoptotic regulatory proteins. Bcl-xL is an anti-apoptotic protein that downregulates apoptosis and promotes cell survival by migrating to the mitochondrial outer membrane, counteracting mitochondrial permeabilization and the subsequent cytochrome c release. Furthermore, Bcl-xL also plays an important role in mitochondrial bioenergetics by modulating mitochondrial fusion and fission, increasing total mitochondrial biomass, and enhancing the efficiency of ATP synthesis by decreasing a proton leak within the F1FO ATPase. This results in an improvement of cellular metabolism and prevention of oxidative stress.[50] Supporting these facts, it has been demonstrated that peripheral blood mononuclear cells (PBMCs) obtained from centenarians are resistant to apoptosis induced by 2-deoxy-D-ribose, an agent that interferes with cell redox status and mitochondrial membrane potential, thanks to Bcl-xL regulation.[51]

The extrinsic (receptor mediated) apoptotic pathway is induced by death receptors activated by their ligands. The binding of Fas ligand to Fas receptor results in the binding of the adapter protein FADD which then associates with procaspase-8 via dimerization of the death effector domain. At this point, a death-inducing signaling complex is formed, resulting in the auto-catalytic activation of procaspase-8. In the immune system, extrinsic apoptosis is required for lymphocyte development and homeostasis. The main forms of Fas are the membrane (mFas) and the soluble (sFas) forms, generated by alternative splicing of the primary transcript. A series of experiments revealed that lymphocytes from centenarians are able to balance the production

of proapoptotic (mFas and FasL) and antiapoptotic (sFas) molecules, whose proportions are likely to be crucial for the well-preserved immune functionality at the extreme limits of human life.[52,53]

The general picture that emerges is that centenarians have a very fined tuned control of apoptosis, upregulating extrinsic apoptosis in order to eliminate damaged cells by environmental insults and, downregulating intrinsic apoptosis thus sparing cells that have not been exposed to genotoxic substances or other challenges.[48]

23.4.4 Stem cell pluripotency

Stem cell exhaustion is one of the hallmarks of aging, which is when stem cells are no longer able to replenish tissues, and to sustain tissue function due to a progressive decline in their regenerative potential.[72] Yamanaka and coworkers demonstrated that the introduction of OCT3/4, SOX2, KLF4, and c-MYC (OSKM) could convert adult cells into induced pluripotent stem cells (iPSCs).[73,74] These cells have the capacity to self-renew and to give rise to every cell type of the adult body.

Centenarians show an outstanding ability to maintain homeostasis along with a fast recovery. Interestingly, Bcl-xL, which is overexpressed in centenarians, has also been reported to be able to enhance the efficiency of OSKM-mediated iPSCs generation from adult donor PBMCs.[75] In fact, we verified that PBMCs obtained from centenarians' samples displayed higher pluripotency-related gene expression when compared with young individuals and septuagenarians.[54]

Accordingly, fresh fibroblasts obtained from centenarian donors who were extremely healthy until an advanced aged were successfully converted into iPSCs and then differentiated into neuronal cells. These results suggest that centenarians represent a valid super-control for use in studies of late-onset diseases, as well as in longevity research.[55] In a similar fashion, iPSCs generated from both senescent and centenarians' cells have been shown to reset telomere size, gene expression profiles, oxidative stress, and mitochondrial metabolism.[56]

One step further, these centenarian derived-iPSCs are able to re-differentiate into fully rejuvenated cells.[56] Recently, the same achievement has been reproduced with supercentenarian derived-iPSCs.[57] Taken together, the ability of centenarian cells to maintain pluripotency might explain disease resistance and could be a valuable tool to uncover the underlying mechanisms of extreme longevity.

23.4.5 Healthy lifestyle

A healthy lifestyle including increased exercise and reduction in food intake and obesity, can help to maintain health span. Genetic considerations are, no doubt important,[54] but a healthy life style is also of utmost importance. In fact, countries, or areas, who have a high proportion of centenarians are the ones with healthier lifestyles, that

is Okinawa, Sardinia, etc. Factors such as diet, education and physical activity throughout postnatal life have a cumulative effect on mortality,[59] and conditions during early life and parental health also have a large influence.[76]

Heritability estimates of longevity suggest that about one-third of the phenotypic variation associated with the trait is attributable to genetic factors, and the rest is influenced by epigenetic and environmental factors. As such, twin studies have revealed that lifespan genetics is around 25% heritable,[77] suggesting that 75% is from modifiable environmental factors. These factors include smoking, physical inactivity, and high alcohol intake, which, according to large multi-cohort studies in high-income countries, imply 4.8, 2.4 and 0.5 years lost respectively.[78] Sedentary behavior is especially common among older people, who spend, on average, almost 10 waking hours in an immobile posture.[60] In fact, lower levels of physical capability in the fifties and inability to perform capability tests are associated with higher rates of mortality. Even at this relatively young age, these measures identify groups of people who are less likely than others to achieve a long and healthy life.[59]

Calorie restriction (CR), a nutritional intervention of reduced energy intake but with adequate nutrition, has been shown to increase health span and lifespan in rodent models. The studies performed in rhesus monkeys only revealed increased health span together with major improvements in health in food-restricted animals, with reduced incidence of plasma triglycerides, diabetes, cardiovascular disease, sarcopenia, and brain atrophy, which are the most relevant health parameters in aging humans.[61,62] Regarding humans, accumulated data from observational and randomized clinical trials, indicates that CR in humans results in some of the same metabolic and molecular adaptations that have been shown to improve health, and stunt the accumulation of molecular damage in animal models. In particular, moderate CR in humans ameliorates multiple metabolic and hormonal factors that are implicated in the pathogenesis of type 2 diabetes, cardiovascular diseases, and cancer, the leading causes of morbidity, disability and mortality.[63] Similarly, intermittent fasting in humans improves health and counteracts disease processes through activation of adaptive cellular stress-response signaling pathways that enhance mitochondrial health, DNA repair, and autophagy.[64]

Finally, lifestyle, personality and mental status also play important roles in successful longevity. 285 centenarians from the Georgia Centenarian Study who had volunteered, traveled, given public talks, and balanced their checkbooks, were more likely to show relatively high mental status scores (i.e., MMSE $>$ 17). Participants with high levels of emotional stability, extraversion, openness, and conscientiousness, and with high levels of an engaged lifestyle, were more likely to show relatively high mental status scores, whereas participants with low levels of emotional stability, extraversion, openness, agreeableness, conscientiousness, and with low levels of an engaged lifestyle, were more likely to show relatively low mental status scores (i.e., MMSE $<$ 18).[65]

Taken together, regular physical activity, a well-balanced diet, an engaged lifestyle, and personality traits seem to be the lessons we should learn from our centenarians.

23.5 Exceptional homeostasis in exceptional aging

Homeostasis is defined as a self-regulating process by which a living organism can maintain internal stability while adjusting to changing external conditions. Homeostasis is not static and unvarying; it is a dynamic process that can change internal conditions as required to survive external challenges. Homeostatic exceptional regulation produces both a finer level of control and a greater flexibility that enables the organism to adapt to changing environmental conditions. The health and vitality of the organism can be said to be the end result of homeostatic regulation of the internal environment. Conversely, it follows that disruption of homeostatic mechanisms is what leads to disease.[79]

Aging and longevity are controlled by a multiplicity of molecular and cellular signaling events that interface with environmental factors to maintain cellular homeostasis. Modulation of these pathways to extend lifespan, identified the cellular machineries and networks of protein homeostasis (proteostasis), and stress resistance pathways as critical players in the aging process. Because practically all cellular functions are performed by proteins, the proper balance and integrity of the proteome is of primary importance. To maintain protein homeostasis, cells must ensure that all protein species fold and assemble efficiently during synthesis, and preserve their functionality in a wide range of environmental and metabolic conditions. Proteostasis is maintained by a complex network of factors including molecular chaperones, and their regulators, as well as the machineries of proteolytic degradation (the ubiquitin–proteasome system and autophagy). A decline of proteostasis capacity during aging leads to dysfunction of specific cell types and tissues, rendering the organism susceptible to a range of chronic diseases,[80] therefore, proteostasis maintenance might ensure healthy aging.

Stress resistance pathways refers to adaptive homeostasis, that is "the transient expansion or contraction of the homeostatic range for any given physiological parameter in response to exposure to sub-toxic, non-damaging, signaling molecules or events, or the removal or cessation of such molecules or events."[81] Adaptive homeostasis enables biological systems to make continuous short-term adjustments for optimal functioning despite ever-changing internal and external environments. Initiation of adaptation in response to an appropriate signal allows organisms to successfully cope with much greater, normally toxic, stresses (term usually called hormesis). These short-term responses can be initiated by oxidative stress, exercise-induced adaptation, caloric restriction, osmotic stress, mechanical stress, immune response and even emotional stress. A dysregulation in adaptive homeostasis unfortunately appears to manifest with aging, especially in the last third of the lifespan,[82] while successful maintenance of adaptive homeostasis results in extended longevity.[83]

23.6 Centenarians beyond 120?

The aim of prolonging lifespan for as much as possible, provided it is with an acceptable health span, has been a desire of humankind for centuries. The most remarkable increase in lifespan in recorded history occurred in the 20th century. At the beginning of that century, the life expectancy of western countries was around 40 years but at the end of the century it rose to 83 years for men and 85 for women. When one deals with the maximum lifespan, there seems to be a limit that can be placed between 115 and 120.[26] All scientific evidence at the time of writing these words, indicates that this seems to be the maximum lifespan we can achieve. The same can be said of, for instance, laboratory animals like mice that have not been shown to live over three years. Invertebrates like *D. melanogaster* have a maximum lifespan of around 60–70 days. So far, no molecular or lifestyle intervention has been shown to increase the life of a mouse to, say, ten years or that of a *Drosophila* to one year, and the same can be said of humans.

Sir Karl Popper, in The Logic of Scientific Discovery, showed that from the purely logical viewpoint it is impossible to foresee when a scientific revolution will take place. A clear example is what happened in physics towards the end of the 19th century. Lord Kelvin famously stated that physics was almost finished and that the majority of the understanding of nature from the physical viewpoint was almost known. Then, in only five years, the work of two geniuses changed the panorama completely. The first was the Quantum Theory proposed by Max Planck in 1900 and then the Relativity Theory formulated by Albert Einstein in 1905 and the whole picture of physics changed.

The ideas of generating long-living individuals have burgeoned (see for instance the front page of Time Magazine in February 22, 2015 stating literally "This baby could live to be 142 years old"). This is not likely to occur. However, if a scientific revolution in the sense similar to the one that happened in physics 120 years ago takes place and it must be emphasized, we cannot foresee when that will happen, then our life expectancy might be extended to hitherto unforeseen limits. But for the moment, we must accept that the limit of maximum lifespan is around 120 and just devise lifestyles and attitudes towards life to make it a long and especially a happy one.

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SECTION 4

The future and innovation in aging

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CHAPTER 24

Aging support with socially assistive robots

Jorge Dias[1,2] **and João Sequeira**[3]
[1]Institute of Systems and Robotics, University of Coimbra, Coimbra, Portugal
[2]Center for Autonomous Robotic Systems, Khalifa University, Abu Dhabi, UAE
[3]Institute of Systems and Robotics, IST—University of Lisbon, Lisbon, Portugal

24.1 Introduction

The aging society is growing in numbers and the majority in the comfort of their own homes. However, this does not mean they can withstand aging symptoms and the gradual degradation of functional abilities. To overcome these challenges, innovative technological solutions are emerging to sustain elderly into remaining in their homes for longer period of time, with independence, autonomy, and quality of life, while enabling better and personalized care. Within this scope, we present a Bayesian User Model to infer user attributes from data available in a community of distributed social robotic systems to support elderly people. In recent decades, there have been very significant demographic changes in developed countries, reflecting a worrying trend of the aging populations.[1,2] This phenomenon has a direct impact on age composition.[1] As populations age, the triangular population pyramid of 2002 will be replaced with a more cylinder-like structure in 2025 (see Fig. 24.1.). The consequences are clear, an increasing older population means an increasing demand for care, but a diminishing ratio of an active population as well.

Against this backdrop, there is an urgent need to create solutions that help mitigate the side effects of aging and to innovate care. Policies have been created to promote the concept of Active Aging.[2] Newer generations of elderly people in developed countries are embodying this concept, craving to maintain their own autonomy for longer, a positive factor towards

[1] Aging Composition is, the proportionate numbers of children, young adults, middle- aged adults and older adults in any given country – which is an important element for policy-makers to take into account. Population aging refers to a decline in the proportion of children and young people and an increase in the proportion of people age 60 and over.

[2] Active Aging applies to both individuals and population groups. It allows people to realize their potential for physical, social, and mental well being throughout the life course and to participate in society according to their needs, desires and capacities, while providing them with adequate protection, security and care when they require assistance

Aging
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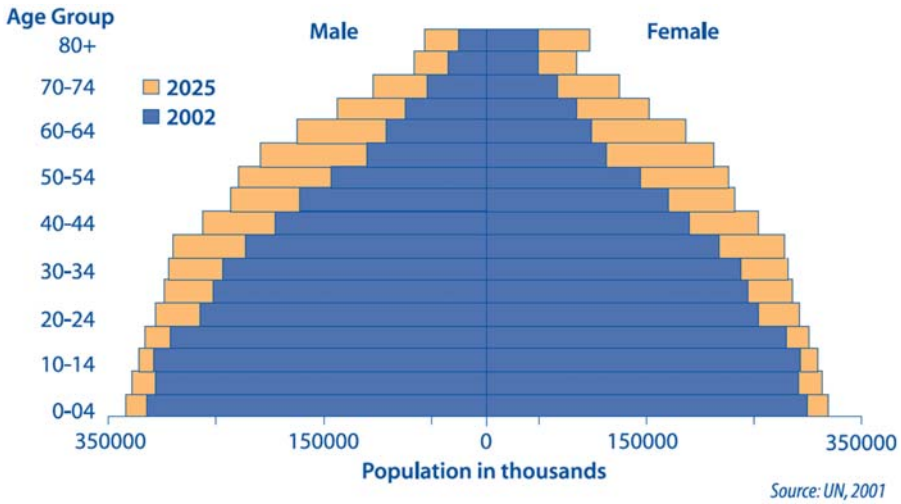

Figure 24.1 Global population pyramid in 2002 and 2025 (data from WHO, 2002)[2]: The population in thousands divided per age ranges in two different epochs. As the proportion of children and young people declines and the proportion of people age 60 and over increases, the triangular population pyramid of 2002 will be replaced with a more cylinder-like structure in 2025.

the sustainability of care, as it promotes a reduction in care consumption. Nonetheless, enabling people to grow old in the comfort of their homes,[3] comes with a challenge too.

Loneliness is a recurring condition. Despite the fact we are living in an age where connectivity is thriving, a recent study[4] shows that we are lonelier than ever before. No other age group feels this problem more than the elderly.[3] Beyond the mental effects of the insufficiency in personal relationships, loneliness reflects also in one's health. In fact, the same study[4] shows the existence of a greater risk of cognitive and physical decline (Fig. 24.2.), which is often manifested in alterations of functional attributes, specifically noticeable in elderly's abilities to perform activities of daily living. This means that loneliness has the potential to be a catalyst of elderly decline and, consequently, increase the need for assistance from a family caregiver or other source of long-term care. Solutions supporting the concept of active aging should aim to support elderly autonomy, independence and quality of life, whereas the path to innovation should find a way to bridge the gap between these traits and the decline of functional attributes.

[3] Even when a senior is being taken care of by family caregivers, T. Byram Karasu, MD, from the department of psychiatry and behavioral sciences at the Albert Einstein College of Medicine, says that there is often little attention paid to deep, engaging communication between a senior and the rest of the family. The changes listed above are factors, but caregivers are usually so worn out from juggling their day-to-day responsibilities that they have little time or energy left for truly meeting a senior's emotional and social needs.

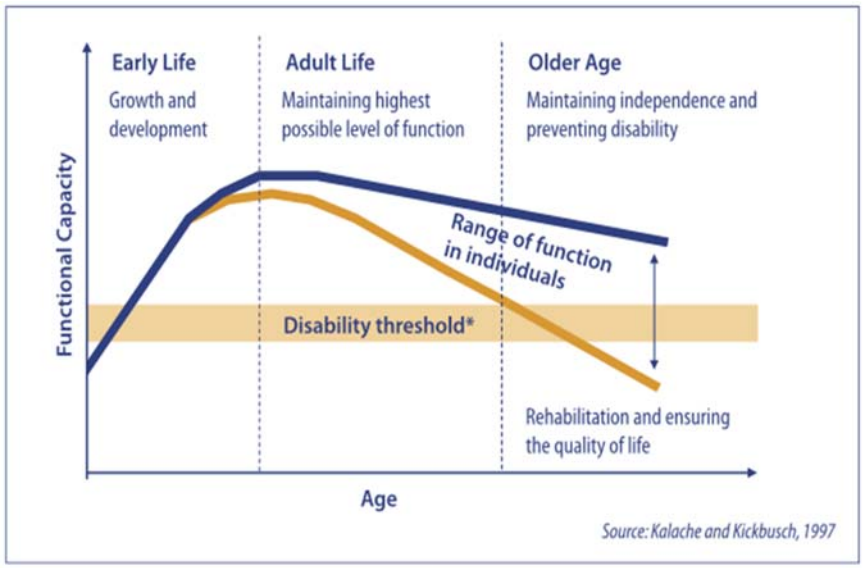

Figure 24.2 Maintaining functional capacity over the life course (from WHO, 2002)[1]: The graph shows the typical functional decline with age (yellow) and the expected functional decline if an elderly person is properly rehabilitated (blue).

24.1.1 Social robotics tools for demanding societies

Robots have shown to challenge the dichotomy between living beings/artificial objects (at least from an epistemic point of view): people tend to recognize the robots as intentional agents even if they are not living beings. Therefore, one of the effects of robots on human beings is triggering humans' natural tendency to attribute intentional states to artificial objects.[5,6]

Human biology and related psychological aspects are thus being acknowledged to have an active role in the acceptance of these new artificial beings. Moreover, novel usages for these novel tools are also dependent on how well humans know themselves.

24.1.2 Where are we in social robotics?

The field of social robotics is currently experiencing exponential growth and development worldwide, where significant progress is being made; there are numerous projects in this field and various applications in different social domains. Some of the robots proposed in the literature have a dual purpose, targeting different audiences (elderly and children). Most, if not all, share common goals, namely, wellbeing, moving robots outside labs, multimodal HRI, long-term HRI, understanding social environments, etc.

A short list of European projects involving social robots includes: (1) MOnarCH, edutainment for inpatient children in an oncological hospital, (2) GrowMeUp, personal assistant robots using cloud computing and machine learning techniques, aiming at establishing positive long-term relationships (see Figure 4), (3) Aliz-E, artificial intelligence for small social robots that interact with children, (4) GIRAFFPlus, a telepresence robot for remote monitoring, (5) CHRIS, safe human-robot interaction within selected application domains, (6) LIREC, building long-term relationships with artificial companions, (7) Cogniron, development of cognitive companion robots, (8) HUMAVIPA, endowing humanoid robots with audiovisual abilities such that they exhibit adequate behavior when dealing with humans, (9) HRIAA, robots with social abilities, personality and emotions, using verbal, non-verbal, and para-verbal communication, (10) STRANDS, long-term deployment of intelligent mobile robots in dynamic human environments, (11) DREAM, for autistic children, (12) CompanionAble, personal assistant for remote monitoring and aid memory services, (13) Alias, personal assistance in domestic and care homes, (14) Accompany, HOBBIT, ROBOT-ERA, RAMCIP, and SocialRobot, personal assistant robots, some with anthropomorphic features, including domestic use applications. In this short list, a bias towards the elderly population can be identified, which may be due to most elders liking robots,[7] and that elderly patients take more liking to a robot that is smaller and with less humanoid features.[8]

Zoomorphic robots have mostly been used to interact with elderly patients and results have shown to be similar to those obtained with domestic animals.[9] Results show an increase in social activity, and less behaviors of aggression, agitation, as well as of depressive symptoms.[10,11] Nutritional intake was also improved and the overall need for medication and medical follow-ups were reduced. At this moment, studies have not yet shown clear effects on patients' cognitive function.

In 2009, the US Food and Drug Administration approved the PARO (see[12,13]) robot as a class 2 medical device, for use with the elderly. This robot is currently being used in multiple countries such as Germany, Denmark, and Japan. Among several other studies, PARO was also used for eleven days at a pediatric hospital in children aged two to fifteen, having shown improvements in the field of communication.[14]

Friedman et al. conducted a study with Sony's AIBO, the first consumer robot of its kind to be offered to the public.[15] They looked into understanding people's relationships with AIBO, by analyzing the spontaneous postings in online AIBO discussion forums. The results showed that AIBO psychologically engaged this group of participants, particularly by drawing conceptions of technological essences, life-like essences, mental states, and social rapport.[16]

Current social robots include several commercial prototypes, some of which were already deployed in real environments. The Chelsea and Westminster hospital has

Figure 24.3 Casual interaction or humanization? (from MOnarCH project,[17])

been using a NAO robot "to assess whether these robots could help combat the social isolation experienced by many inpatients in hospital wards." (see[17]).

The case of SoftBank Robotics' Pepper, using as auxiliary staff/receptionist at Ostende Hospital (see[18]) is an interesting example of technology that is still under development but, nonetheless, is profiting from its public deployment. Pepper has also been "employed" at a maternity ward at a Belgium hospital, as an attempt to improve healthcare and putting a smile on patients' faces.[19] Other applications of this robot include very simple receptionist tasks in commercial environments.

Long term experiments in non-lab environments have been recognized to yield distinctive conclusions on human-robot integration. Quoting[19], p. 9, "... laboratories are not real environments, especially for service robots ...". Experiments in the MOnarCH project support this idea,[20] and often generate data that opens novel perspectives on the role of social robots. Fig. 24.3 illustrates a fully casual interaction that shows the potential of robots to affect the social behaviors of humans.

Behaviors such as those in Fig. 24.3 have been justified as due to the human biological mechanism that regulate empathy towards inanimate objects (see[21,22]). Even if these are simply a side effect due to novelty, it is the kind of human response that shows the potential of social robots for well being.

24.2 Where is social robotics heading?

Human-robot interaction, a key area contributing to Social Robotics, is evolving fast, namely in terms of human-similar sensing and processing techniques. Even though the progress in sensing, how this information is used is still improving, namely in what concerns the estimation of more complex concepts from social sciences, that is heading towards a better understanding of the human condition and social dynamics is a key step to create socially-skilled robots.

Human profiling, personality traits estimation, and emotion recognition, are topics long studied in social sciences and are undergoing a rediscovery by the engineering sciences. At the core of this rediscovery is a conjecture that artificial entities with social skills similar to humans are easier to integrate (i.e., having humans and social robots forming collaborative intelligence collectives) in normal human societies.

In the personality field, multiple theories are available from social sciences.[23–25] Among the most suitable for computational implementations (following[26]), the "big-five" model,[27], identifies five personality traits that are often considered to accommodate the fundamental features of personality.[28]

Artificial neural networks are being used extensively to identify personality traits. LSTMs and CNNs are used[29] to estimate "big-five" traits from text. Empirically designed CNNs are used[30] for personality detection ("big-five" model) from text data. Similarly,[31] estimates of "big-five" traits from text using several CNN-based architectures[32] reported 95%–99% accuracy for the "big-five" traits, from online text data. It is used as a cascade of ANNs to estimate "big-five" traits from static facial images.[33]

This variety extends to emotions, with extensive research (and commercial, off-the-shelf, packages available, see for instance the Noldus products), namely since the six basis emotions paradigm, from facial expressions, was presented.[34] Using static facial expressions under favorable lighting conditions,[35] recognition rates were reported up to 96%. Extended emotion sets have been used,[36] with 26 emotions and a CNN-based architecture. Motion cues, such as arms, head, and torso movements, can provide relevant info on emotions.[37] Similarly, sound cues may also contribute significantly. Accuracy was reported over 80% when fusing facial and prosody data.[38]

Personality has been referred to as patterns of thought, emotion, and behavior,[39] and a combination of behavioral features and emotions.[40] Recognizing emotions and their contribution to behavioral patterns thus enables the assignment of synthetic personality to robots, making them closer to humans and simplifying the establishment of collaborative intelligence.

Furthermore, connections between personality and people profiling, commonly known as persona,[41] can be established. These are used for decision-making purposes in multiple areas, for example, consumer trends analysis, and may also be used to adjust robot behaviors. Knowing humans and how they react to robots is thus a current trend

in social robotics. Long-term experiments provide valuable lessons.[42,43] Complex interactions can be obtained even if a robot has only basic, scripted, decision-making skills, for example, as provided by finite state machines carefully tuned to induce the adequate perceptions in people, for example, the perception of liveness.[43]

The method and model presented in this article is modular and allows for refining of the existing attribute models by integrating new knowledge based on their information value. Unitary attributes are clustered into a unified user representation, revealing a generalization of different group profiles. The system modular and computational properties allow for the implementation of multiple architectures, such as a team of robots connected to each other, or connecting robots with an external sensor network. In this article we show how to design a set of realistic use-case scenarios with the purpose of demonstrating the network of social robots to support elderly people: (1) perform online learning; (2) analyze and cluster groups of users; and (3) be resilient to faults in system operation. Experiments are run using experimental datasets, obtained from synthetic data and with users operating in real scenarios. Results show convergence to a reference model, while fusing new data asynchronously and independently. Estimation errors tend to zero over time, exhibiting high accuracy when estimating user attributes. The technique shows that is feasible to design social robots with user-adaptive interfaces and distributed inference.

24.2.1 Social robots for aging societies

The research community has been actively pursuing innovative technological solutions that can sustain the elderly to remain longer in their homes (see Fig. 24.4). Within an uprising solution pool,[44–46] robots have a particular appeal. They are mobile, they have artificial intelligence capabilities,[44–47] they often embody a sense of presence,[45–48] and they are able to engage in human-like interactions.[49,50] It won't be long before robots are a solution as popular and innovative as washing machines were early in the 20th century. Behaviors such as the one displayed in Fig. 24.3 are good indicators that people are willing to accept robots and are eager to interact with them.

At present, networked (Cloud) Robotics, the Internet of Things, and Big Data are highly popular keywords. Nonetheless they represent active research areas, and robots are certainly part of them. Robots can connect to clouds in order to deploy and retrieve information.[52,53] Robots can connect to different types of sensors, for example, to capture data from the environment,[54] domotics, a person,[55] wearable, and even connect to other robots.[56] Robots have the ability to sustain a 24/7 operation in elderly homes, presenting an invaluable opportunity because of all the information they can gather and how it can be explored.

The information value is not confined to the robot's boundaries. Imagine a robotic inclusive society where a network of robots operating in elderly homes, harvests

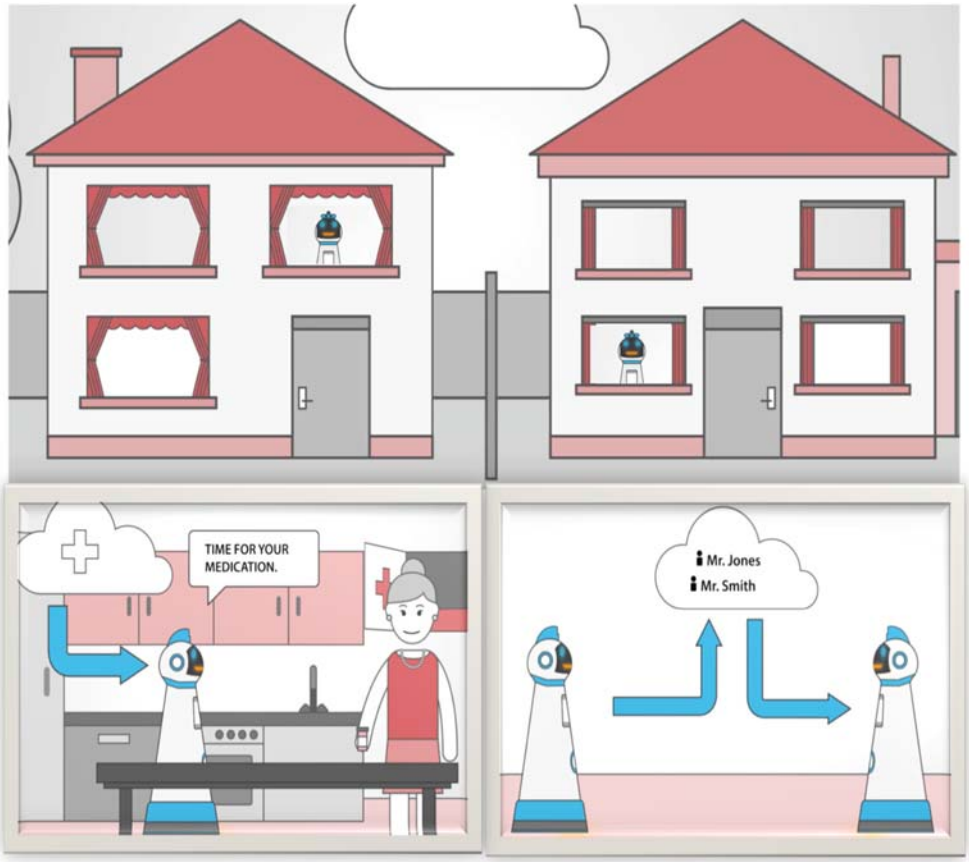

Figure 24.4 The concept of Networked Robots for the Aging Society (from GrowMeUp, 2018[51]): Robots will coexist with people in their homes, be able to know their users and support them in their daily lives, as well as exchanging information with them.

information that could be used by policymakers or care providers to obtain a real-time diagnosis of a given elderly population, identifying preventive care caveats, trending societal challenges and so forth. However, from the robot's perspective, the information could be used to personalize their service provision to an elderly person,[57] to support the diagnosis of functional decline of an older person[58] or use this knowledge to perform tasks that compensate for a given impairment.[59] It is possible that a robot may become to know an elderly better than the elderly knows himself, and therefore be able to actively contribute to his/her autonomy, independence and quality of life.

The information emerges mainly from the elderly and its interaction with a robot and/or the environment in its daily life. However, "a life course perspective on aging recognizes that older people are not one homogeneous group, and that individual diversity tends to increase with age".[2] This research presents an instantiation of the

underlying human uncertainty and diversity. The purpose of the proposed framework is to enable robots to create internal user representations from heterogeneous, distributed and asynchronous data streams. This representation, commonly dubbed user-model, also enables profiling large groups of persons considering their particular attributes. To achieve this goal, we propose to use Bayesian Inference[4].[60,61]

As robots move from factories into homes, the study and optimization of Human-Robot Interaction (HRI) becomes an increasingly important factor. Indeed, while factory technicians are willing to adapt to the characteristics of the robotic equipment they must use, domestic users must accept and adopt these technologies of their own volition. The main issue in ensuring success in domestic settings becomes, ensuring that the users are interested and satisfied by the devices they own.

The issue of technological acceptance has been thoroughly studied in the context of Human-Computer Interaction (HCI). HCI studies the issues that arise from the interaction between a computerized system, such as a computer or smart device, and a human, using their limited interaction modalities: keyboards, touchscreens, occasional voice in-put, etc. In this context, it has long been established that user-adaptive interfaces lead to significantly improved acceptance when compared to non-adaptive ones.[17] Robots, on the other hand, can use any natural communication channel employed by their users, resulting in much higher potential for user-adapted behavior. Thus, it becomes interesting to study the phenomenon of user-adaptivity in the context of HRI.

User-adaptive systems have been shown to be easier to accept by end-users than non-adaptive ones. Thus, in the context of a growing domestic robot industry, it becomes of key importance to study the scientific and technological impact of the employment of these techniques on robotic systems. This article uses user-adaptive techniques on robotic systems with uncertainty, based on data analysis of human-robot interactions. {{{Fig. 24.5}}}

24.2.2 User behaviors under uncertainty—the bayesian user model

Mobile robots can be seen as a platform with a sensor suite. Consider a network of distributed robots, composed of an arbitrary number of sensors, producing different types of data $e_m \in \mathbf{E}$. Let a user be represented by $c_n \in \mathbf{C}$, which stands for any quantifiable or qualifiable user attribute ranging from simple user preferences,[63] for example, input language, to mobility patterns[64] or psychological attributes[65] for example, personality traits.

[4] It belongs to the category of generative algorithms, having unique abilities to deal with the complexity of multimodal state conditionals. They also permit the exploration of the Independent and Identically Distributed (IID) property towards an easy integration of observation variables of different types and an embedded learning scheme - updating models from the recursive computation of new estimates.

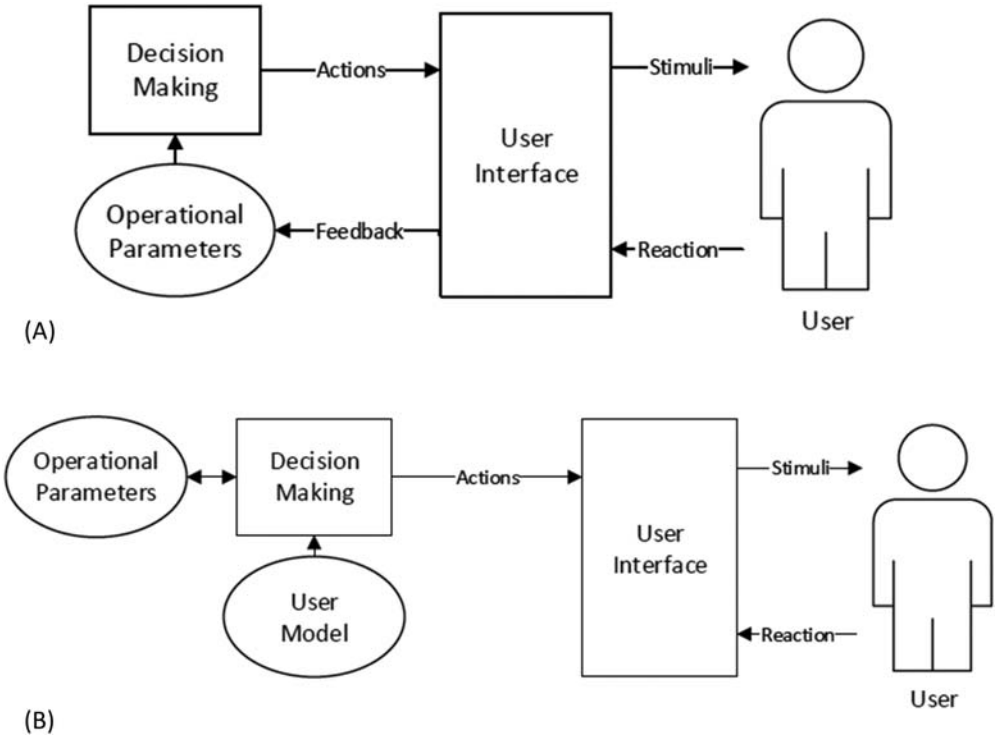

Figure 24.5 (A) General architecture of user-adaptive system. An illustration of the general architecture of an adaptive system that does not rely on pre-defined knowledge of the user. The user's feedback is directly employed in changing the behavior of the system.[62] (B) Model for User-Adaptiveness. The figure gives an illustration of the general architecture of an adaptive system that relies on a model for user-adaptiveness.[62]

The goal is to infer over the user attributes c_n from the asynchronous observation of distributed data streams e_m. Given the uncertain nature of this perception problem, the proposed model explores variable dependencies using conditional probabilities under Bayesian propositions. Therefore, the posterior distribution $P(\mathbf{C}|e_1 \cdots e_m, I_d)$ declares the variable dependency inferring the attribute c_n given the observed data streams e_m and the user identity I_d. Applying the chain rule to the posterior, considering $e_m \in \mathbf{E}$ as independent and identically distributed (IID) variables, we obtain the following decomposition.

$$P(\mathbf{C}|\mathbf{E}, I_d) \propto P(C) \prod_{E_i \in \mathbf{E}} P(E_i, I_d|C)$$

Using a specific variable I_d to identify each user, enables the encoding of attributes of an entire population of users in a convenient format. In fact, projecting all distributions along I_d axis, allows a generalized overview of each attribute with respect to each evidence variable.

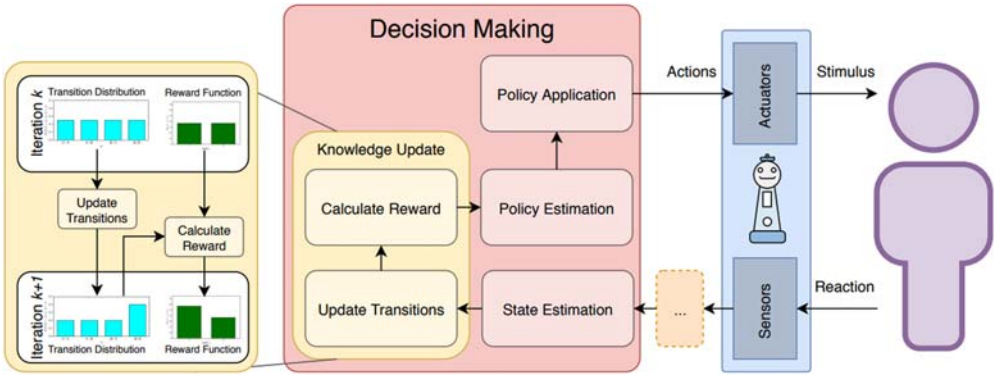

Figure 24.6 ***A more detailed view of the user profiling and human-robot interface.*** The transition and reward functions can be re-calculated each time new information is obtained by the system, resulting in a new policy, which is better suited to the user.[66]

Because all attributes are (IID), and thus computed independently, the system architecture is modular, composed of an attribute module, classifying each c_n. During an interaction with a single user, as in Fig. 24.6, the system is able to infer multiple attributes. By concatenating attributes in a vector, we obtain a unified representation, defined as in the following equation.

$$U_{l=k} = [\hat{c}_1, \hat{c}_2, \cdots, \hat{c}_n], \quad \text{where} \quad \hat{c}_i = \text{argmax}_{c_n \in \mathbf{C}} \quad P(\mathrm{c}_n | e_1 \cdots e_m)$$

Each vector represents a sample in the so-called unified attribute space. An ensemble of vectors $U_{l=k}$ represents the probability density of the user's attribute vectors that the system can infer at one point. To estimate group profiles (inter-user attribute patterns), we apply an Expectation-Maximization algorithm[67] to fit a Gaussian Mixture to the attributes' space representation, resulting in an unsupervised step in the technique.

The definition of the attribute space has two purposes by design. On one end, it has the potential to be used as information enabling a robotic system to adapt its decision-making.[68] On the other end, if one or more attribute modules fail, absent attributes can be derived from the probability density distribution given a known user profile. At any point a user, of which n attributes are known, can be matched to the existing profiles by minimizing a distance metric defined such as:

$$d_i(\mathbf{C}, \Sigma) = \sqrt{\Sigma_{C_j \in \mathbf{U}_{\text{inc}}} \left(C_j - \Sigma_j\right)^2}$$

24.2.3 Online knowledge integration using learning

The elderly population shows dynamics[2] that raise the challenge of updating the attribute representation over time. Classification learning in Bayesian approaches relies on

supervision learning,[69] that is the training samples are required to be labeled. In our framework, we propose a mechanism using Soft Labels,[70] however it is also possible to integrate online supervised learning, that is learning by actively asking users to provide labels during the interaction, called active learning.[71]

The estimation process described in the previous subsection runs asynchronously every time the system receives new evidence. The estimation step gives a new attribute estimate for the posterior distribution $P(C_n|E, I_d)$. The proposed learning mechanism relies on three key elements:

1. A soft label $L_i = \text{argmax}_x \quad P(C_i|E, Id)$;
2. The posterior entropy $h_i = H(P(C_i)) \sim H(P(C_n|E, I_d))$ where H is the entropy function, as defined in[72] having the purpose to weight against uncertainty.
3. The evidence vector E that was used to generate L_i.

These three elements generate the tuple $T_i = (L_i, E, h_i)$. By continuously integrating and fusing local estimates, the system is able to reach a precise attribute model of the population. Each device in the system becomes a weak estimator in an ensemble, feeding local knowledge encoded into weak labels, feeds into a fusion mechanism. User attribute models are encoded as a likelihood of the form $P(E, I_d|C)$. These distributions are iteratively updated as a Gaussian kernel by performing:

$$P(\boldsymbol{E}, I_d|C_i = L_i)_{k+1} = \frac{1}{\Psi}\left(P(E, I_d|C_i = L_i)_k + D\right)$$

where and Ψ is a normalization factor; D is a learning factor function of T_i, such that $\mathrm{D} = P(\boldsymbol{E}, I_d|C_c = L_i)_{\text{observed}} = \mathcal{N}(\mathrm{M}, \Sigma)$, where M is defined accordingly to the evidence received and Σ is a covariance matrix where each diagonal element is defined by entropy $\Sigma_{i,j} = F(h_i)$. Thus, at each update of the likelihood function, the integration of new knowledge into the system allows it to continuously learn as described in Fig. 24.7.

24.3 Results with a team of robots

Consider a network of robots, deployed in different locations. Each location is characterized by having a population of users sharing similar attributes. Let each robot include a number of sensors, generating different types of data to be analyzed. Robots can also connect to external sensors generating other types of different data. The goal is that the robot gets to know the different users that interact with it by creating an internal representation of them. Also, it should be able to associate and autonomously create profiles characterizing groups of persons that share similar attributes, known as population profiles. In order to achieve the proposed use-case, from a cognitive perspective, the robots need to:

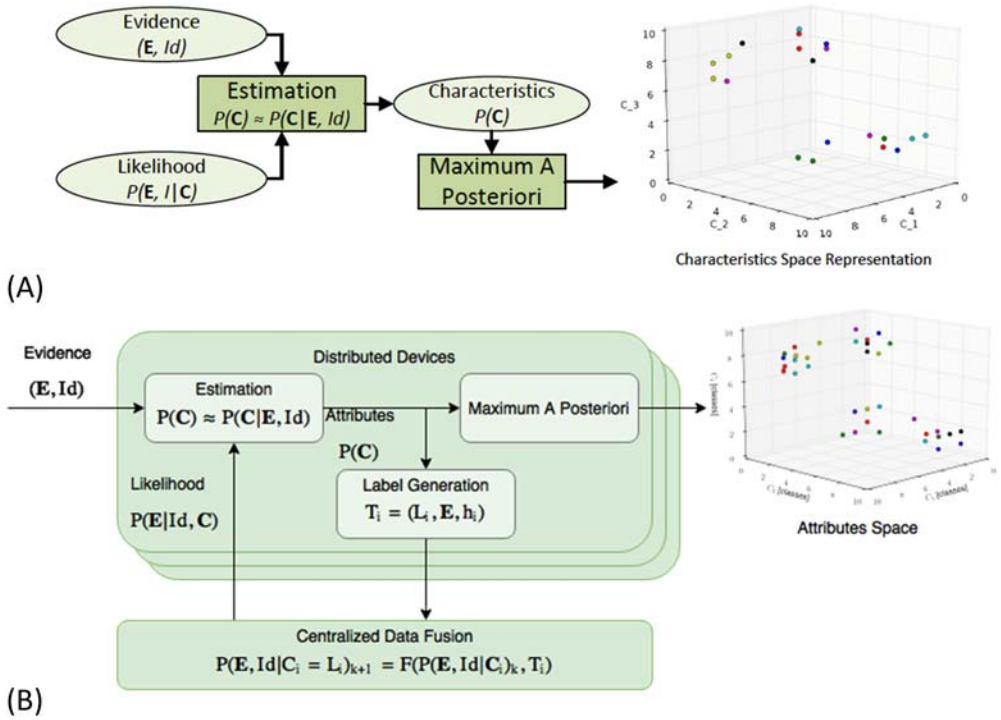

Figure 24.7 Knowledge integration workflow: (A) A block diagram with a single iteration of the knowledge integration workflow. (B) Evidence is captured by sensors and feeds into the estimation step. The estimation generates soft labels and provides the input for computing the unified attribute vector, using a maximum a posteriori. The generated tuple is brought to the data fusion mechanism, which updates the attribute likelihoods and feeds them back to the distributed systems.

- Generate a precise internal representation of the user's attributes from distributed, asynchronous data.
- Collectively cluster, and subsequently identify users from distinguishable profiles.

Given the unfeasible long-term, large-scale study to demonstrate the full range of the proposed research, our main experimental setup is done using a simulator to mimic the use case conditions. For the current use-case, assume that we have a network of 100 robots. Robots include a set of 3 sensors that generate different types of data. Robots connect to two additional types of external sensors, originating a sensor compound per robot of, $E_m = 5$ sensors. Sensor data is simulated through the generation of random numbers $e \epsilon \mathcal{N}$ confined to the interval [0;3] (for computational simplicity). Consider the existence of $C_{(n=3)}$ recognizable attributes, with each attribute varying between 10 mutually exclusive states ($C_n$with $n = \{0, 1, 2, \ldots, 9\}$).

24.4 User's attributes from distributed, asynchronous data

For each experimental trial, we continuously feed random observations to the network. Results that follow correspond to the average results from running 100 trials. Figures only show a maximum of 3000 samples for readability and because after 3000, samples results did not present significant differences.

The total error over time (Fig. 24.8) is obtained as $= \hat{U}_i - U_i$, where $\hat{U}_i$, and U_k are the expected attribute vector and the actually classified attribute vector. Results in Fig. 24.8A show that the error tends to decay to zero after roughly 10^3, iterations, meaning the precision tends to 100% of correctly classified samples. The irregular behavior observed in Fig. 24.8B occurred because attribute models were forced to output uniform probability distributions (maximum entropy) randomly for short periods of time. However, the general error-decaying trend still stands, which shows that the system is resilient to the failure of attribute classifier modules.

In Fig. 24.9A the quadratic error over time is an indicator of when the attribute model (likelihood) converges into a stable distribution over time. We found that after roughly $k = 100$ iterations, the distribution differences over time start to decay to zero, demonstrating attribute model convergence. The same behavior is observed for the fault operation conditions in Fig. 24.9 (b) as attribute model failure generates maximum entropy, and thus the error is not integrated in the existing likelihood distribution. Contextualizing both Fig. 24.8 and Fig. 24.9, it is clearly visible that, after the attribute model converges at $k = 100$, this fact is coincident with the decay of the attribute classification error. We conclude that the system converges into a precise attribute model.

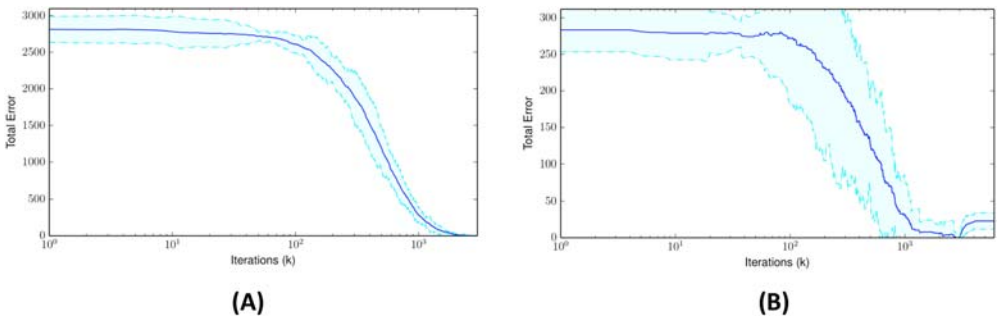

Figure 24.8 Attribute classification error over time: The total error ε evolution over time. (A) Results with all attribute modules working properly. (B) Results when errors injected in attribute module operation, meaning that they were randomly failing for short periods of time. The dark blue line shows the mean error over 100 trials. The light blue represents the corresponding standard deviation.

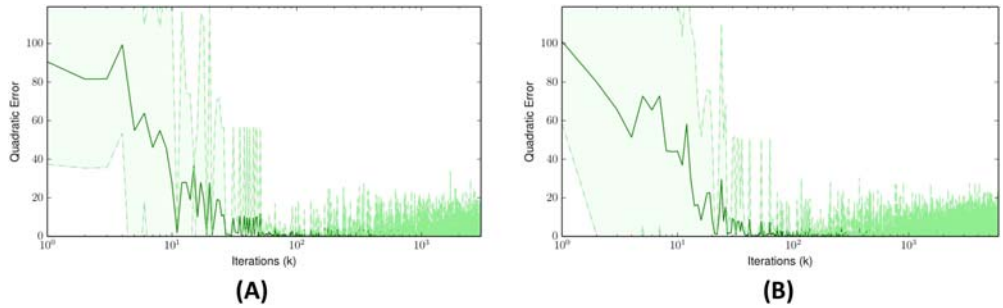

Figure 24.9 Quadratic error of attribute model over time: The quadratic error *Q* evolution over time. (A) Results with all attribute modules working properly. (B) Results when errors injected in attribute module operation, meaning that they were randomly failing for short periods of time. The dark green line shows the mean error over 100 trials. The light green represents the corresponding standard deviation.

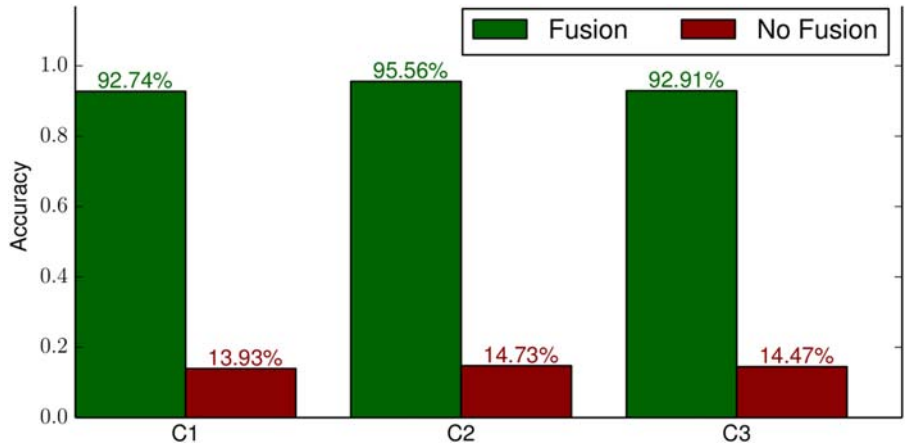

Figure 24.10 Precision results for attribute classification: Results for precision for all considered attributes and the global precision, with and without the fusion mechanism activated. These results are for the case where all attribute modules are working.

This fact becomes clearer, when looking at the precision results presented in Fig. 24.10. The models show an improvement in classification precision of five times, when comparing results with and without the fusion mechanism activated. When the fusion mechanism is not active, the same attribute models are used throughout the trial. Precision results show that the system is able to continuously learn based on data annotated from its own belief states, while using entropy as a measure against uncertainty.

24.5 Collectively cluster users into distinguishable profiles

At each iteration, the system produces a unified attribute vector $\mathbf{U}_k$, one per different user, generating a coordinate in attribute space. Because results from a previous subsection shows the model exhibits the target performance under 3000 iterations, for these tests the system is running over the same time span, showing the evolution of each of the 100 users in the unified attribute space during that period. These vectors are clustered using an Expectation Maximization approach for three different profiles.

The figures present the reference model, at the evolution at different iteration steps. Fig. 24.11 shows that the 100 users were known to belong to three different profile categories, which acts as the reference profiles. Initially, the robot does not have knowledge about any user; it is only able to classify attributes with low confidence (as seen in Fig. 24.10). As time advances and as the attribute models are refined, the robots become familiar with the users, enabling them to capture the subtleties of their attributes. Therefore, upon convergence, the robot is able to profile them accurately, as seen in the next Fig. 24.12.

Fig. 24.12A and B corroborate the analysis from Fig. 24.11 with an analysis of the KLD[73] measurement that measures the difference between two probability distributions $p(x)$ and $q(x)$. By the 1000th iteration, the KLD starts tending to zero, meaning that the obtained clusters have converged to the reference. This shows an ability of the proposed framework to group different users into profiles that share similar attributes. In Fig. 24.12B, we simulated a failure on half of the modules around $k = 1500$, taking the system under 100 iterations for the system to converge. Because some attributes are no longer classifiable, the error stabilization occurs with a bigger KLD when compared to the case when all attribute modules are operational.

24.6 Discussion

Having humans and AI-powered robots collaborating has already been recognized to require people "to do new and different things" and "to do things differently." Extending this notion to social robotics requires nudging people not to fear robots, which is likely to be a lengthy process. However, slow integration of robots may yield novel perspectives on the related ethical issues.

In summary, we have demonstrated that our system has the ability to create precise representations of people through probabilistic models of their attributes. Despite numerous approaches existing that encode user information, literature shows they rely almost solely on pre-defined profile templates. Our framework allows robots to update their internal user representations by exploring a soft-labeling methodology. This enables the robot to automatically annotate observations based on its own internal beliefs, using entropy as a measurement of confidence against data uncertainty. Results

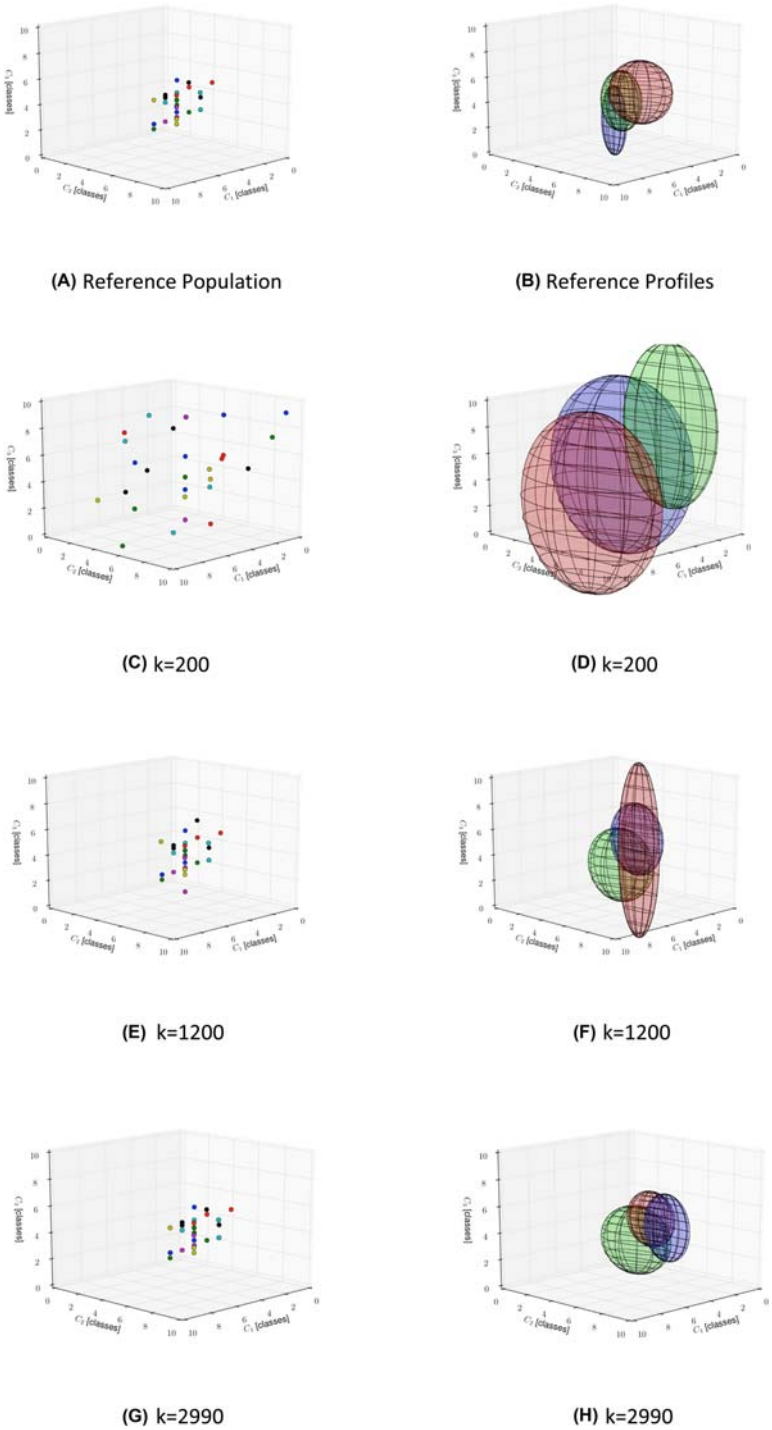

Figure 24.11 Profile clusters generated during one trial: (A,C,E,G) the populations of vectors. (B,D,F,H) the generated profile clusters after the application of the expectation-maximization algorithm.[62,66]

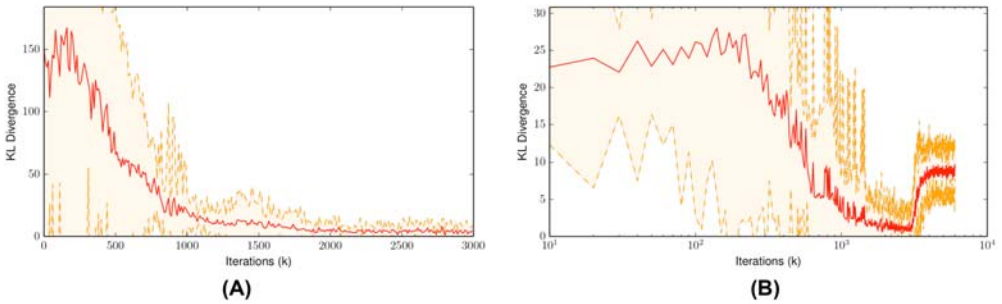

Figure 24.12 Kullback–Leibler Divergence (KLD) of each profile cluster to the reference profile: The graph shows the KL distance[73] measuring the distance of the generated profiles against a reference profile, over time. (A) Results with all attribute modules working properly. (B) Results when errors injected in attribute module operation, meaning that they were randomly failing for short periods of time.

show that the system has the ability to recurrently update the user model, maintaining an accurate attribute classification over time. The system already showed direct impact when applied to a multi-objective adaptive social robotic system.[74] Although we have focused on social robots, the framework is clearly applicable with other technologies, if we consider that a robot is nothing but a platform with a sensor suite. In fact, the properties of the Bayesian formulation allow extending or transferring the BUM to many other applications that can explore data from different types of sensory data like driver profiles,[75] gaming,[76] or sports analytics.[77] The system is also able to create profiles (inter-user relations) by means of a unified attribute space. Beyond the advantages elicited in the results section, this domain transposition enables the study of populations by identifying attribute dominances, similarities, or any type of statistical analysis that one may see fit.

As for any learning system, its precision depends on the available data and the training procedure. The system acts as a semi-supervised machine, which becomes fully autonomous once the seed attribute models are defined and the system starts its operation. This may pose one critical contradiction, if the initial attribute model is completely misleading. It means that the system's initial beliefs will be false positives, leading the model to converge on wrong assumptions. Therefore, one has to ensure a reasonable assumption for the initial attribute model specification, including a correct choice of the probabilistic representation. One other caveat of the proposed formulation may be found on the Expectation-Maximization step, where the reliability of results is tightly correlated with the number of components of the Gaussian Mixture Model. Our results are shown with a fairly accurate choice of the number of distinct population profiles, as we know beforehand the number of different users. If this is not the case, an educated guess or a number of trials will be required to fine-tune a selection of user profiles.

We believe social robots and the Bayesian User Model framework allows for novel adaptive systems that aim to capitalize on comprehensive knowledge of their users, providing means for rapidly adapting to new contexts and users at a moment's notice. Moreover, we believe it has the potential to create a novel framework for the elderly population by profiling and diagnosis of behaviors that can improve and augment the social support with robotic technologies.

Acknowledgments

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CHAPTER 25

Machine learning in the context of better healthcare in aging

Jorge Henriques[1], Caio Ribeiro[2], Teresa Rocha[3,1], Simão Paredes[3,1], Paulo de Carvalho[1], Ana I. Duarte[4,5,6], Alex A. Freitas[2], Teresa Cunha-Oliveira[4] and Francisco B. Pereira[3,1]

[1]CISUC, Centre for Informatics and Systems of University of Coimbra, DEI, Coimbra, Portugal
[2]School of Computing, University of Kent, Canterbury, United Kingdom
[3]Polytechnic of Coimbra, Coimbra Institute of Engineering, Coimbra, Portugal
[4]CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[5]Institute for Interdisciplinary Research (IIIUC), University of Coimbra, Coimbra, Portugal
[6]CIBB - Center for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

25.1 Introduction

Aging is a universal, natural biological process associated with the passage of time that initiates after the organism has attained its maximum reproductive capacity and it impacts health and fitness, both being genetically determined and environmentally modulated.[1] With aging and its related progressive accumulation of cellular and molecular damage, the organism suffers progressive physiological changes that lead to senescence, decline of biological functions, and reduced capacity to adapt to metabolic stress, increasing the risk for disease and ultimately death.[2,3] Indeed, the progressive metabolic dysfunction (namely the downregulation of glucose metabolism and mitochondrial energy production) has long been recognized as a prominent feature of aging that affects nearly all individuals,[4–6] and ultimately constitutes the primary risk factor for cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases,[7–12] among other major human pathologies, and increases the risk for functioning loss, disability, frailty, and imminent death.[13] To further aggravate this scenario, as people age they are more prone to undergo simultaneous comorbidities.[2] This renders human aging a double-edged sword, by representing one of the major socioeconomic achievements and privileges of the last century, but also as a major social, economic, and medical challenge.[2] The World Health Organization (WHO) estimated that 1 billion people were aged 60 and older in 2019, a number that will rise to 1.4 and 2.1 billion by 2030 and 2050, respectively.[2]

Different body functions can change at different rates over time, and the rate of biological aging varies among different individuals,[14] resulting in a low correlation between chronological age and functional loss or tissue deterioration. To further

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complicate this issue, it is well known that besides genetic factors, the (healthy) aging process relies also on environmental factors, gender, ethnicity, and socioeconomic status, all of them exerting their silent influence since the early stages of aging.[2] Knowledge of the specific rate of biological aging would allow for the prediction of a person's longevity and risk for disease, to determine the need for intervention and give insights into how the consequences of aging can be counteracted using drugs, diet, or exercise. This could be implemented based on reliable indicators associated with healthy aging, which are called biomarkers and need extensive validation. The American Federation for Aging Research defined a set of criteria for biomarkers of aging, in order for them to serve as reliable indicators of biological age. Briefly, a biomarker: (1) should predict a person's rate of aging relative to their total life span independently of (and better than) chronological age, (2) must report a physiological process underlying aging and not the effects of disease, (3) must be able to be tested repeatedly and reproducibly without harming the person, and (4) should work both in laboratory animals and in humans to allow for pre-clinical research.[15] Validation of these biomarkers would allow for testing intervention strategies without the need to follow each patient throughout an extended period of their life. Nine tentative hallmarks of aging were proposed, including genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient-sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.[11]

Phenotypic and molecular biomarkers that have been used to study the health of older populations include clinical measures associated with the cardiovascular system, metabolic processes, inflammation, immunity, and infection, the central nervous system, activity of the hypothalamic pituitary axis, sympathetic nervous system, organ function, oxidative stress, antioxidant defenses and genetic markers.[16] More recently, molecular predictors of biological age were proposed, including epigenetic clocks, telomere length, transcriptomic, proteomic, metabolomics-based, and composite biomarker predictors.[17] All of these biomarkers should be properly explored in order to extract meaningful knowledge that may contribute to properly addressing the aging issues. In this context, machine learning (ML) approaches might be very effective to deal with the complexity and nonlinearity of this data.

25.2 Machine learning overview

Massive amounts of age-related biological data exist nowadays. One remarkable example of publicly available data is the Human Aging Genomic Resources, a collection of databases with information for the study of the biology and the genetics of aging.[18] Given the amount, complexity and variability of biological processes, assessing the

predictive power of biomarkers of biological aging or aging-related diseases is challenging. Additionally, the available information increases at an extremely fast rate.

In this scenario, it becomes mandatory to rely on sophisticated and robust computational tools to process and analyze the data. ML comprises a suite of statistical methods and tools that are able to deal with a large amount of heterogeneous data and unveil relevant hidden patterns.[19] The knowledge extracted from raw data contributes to gain a better understanding regarding the problem being addressed, as well as to create computational models that are able to make accurate predictions for future events.

The point of departure for any ML project is to collect and preprocess a sample of examples that describe/are representative of the situation under study. Information in this dataset can have many different types. The most common examples are numerical values (e.g., gene expression data or biomarkers), text (e.g., genome sequencing), images, sound or video, although several other possibilities exist.

The most common ML approaches are supervised and unsupervised learning, even though other variants can be considered. The distinction is related to the information provided by the examples that comprise the dataset. Learning is supervised if the dataset instances are labeled, for example, if a specific gene expression result is identified as belonging to a given disease or if an X-ray image is marked with a diagnosis. Conversely, unsupervised learning methods are applied when the dataset only contains unlabeled raw data.

ML algorithms are supplied with the dataset instances and autonomously extract useful knowledge, by unveiling relevant hidden patterns that may exist in the data, inferring relationships between features/attributes describing the examples or creating predictive models that can be used in future situations.

Unsupervised learning is particularly challenging, as it deals with unlabeled instances. In this case, the major goal of learning is to unveil and understand interactions between the instances of the dataset and the features that describe these examples. Clustering is the most common unsupervised approach, and it aims at organizing instances into different groups. The goal is to have a set of relatively distinct clusters, each one comprising a subset of similar (i.e., homogeneous) examples. Even without a label, the subset division and organization of the data will lead to a better understanding of the problem being addressed. Different strategies for clustering may be applied according to the specific goal of the problem under analysis. Methods can be either hierarchical or non-hierarchical. Moreover, cluster discovery is usually guided by distance, density, or a specific distribution model (e.g., a Gaussian distribution).[20]

In supervised learning, the examples are labeled. This way, the goal of the computational method is to learn a model that relates the independent variables (features or attributes) describing the instances with the corresponding dependent response. Labels can be either discrete/categorical or continuous, which subdivides supervised methods,

respectively, into classification or regression. The process of fitting such a model is called "training" and there are many possible algorithms for this task, ranging from straightforward classical methods, such as linear regression, to modern approaches like decision tree ensembles, support vector machines, or deep neural networks. After training, the predictive accuracy of a model is assessed with new unseen data. It is vital to rely on data unobserved during training, as this will certify the generalization ability of the model.

25.3 A review of machine learning applications for aging research

ML methods are increasingly being applied as relevant data analysis tools to assist aging-related research.[21–23] As referred to previously, the aging process brings a broad decline in organ function that increases the risk for chronic diseases, especially those affecting the immune system (such as decreased immune response and immune memory, and increased chronic inflammation)[24] and the central nervous system (including dementia and its most common form, Alzheimer's disease).[25–27] Numerous studies tried to establish indexes of biological age based on cell numbers, gene or cytokine expression, blood and leukocyte epigenetics, telomere length, or genetic predisposition, that could be correlated with health, disease or even all-cause mortality and might thus constitute reliable biomarkers.[28–36] However, their generalized use in clinics appears to be hampered by the lack of a direct correlation between the biological alteration upon aging (for instance in telomere length, epigenetic clock or immune gene expression)[28,29,33,34] and the specific physiological processes that are affected.

Conversely, others suggested that age-related changes in the immune responses could be more relevant in this context.[24] In this regard, studies demonstrated that the increased levels of the inflammatory C-reactive protein, interleukins-6 and -1ß, and TNFAR1 were correlated with a higher cardiovascular risk and all-cause mortality in older adults.[35,36] Furthermore, aging has been increasingly shown to dramatically affect both adaptive and innate immune mechanisms, including the decrement in the proliferation, function, and migration of immune cells,[37–39] in T-cell receptor diversity,[40] antibody secretion,[41] phagocytic and cytotoxic mechanisms,[42,43] alongside the dysregulation of cytokines and chemokines.[38,44] This is further reinforced by the aging-mediated changes in human antibody production[45] and the consequent effect on humoral immunity.[37,46,47]

Therefore, it is plausible to hypothesize that the increase in immune age may render individuals more prone to autoimmune diseases or even increase the severity of acute inflammatory disorders, thus pointing towards a possible cross link between aging and autoimmune diseases.[24] In this perspective, the immune age may constitute a highly relevant biomarker of immune function in health and disease, that is a person

with an accelerated immune age (or an older immune than chronological age) might be at a higher risk for autoimmune, autoinflammatory, and acute disease. [24]

Accordingly, by using the antibody binding profile from individuals, these authors established a ML model that provides a high correlation between the immune and the chronological ages.[24] Specifically, they found that aging was correlated with a strong increment in the number of N-terminal di-serine motifs and the subsequent binding of antibodies, which may in turn stimulate auto-antibody production and the autoimmune processes that often occur upon aging.[24] The authors emphasize that the N-terminal di-serine motifs may be relevant in aging studies; however, this parameter may only partially predict the chronological age and, therefore, may not be sufficient to constitute an age-related predictive biomarker.[24]

Hence, besides its relevant impact on health and disease onset/progression, such an immune age index could also be useful to evaluate the therapeutic efficacy of anti-senescent/-immune disease interventions,[38,48–50] and to establish preventive strategies that slowdown the immune aging of individuals (such as physical activity or vitamin E supplementation)[51], culminating in longer and healthier lives.[24]

In the next sections of this chapter, we present two novel approaches of the application of ML methods in the area of aging research. The first contribution was developed under the myHeart project and describes a telemonitoring system aiming at creating personalized, interpretable and dynamic models for cardiovascular risk assessment of acute events, namely death and re-hospitalization.

The second contribution presents an ongoing project dealing with data from the English Longitudinal Study of Ageing. The study aims at applying supervised learning algorithms to the collected data to accurately predict ten age-related diseases.

25.4 Telemonitoring data mining for hearth failure management

The employment of information and communication technologies, and personal health systems (pHealth), might be particularly important. The patient is at the center of the health delivery process and, through remote monitoring and management applications, the continuity of care at all levels of healthcare delivery can be a reality. In particular, this scenario introduced a great interest in telemonitoring solutions, enabling remote monitoring by means of nonintrusive sensors, for continuous access to multiple data sources, including physiological signals, providing professionals with a global and reliable view of the patient's condition.

Based on the telemonitoring system developed under the myHeart project, this section reports part of the algorithms and respective achieved performances using biosignals gathered with the MyHeart wearable sensor device (vest). Two main applications are addressed: (1) Diagnosis (classification) of heart failure arrhythmias, and (2) Prognosis (prediction) of heart failure decompensation. The first was implemented to

run in a home-based environment, where electrocardiogram (ECG) signals are acquired. The second uses the historical information of the patients, mainly consisting of daily measurements, such as blood pressure, weight and heart rate. Regarding the latter, prediction models are currently being improved and validated with new data, in the context of the lookAfterRisk project, which combines the development of risk prediction models with home-mobile technologies, to improve the management of myocardial infarction patients. The scientific goal is the use of computational intelligence methodologies for the development of personalized, interpretable and dynamic models in the cardiovascular risk assessment of acute events, namely death and re-hospitalization.

25.4.1 Heart failure condition

Heart failure (HF), also known as congestive heart failure, is a cardiovascular condition in which the heart cannot pump enough oxygenated blood to meet the needs of the body's organs, occurring because the heart muscle is damaged or overworked. Initially, symptoms may only occur when a patient is exercising, however, over time, breathing problems and other symptoms may occur even at rest.[52]

Heart failure has become a major public health concern, and the cause of considerable morbidity and mortality. [53] In fact, HF is a growing epidemic for which, despite clinical advances, mortality rates continue to be high. The progression of HF creates recurrent hospitalizations (acute decompensation events) and, even though the symptoms are reduced, the patient's cardiac function continually deteriorates,[54] as illustrated in Fig. 25.1.

Although the development of new therapeutic approaches can prolong life and shorten hospital stays,[55] these patients will require re-hospitalization and have, unfortunately, a poor prognosis. Approximately 20%–30% of the patients are readmitted after 30 days, and nearly 50% are readmitted before 6 months. In Europe, more than 8 million people are affected, and with the advancing age and longer life span, these numbers are likely to increase drastically.[56]

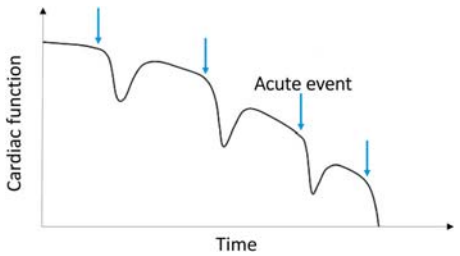

Figure 25.1 Contribution of acute events to the progression of heart failure (HF). With each admission of acute HF, there is a short-term improvement, however, the patient leaves the hospital with a further decrease in cardiac function.

Prevention offers the opportunity to systematically fight the origin of cardiovascular diseases, enabling clinical professionals to play a proactive role in daily care, by implementing more effective and personalized therapies. By means of telemonitoring systems, it is possible to assess the patient's HF status and the earlier recognition of hemodynamic deterioration that would not be feasible in common clinical practice. Theoretically, the adjustment of treatments and actions, prior to the development of symptoms or decompensation, can be potentially applied, thus preventing hospitalizations and therefore contributing to the reduction of healthcare costs.

25.4.1.1 myHeart study

The telemonitoring myHeart trial was a clinical observational study carried out with 148 patients from six clinical centers in Germany and Spain. The trial had an enrollment phase of 9 months, with 12 months of patient follow-up. These patients were in NYHA class II (slight limitation of physical activity, comfortable at rest), predominantly male (70%) and over 60 years old (63.8 ± 12).[57]

In the context of HF management, the acquisition of electrocardiogram signal (ECG) is of major importance, providing key information about alterations in the cardiac electrical conduction, to assess rhythm disturbances, and ultimately, the cardiovascular status of the patient. In addition to the ECG signal, other telemonitoring measurements have usually been acknowledged to be relevant for the HF management. These include body weight (BW), blood pressure (BP), heart rate (HR), respiration rate (RR) and fluid contents that are collected periodically, typically on a daily basis.[58]

25.4.2 Algorithms for heart failure management

25.4.2.1 Diagnosis of heart failure arrhythmias

The input consists of the ECG signal, obtained from wearable sensors, being the application divided into three main modules, as illustrated in Fig. 25.2: (1) preprocessing, including QRS detection and ECG segmentation, (2) feature extraction from ECG, (3) classification (diagnosis) of major arrhythmias, and atrial fibrillation (AF), ventricular tachycardia (VT), and premature ventricular contractions (PVC). The accuracy of a classifier is, obviously, highly dependent on the number of classes to be categorized. Clearly, with only two classes, each classifier is able to provide a superior classification result, due to the lower complexity of the problem. This fact justified the design of a distinct classifier (neural network) for each specific task (AF, PVC and VT).

25.4.2.1.1 Preprocessing

The first step comprises several phases: removal of baseline wander, reduction of noise, R peak detection, and main wave's delineation (ECG segmentation). The methods implemented are based on digital wavelet transforms.[59] and multiscale morphological operators.[60] In effect, the cyclical behavior of the ECG signal, together with its spectral

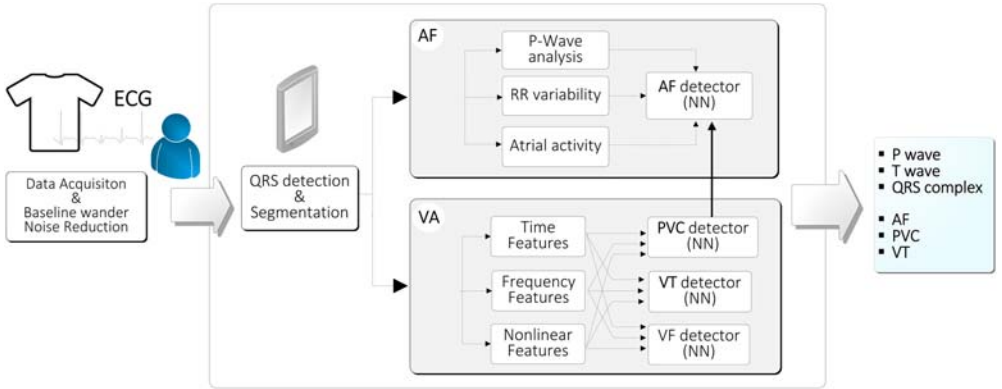

Figure 25.2 Electrocardiogram analysis toolbox, as a component of the myHeart heart failure management system.

components, reflected in the different frequency bands and scales, can be distinguished using multi-resolution decomposition by means of wavelet transforms, or by applying morphological transforms at different scales. As a result, this ECG analysis addressed:

- the baseline wander and noise removal using a wavelet approach[61];
- the R peak detection performed with the algorithm developed by[62];
- the segmentation through the method proposed by Sun,[60] with some adaptations.[61]

25.4.2.1.2 Feature extraction

Atrial fibrillation detection—The proposed strategy makes use of the three principal physiological characteristics of AF: absence of P waves before the QRS-T complex, a sawtooth like pattern along the cardiac cycle corresponding to atrial activity, and the irregularity of the RR intervals. This knowledge-based approach has the advantage of increasing interpretability of the results to the medical community, while improving detection robustness.[63,64]

Ventricular tachycardia detection—The approach assumes that the fundamental differences of normal rhythm and VT can be discriminated via time ECG shape, together with power spectral density analysis.[65–67]

Premature ventricular contraction detection—The proposed algorithm assumes a patient-specific approach. The method uses characteristics of the QRS complex of each beat and compares them with those of neighboring beats of the same patient, as well as compares the QRS morphology of normal and abnormal beats.[68]

25.4.2.1.3 Classification

The proposed classifiers consist of feedforward neural networks with sigmoidal type activation functions. The parameters, that is the weights and the bias, were trained using the Levenberg-Marquardt algorithm.[69]

25.4.2.2 Prognosis of heart failure decompensation

The prediction strategy assumes that trends of physiological data, common to patients with similar disease progression, may have prognostic value in the prediction process. Moreover, it is assumed that hemodynamic changes, able to characterize the occurrence of a given event, can be captured by variations of biosignals' evolution (trends).

The framework behind the prediction approach is illustrated in Fig. 25.3. Given the current telemonitoring data $X(t)$ (measurements daily collected), the prediction of a specific cardiac event is supported on a similarity analysis using the historical dataset.[70,71] The process starts by defining the historic patterns P_i that describe the dynamics of the clinical events to be detected. These patterns can be knowledge-driven, based on clinical evidence, through the definition of specific behaviors (such as trends, offsets and sudden variations). Alternatively, data-driven approaches can be applied in a knowledge extraction procedure: a pattern discovery process by clustering techniques can be employed, grouping data into subclasses that reflects similar patterns (in the present situation, decompensation, or normal condition). In this case, data resulting from the myHeart telemonitoring study[57] was employed. The records are composed of biosignals collected on a daily basis (BP, HR, BW, and RR), before the occurrence of one event, together with its prediction (decompensation heart failure and normal condition).

From the research and methodological perspectives, two major issues should be addressed:[70] (1) how different variations in time series (trends) can be captured, compared and grouped; (2) how these variations can be used in the required prediction (decompensation or normal condition).

25.4.2.2.1 Time series clustering

The former is based on a similarity search scheme between a time series that combines the Haar wavelet decomposition, in which signals are represented as linear combinations

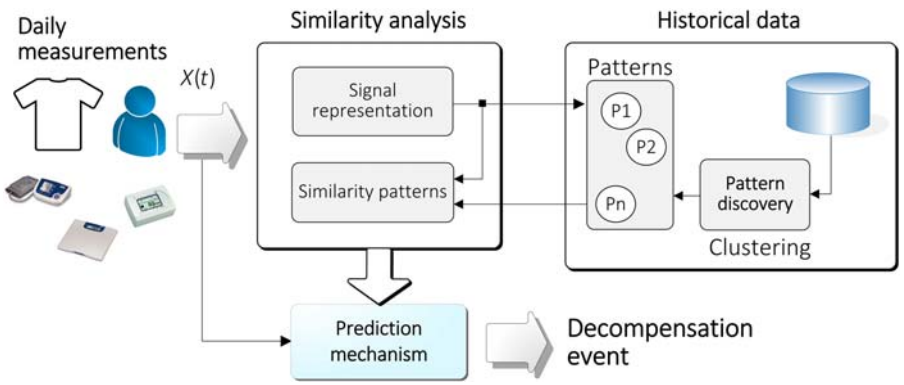

Figure 25.3 Proposed approach for the prediction of heart failure decompensation events.

of a set of orthogonal bases, with the Karhunen-Loève transform, which allows for the optimal reduction of that set of bases. Supported on the localization property of the wavelet basis, and using a reducing operation, the bases that significantly reflect the dynamical patterns of the time series under analysis are chosen. The trend similarity measure is then indirectly calculated by means of the coefficients obtained in time series description by using a distance measure.[72]

25.4.2.2.2 Prediction approach

Essentially, the prediction strategy is inspired by a case-based reasoning principle, where the main hypothesis is that the future evolution of similar conditions that occurred in the past can be used in the prediction of the current condition.[73] The prediction strategy assumes that the new prediction is computed by a majority vote of the M predictions previously identified in the historic dataset. It is important to note that the prediction scheme does not require the computation of an explicit model (such as a regressive one or a neural network classifier). In effect, what is involved is a trend similarity analysis followed by a nearest neighbor procedure: the present case is compared with others available in the historic data, and the most similar cases are used directly in the prediction process.

25.4.3 Experimental results

25.4.3.1 Diagnosis of heart failure arrhythmias

Although algorithms have been run with the collected ECGs, it was not possible to adequately assess their performance (e.g., by means of sensitivity and specificity values) since no ECG annotation was performed by experts.

25.4.3.1.1 Segmentation

The present methodology was employed to segment the ECG, and to determine the RR interval, the QRS width, the PR interval, the PQ interval, and the QT interval. Fig. 25.4 depicts one example of the segmentation parameters obtained from a typical patient along 192 sessions. The parameters presented for each session result from the average of all values acquired during the session (a session is performed for 5 min).

25.4.3.1.2 Premature ventricular contractions and atrial fibrillation episodes

Two illustrative examples relative to PVC detection and AF classification are presented in Fig. 25.5. As can be observed, the algorithms identified several PVC and AF episodes (the maximum number was 3 episodes in a session).

25.4.3.2 Prognosis of heart failure decompensation

The strategy was evaluated using daily measurements acquired during the myHeart telemonitoring study (heart rate, respiration rate, body weight and blood pressure). From

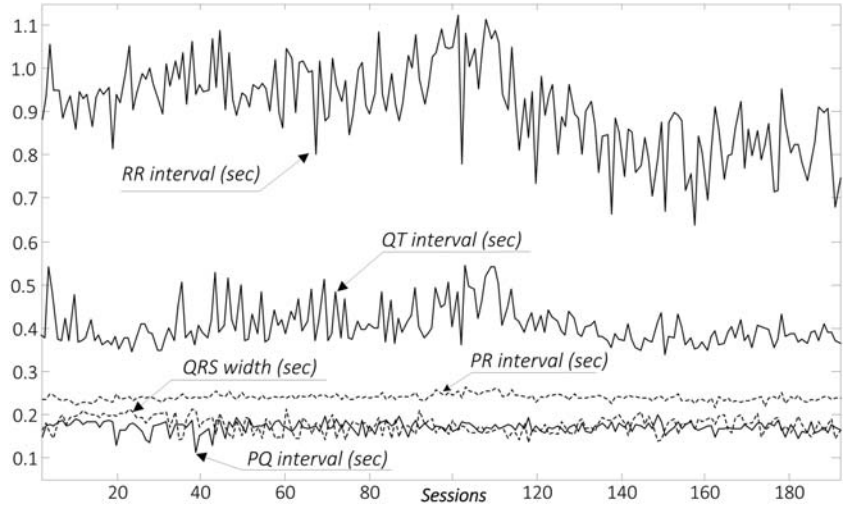

Figure 25.4 Typical electrocardiogram segmentation results (myHeart trial). The parameters presented for each session result from the average of all values acquired during the session (5 min).

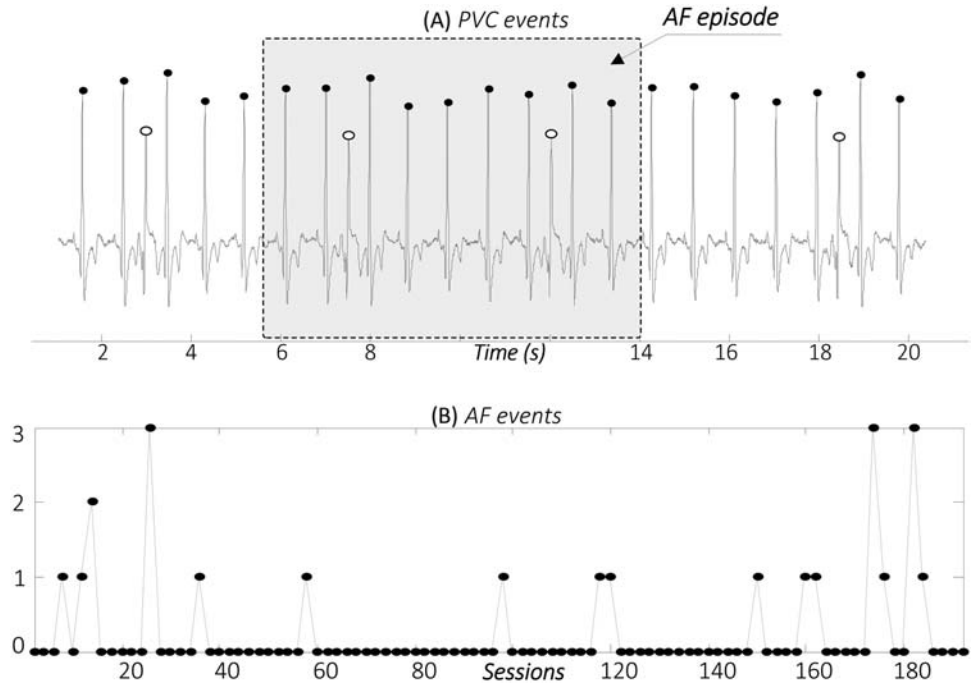

Figure 25.5 Illustrative examples of premature ventricular contractions (PVC) and atrial fibrillation (AF) detection results from myHeart database. (A) PVC detection: symbol (•) denotes a normal electrocardiogram, while symbol (○) denotes a PVC episode; (B) AF detection: The parameters presented for each session result from the average of all values acquired during the session (5 min).

the 148 patients recruited, 41 were considered analyzable, that is, with more than 30 days of telemonitoring measurements (15 decompensation events and 26 normal conditions).

In the prediction scheme, two key parameters had to be specified: (1) N: the duration of the time series (template), that is the number of days considered relevant before the occurrence of an event; (2) M: the number of patterns (or number of neighbors) considered in the prediction mechanism. With respect to the number of days before an event, the best results were obtained with $N = 4$ or $N = 8$, which are perfectly in accordance with clinical literature. With respect to the number of neighbors, the obtained results suggested that lower numbers are preferable ($M = 1$ or $M = 2$).

Regarding the validation assessment, a leave-one-out cross-validation approach was followed, achieving sensibility and sensitivity values of 0.87 and 0.68, respectively.

25.4.4 Discussion

The integrated ECG analysis platform, developed as proof of concept of the myHeart heart failure management product, allows for the detection of the most significant cardiac arrhythmias in HF management, namely AF, PVC, and VT. When applied in a real home telemonitoring environment, a true validation could not be performed through the signals collected by wearable ECG sensors (myHeart database was not annotated), although the achieved results suggest the feasibility of the algorithms.

On the other hand, this chapter assessed the prognostic value of a particular set of daily measurements collected during the myHeart trial, in the prediction of HF acute decompensation events. Although it can be argued that the number of patients and events were limited (41 patients), the obtained results show that the proposed trend similarity detection and prediction scheme may be appropriate for the early detection of heart failure decompensation.

Future directions of work, currently being explored in a collaboration with Centro Hospitalar de Leiria, Portugal, and with the commitment of the Portuguese Society of Cardiology, is the development of dynamic strategies, supported on tele-home monitoring solutions, able to continuously incorporate new information into cardiovascular risk assessment models, to improve the accuracy of such models. These will be applied at hospital admission, in patients with a first episode of acute myocardial infarction and will be continuously updated when the patient returns home.

25.5 Machine learning for the English Longitudinal Study of Ageing

The English Longitudinal Study of Ageing (ELSA) is an ongoing study that collects data from thousands of participants living in the UK every two years, focusing on individuals over 50 years of age. The study collects data from the participants about a range of subject areas, such as economy, health and wellbeing.[74]

In this case study, we used a longitudinal dataset created from the "Nurse-data files" in ELSA, collected when a health professional visits the ELSA participants in their household every two waves of the study (every 4 years, as ELSA waves happen in 2-year intervals). We used data collected from 2004 to 2016 (waves 2 through 8), spanning 12 years of the participants' lives. This ELSA-nurse dataset contains a range of health or biomedical variables, such as blood samples, and results of tests performed by the health professionals, such as mobility exams. We used this data to predict the value of the target (class) variable at wave 8 (i.e., a supervised learning problem).

The dataset created for this study had 7097 instances (ELSA participants) and 45 different variables, out of which 41 had repeated measures (making our dataset longitudinal), which generated in total 140 predictive features (counting each measure of a variable as a separate feature). The 10 classes to be predicted correspond to 10 age-related diseases, with binary values indicating whether or not the individual reported being diagnosed with that disease in the latest wave of the study.[75]

25.5.1 A brief overview of random forest classifiers

The classification problem of supervised ML consists of creating prediction models from training data containing class labels, then using these models to predict the class labels for previously unseen testing data. In this case study, we applied Random forests (RFs), a popular type of classification algorithm.[76]

Random forests are a type of ensemble classification algorithm which combines the predictions of many different decision trees (e.g., via majority voting) to get more accurate and more stable predictions. This requires the trees in the ensemble to not only be reasonably accurate but also diverse, that is they should make different types of prediction errors, so that the majority vote is more likely to be correct.

Random forests use two main strategies to learn diverse decision trees. First, each decision tree in the ensemble is learned from a randomly sampled subset of the training instances. Second, for each node of the decision tree, the algorithm randomly samples a subset of the predictive features to use as candidates for splitting the data, and the best among those candidate features is selected.

Random Forests have performed remarkably well in benchmark experiments[77,78], comparatively to other powerful classification algorithms, and it has the advantages of being simple, fast and retaining some interpretability in its learned models, which is an important advantage in health-related applications.

25.5.2 Preparing the English Longitudinal Study of Ageing-nurse data for the classification task

25.5.2.1 Data-driven missing value replacement

Longitudinal studies often generate datasets with many missing values.[79] That is because, in addition to the usual reasons for a feature's value being missing,

Table 25.1 The missing value replacement (MVR) methods that compose the data-driven MVR approach.

MVR method	Description
Global mean/mode	Mean (or mode, for nominal features) value over all available measurements.
Age-based mean/mode	Mean/mode value over all measurements from individuals with the same age as the respondent with a missing value for that feature.
Previous observation carried forward	If the value for the previous wave's measurement of a feature is known for an individual, carry it over to the missing value in the current wave.
Mean/mode of previous and next observations	If the values of both the previous and the next wave's measurements of a feature are known, calculate their mean/mode and use the result to replace the missing value in the current wave.
K-nearest neighbors	Find the K-nearest instances to the current instance, based on all measurements of the current feature, the age and gender of the respondents, which have a known value for the current feature. Replace the missing value in that feature by the mean/mode of the values of the neighbors' measurements.

respondents may leave an ongoing study or join at a later wave (meaning all the values for such individuals will be missing in the waves they did not participate in the study).

There are several methods for missing value replacement, but no single method is the best choice for all types of features and datasets. Thus, we used a feature-wise data-driven approach to choose the best method from a set of five methods (Table 25.1), representing statistical techniques, ML algorithms, and techniques devised specifically for longitudinal data.

For each feature, first our data-driven MVR approach calculates the average estimation error for each of the five methods in Table 25.1, over the known values of that feature. Next, it orders those methods in increasing order of their estimation errors, and then it applies these methods in that order until there are no missing values left in that feature. Thus, for each feature, each missing value is replaced using the MVR method that had the smallest estimation error for the known values of that feature.[80]

25.5.2.2 Adapting the random forest algorithm to longitudinal data

The standard RF algorithm does not cope directly with the temporal information of longitudinal data, as is the case with most ML classification algorithms. Hence, we adapted a part of the RF algorithm to address this issue, adding a bias to make the

classifier favor more recent information. This adaptation is based on the intuitive notion that features measured closer to the class (target) wave are more closely related to the target value (i.e., whether the individual was diagnosed with a disease in wave 8).

The proposed adaptation is a lexicographic bi-objective approach, applied to the node split function of the decision trees that compose the RF. A decision tree splits the data based on the values of a selected predictive feature. Normally, the selection of the feature to be used in a split is based solely on the reduction of class impurity in the dataset that the split would cause, using a metric like the information gain ratio or other similar metrics. In our adaptation, this decision becomes bi-objective. In addition to the information gain ratio (primary objective to be maximized), as secondary objective to be optimized we use the time-index of the selected feature, favoring more recent features over those measured further in the past. Importantly, a feature's time-index only comes into play as a tie-breaking criterion over features considered as candidates for the split of a node that have virtually the same information gain ratio. We call this a lexicographic bi-objective approach, since the two objectives are optimized in a clear and predefined order of priority. After a series of experiments,[75] we observed that the classification models generated with this adapted RF algorithm generally had better predictive accuracy than a standard RF algorithm.

25.5.3 Computational results

25.5.3.1 Predictive performance results

We trained RF classifiers with the lexicographic bi-objective split approach using the previously described dataset, with all missing values in the original predictive features replaced by estimations. The classifiers were evaluated using a well-known, 10-fold cross-validation, where the dataset is divided into 90% training and 10% test data for each iteration, until all instances have been used in testing once. The training data is used to create the classification model, then the model is used to predict the class label for each test instance, and we compare the predicted and actual values to measure predictive performance.

All 10 datasets have a class-imbalance problem, as we have much fewer examples of individuals diagnosed with the age-related disease (target variable) than of healthy individuals. Thus, we undersampled the training data, in all experiments, using the Balanced Random Forest method.[81] Briefly, this means the algorithm learns from training data with the same number of positive and negative examples, and is then tested with the imbalanced (unchanged) test data. Then, we calculate performance metrics for each classifier, which indicate how well the model predicted the class of the instances in the test datasets. Table 25.2 reports the average values of the following metrics over the 10 test sets of the cross-validation:

Table 25.2 Random forest's performance. Values averaged from the 10-fold cross-validation.

Dataset (IR)	Sensitivity	Specificity	GMean	AUC
Arthritis (1.35)	0.624	0.752	0.683	0.744
High BP (1.49)	0.648	0.548	0.596	0.681
Cataract (2.06)	0.680	0.683	0.682	0.732
Diabetes (6.5)	0.698	0.780	0.735	0.808
Osteoporosis (9.85)	0.681	0.810	0.742	0.810
Stroke (15.86)	0.684	0.729	0.706	0.752
Heart Attack (16.7)	0.659	0.725	0.690	0.752
Angina (26.41)	0.636	0.727	0.680	0.742
Dementia (59.96)	0.592	0.664	0.612	0.703
Parkinson's D. (160.3)	0.628	0.750	0.685	0.739

Sensitivity (True Positive rate): the ratio of majority class instances (healthy individuals) that were correctly classified by the model.
Specificity (True Negative rate): the ratio of minority class instances (individuals diagnosed with the target disease) that were correctly classified by the model.
GMean: The geometric mean between Sensitivity and Specificity, a performance measure that puts an equal weight in correctly classifying instances of each class.
AUC: The area under the ROC curve, a popular performance metric that takes into account the trade-off between maximizing the True Positive rate and minimizing the False Positive Rate.

Each row in Table 25.2 corresponds to one of our 10 target variables, the age-related diseases, ordered by their class imbalance ratios (IR). The IR value indicates how many majority class instances there are in the dataset for each minority class instance. For example, in the Dementia dataset, for each respondent who reported being diagnosed with Dementia (negative class), we have about 60 that did not (positive class).

25.6 Feature importance analysis

One advantage of the RF ensemble classifier is that its models are more interpretable than those created by fully "black-box" algorithms, such as Neural Networks and Support Vector Machines. Individual decision trees are easily interpretable, but as a RF has a large number of trees (usually over 100), we can instead calculate feature importance metrics to get a sense of how effective each predictive feature was in the model.

This interpretation analysis can identify general trends, and spark further research, from the results of the classification model. This is an important contribution, in addition to the classification model itself. Understanding which features were useful in predicting the target variables can lead to a better understanding of the relationships between those features and the classes, potentially leading to novel biomedical knowledge. In this case study, we are looking at biomedical variables (features), most of

which were measured at different time-points of the study, Hence, we aim at identifying which of those features were instrumental in the classification of the study's participants as healthy or unhealthy, for each of the 10 target age-related diseases.

Table 25.3 shows the features included in the set of 5 best ranked features used as predictors for each of the ten age-related diseases used as class variables, considering the different measurements of a feature (in different waves) as the same base feature. In each cell of this Table, a "✓" symbol indicates that the feature in the corresponding row is one of the five best ranked features for the age-related disease in the corresponding column. These results were obtained by running the RF algorithm with the aforementioned lexicographic bi-objective approach for selecting the best feature at each tree node. Note that, for some age-related diseases in Table 25.3, the number of

Table 25.3 Features that occur among the five best-ranked features used as predictors for at least one of the 10 age-related diseases used as class variables.

Feature	Angi.	Artr.	Cata.	Deme.	Diab.	HBP	He. At.	Oste.	Park.	Stro.
chestin	✓		✓				✓	✓		
sex			✓		✓	✓				✓
eyesurg						✓		✓		✓
hasurg	✓		✓			✓				
Igf1							✓		✓	✓
mmstre	✓				✓				✓	
bmiobe				✓					✓	
clotb				✓				✓		
fglu			✓			✓				
indager		✓			✓					
mmlore		✓		✓						
mmssre					✓					✓
trig	✓					✓				
cfib		✓								
chol		✓								
diaval		✓								
hdl	✓									
hgb									✓	
hscrp				✓						
ldl									✓	
mmcrre				✓						
mmgsnavg			✓							
pulval							✓			
wtval							✓			

cells in the corresponding column with the symbol "✓" is less than 5, because two measurements of the same feature (in different waves) are among the five best features for that age-related disease. The features are shown in decreasing order of the number of age-related diseases that they are associated with, and as a tie-breaking criterion, features are listed in alphabetical order. The meaning of the features shown in this table is described in Table 25.4.

As shown at the top of Table 25.3, there are two features which were among the five strongest predictors for four out of the ten age-related diseases, namely chestin ("Lung function: any respiratory infection in last three weeks") and sex. There were also four features which were among the five strongest predictors for three age-related diseases, and seven features which were among the strongest predictors for two age-related diseases. Overall, this shows that several features are strong predictors for multiple age-related diseases, contributing to a more holistic study of the aging process, instead of studying just one age-related disease at a time as usual.

Table 25.4 Description of the best-ranked features shown in Table 25.3.

Feature	Description
bmiobe	Body mass index grouped according to WHO definitions
cfib	Blood Fibrinogen level (g/L)
chestin	Lung function: Whether had any respiratory infection in last 3 weeks
chol	Blood total cholesterol level (mmol/L)
clotb	Blood sample: whether has clotting disorder
diaval	Mean diastolic blood pressure
eyesurg	Whether have a detached retina or had eye or ear surgery in the past 3 months
fglu	Blood glucose level while fasting (mmol/L)
hasurg	Whether had abdominal or chest surgery in the past 3 months
hdl	Blood High-density lipoprotein (HDL) level (mmol/L)
hgb	Blood hemoglobin level (g/dL)
hscrp	Blood C-reactive protein (CRP) level (mg/L)
ifg1	Blood insulin-like growth factor (IGF-1) level (nmol/L)
indager	Age of the respondent
ldl	Blood LDL cholesterol level (mmol/L)
mmcrre	Single chair rise test outcome
mmgsnavg	Mean grip strength with non-dominant hand
mmlore	Leg raise (eyes open) test outcome
mmssre	Outcome of side-by-side stand test
mmstre	Outcome of semitandem stand test
pulval	Pulse pressure
sex	Sex of the participant
trig	Blood triglyceride level (mmol/L)
wstval	Mean waist (cm)

25.7 Conclusion

The aging of the world's population creates a new distribution of health expenditure, with a significant change towards chronic diseases and other aging-related diseases (e.g., cardiovascular and neurological diseases). These changes cause unaffordable costs for health systems. At the same time, there is an unprecedented amount of age-related biological data being collected and made publicly available. This scenario, coupled with the renewed interest and advances observed in the area of ML, creates a decisive opportunity for the development and application of effective computational methods, aiming at unveiling the real potential of the collected data and, therefore, to extract meaningful knowledge from it.

The two examples described in this chapter are remarkable situations where biological aging-related diseases can benefit from the application of ML-related techniques: the first example describes a home telemonitoring environment for the early detection of heart failure, whereas the second situation relies on the analysis of real data to early predict the potential development of multiple age-related diseases.

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CHAPTER 26

The future of integrated care in aged individuals

Alexandre Lourenço[1,2,3], Maja Furlan de Brito[4,5] and Bárbara Gomes[4,5]
[1]Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[2]NOVA National School of Public Health, Public Health Research Centre, Universidade NOVA de Lisboa, Lisbon, Portugal
[3]Comprehensive Health Research Center (CHRC), Lisbon, Portugal
[4]University of Coimbra, Faculty of Medicine, Coimbra, Portugal
[5]King's College London, Cicely Saunders Institute of Palliative Care, Policy & Rehabilitation, London, United Kingdom

26.1 Introduction

Health systems have evolved over time. We can find the first public hospitals appearing during the Christian period in the Byzantine Empire by the end of the 4th century. Medieval European and Islamic societies contributed to the dissemination and development of the concept. After the 16th century, European exploration globalized hospitals around the world. Until the 20th century, the idea of a hospital encompassed hostels for travelers, dispensaries for poor relief, clinics and surgeries for the injured, and homes for the socially excluded (e.g., blind, disabled, elderly, mentally ill). Aristocrats and the wealthy were treated at home.

It is only after the World Wars, and mostly the second World War that acute care gained terrain and the hospital started to resemble what it is now. The increased differentiation and technological development helped develop the hospital as the central hub of healthcare. It changed our lives. The hospitals are the new cathedrals where our main life events are taking place and miracles happen. We are born at the hospital, treated at the hospital, and die at the hospital. As a scientific center, the hospital concentrates the most advanced technology, and for the first time, the poor and the wealthy are treated at the same place.

The geometric knowledge increase is followed by differentiation. Medical practice ramifies in a plethora of specialties and subspecialties that gain more and more interest moving healthcare from the individual to the organ and the biomedical process. Hospital service delivery follows professional views, and it is verticalized in different medical specialties' that have trouble providing a common approach to the patient.

Increased differentiation has boosted complexity and costs. Peter Drucker famously called hospitals "the most complex form of human organization we have ever attempted to manage."[1] Economic growth sustained the improvement in coverage and scope of health services. Far from infinite, fiscal constraints arose and inputted additional pressure

Aging
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to improve health system efficiency and cost-sharing. The professional bureaucratized organization faces managerialism that introduces the cost-containment and productivity-based policy agenda. Nonetheless, intense professionalization and institutionalization resisted top-down or radical change, evolving instead through an incremental pattern of change that reproduces the professionalized status quo.[2]

At the same time, the escalating complexity and heterogeneity of healthcare delivery systems has led to increased fragmentation, making the health system more problematic to use. The focus moved from a system initially based on the care for the most fragile, to the organizational needs.

26.2 The current model is more and more inadequate

High-income countries' health systems continue to guarantee an improvement in the health status and longevity of their populations.[3] However, critical fiscal challenges remain, and those countries are trying to keep costs under control. The pressure is growing to ensure better equitable access to quality of care. On the other hand, we know that a significant portion of health spending is, at most, ineffective and at least a waste.[4] Efficiency gains can help contain costs and free resources to improve access and the scope of services. Specific reforms vary between countries, but there is a growing perception of the need to review the delivery models[5] and financing,[6] to create better efficiency of the resources used and ensuring universal access to healthcare.

The focus may be on the optimization and flexibility of the organizational model, driven by the development of innovative solutions,[7] that reduce waste and maximizes knowledge to improve healthcare and the experience of the citizen in its use. But is it enough? As Peter Drucker said, "there is nothing so useless as doing efficiently that which should not be done at all."[8]

The delivery of health services is the perceptible health system branch. In addition to being the interface to the population, it is the operational "arm" of the health system to improve the population's health levels, not only in average terms but also in an equitable way.[9] The archetype of the organization of health services will be a model that allows achieving this purpose efficiently.

The current delivery model was developed to manage acute disease and episodic cases, and as a consequence, relies heavily on acute care. According to each country, primary and long-term care or other community services were also developed but never threatened acute services' dominance. As a result, we have a fragmented health system limited in its ability to provide people-centered care. Suppose we also considered the failure to integrate social services. In that case, we realize that the categorization of welfare services is far from real-life problems that are nearly always messier, more complex, harder to define and more difficult to resolve than this. Put simply; people do not live their lives according to the categories we create.[10]

The current model and living conditions improvement allow us to live better and longer. But as a consequence of its success, we are facing population aging with increased prevalence of chronic diseases. In addition, lifespan does not equate to health span.

If we do not redefine how we provide care, the gap between the current model and the population's needs will widen. It is not only a matter of minor adaptations. We need to reframe care according to the individual needs and life cycle. We need to change the question from "how might we cooperate across boundaries?" to justify why we are not.[11]

26.3 The avoidable suffering

Improving health through avoidance of suffering is an essential component of high-quality health systems.[12] Still, the relief of suffering remains one of the most neglected dimensions of global health.[13] Published in 2019, the first worldwide projections of the future burden of serious health-related suffering, as defined by the Lancet Commission on Palliative Care and Pain Relief,[14] have shown an escalating burden in all world regions.[15] Combining WHO mortality projections (2016–60) with estimates of physical and psychological symptom prevalence in 20 life-threatening conditions, serious health-related suffering is predicted to almost double by 2060. By 2060, an estimated 48 million people will die with serious health-related suffering. This compares with 26 million people in 2016. The fastest increases will occur in low-income countries, among older people ($\geq$70 years), and people with dementia. Over 22 million more people aged $\geq$70 years will experience serious health-related suffering in 2060 compared with 2016, a 183% increase (Fig. 26.1). The global burden of serious health-related suffering in dementia will increase from 1.5 million in 2016 to almost 6 million in 2060, a fourfold increase. Without improved health systems and new care models, we will fail to relieve the suffering of millions of people. This will increase pressure on already vulnerable health systems, placing a greater burden on families and informal care. Immediate global action for stronger health systems to prevent avoidable suffering is therefore an ethical and economic imperative.[15]

26.4 An integrated care approach

Healthcare is not ready to deal with the complex impact of aging and the associated rise in suffering. It needs to swiftly adapt service delivery to a more integrated approach that joins different care levels, but also social care and community players like municipalities, fostering collaborative work with academia, business companies and civil society.[16]

There are four areas that are critical for this transformation towards integrated care to occur: (1) healthy aging and disease prevention, (2) patient-centeredness and multimorbidity, (3) dementia, and (4) palliative, end-of-life and bereavement care.

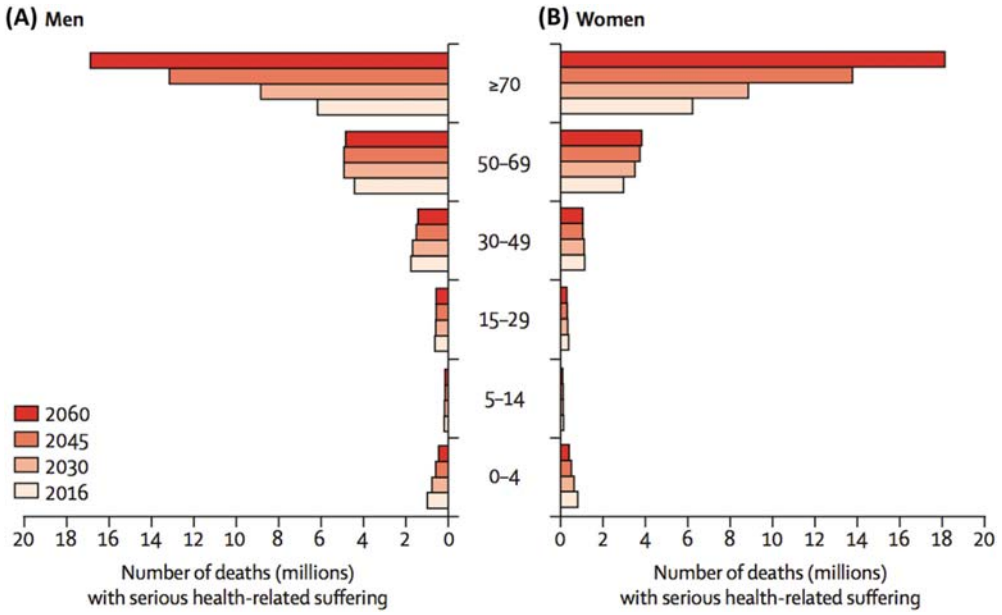

Figure 26.1 Changes in the number of people dying with serious health-related suffering for global population stratified by age-group and sex. *Reprinted from Sleeman KE, de Brito M, Etkind S et al. The escalating global burden of serious health-related suffering: projections to 2060 by world regions, age groups, and health conditions. Lancet Glob Health 2019;7(7):e883–e892, Copyright (2019), with permission from the author.*

26.4.1 Healthy aging and disease prevention

There have been significant advances in people's health and life expectancy in recent decades. Advances in healthcare keep people alive while controlling their conditions, leading to growing numbers of people surviving with chronic illness. As a consequence, the proportion of older people is rising leading to a higher number of those with chronic health problems because of accumulated exposure to chronic disease risk factors over a lifetime (Fig. 26.2).

Knowing that our health span depends heavily on socioeconomic factors (40%), health behaviors (30%), healthcare (20%), and physical environment (10%),[18] it is more consensual to move from a simplistic healthcare care approach to a population health approach. A population health approach focuses on interrelated conditions that influence the health of populations, identifies variations in observed patterns, and uses the resulting knowledge to inform policies to improve the health and well-being of populations.[19]

This complementary view of public health and healthcare—population health management, allows for a more practical approach of the motto "health in all policies" and the application of the public health three P's:

- Promotion of health in a population,

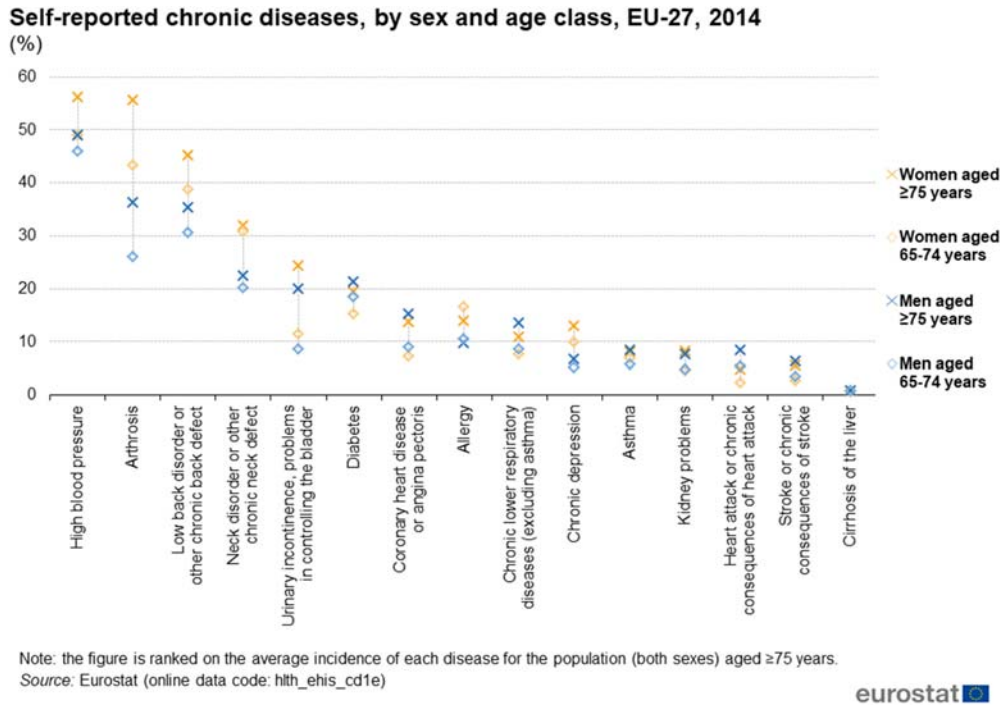

Figure 26.2 Self-reported chronic diseases, by sex and age class, EU-27, 2014 (%)[17]. *Eurostat. Persons reporting a chronic disease, by disease, sex, age and educational attainment level. online data code: HLTH_EHIS_CD1E last update: 21/07/2021 10:00; 2016.*

- Prevention of disease in a population,
- Protection of the health of a population.

Disease prevention includes: (1) primary prevention, which is concerned with eliminating risk factors for a disease; (2) secondary prevention, which focuses on early detection and disease treatment (subclinical and clinical); and (3) tertiary prevention, which attempts to eliminate or moderate disability associated with advanced disease.

A greater vertical alignment and horizontal integration between community health and social care, by more effective joint working between public health and local government, and more effective partnerships between the public, private and voluntary sectors, will promote a more person-centered care, with services that are integrated across the patient life cycle.

26.4.2 Patient-centeredness and multimorbidity

A key challenge in meeting the needs of older people is the involvement of the multiplicity of health and social care providers. This does not necessarily translate into

poorer care, however it may increase the responsibility and the burden on the older person and their family caregivers to coordinate the fragmented and possibly discontinuous care. In this light, patient-centeredness is considered a core aspect of improving healthcare quality.[20] Patient-centered care is respectful of and responsive to individual patient preferences, needs and values, and ensures that the clinical decisions are guided by the patient's values.[20] The active mechanism through which patient-centeredness happens, triangulates three care qualities: the accessibility, the coordination and the continuity of care. Together, they improve the patient experience of navigation through the health system.[21]

Putting the patient in the center requires two conditions; (1) respect for patient's needs and preferences, and (2) empowering patients and their family caregivers through the provision of timely and adequate information and support to enable them to make decisions about their own care. That said, future healthcare services need to be built around the patient and their family, rather than them looking for ways of adapting their life to the services they need.

In an aging society, more people have multimorbidity than a single disease, with more than 50% of older people having three or more chronic diseases and most people coming to a multimorbid stage by the age of 65.[22,23] The majority of the burden of multimorbidity falls onto primary care,[24] which needs to be redesigned, shifting from a disease-centric to person-centric provision of care. Primary care needs to become the leader in bottom-up as opposed to top-down approaches, engaging all stakeholders towards optimizing each personal health experience throughout a multimorbidity trajectory until death. This requires being sensitive at key transition points in health status and changes in care goals.

Following the preferences of older people with multimorbidity means responding to the fact that about half of them would prefer to be cared for and die at home.[25–27] Although favorable for the relief of pressure on hospitals, turning to community care providers as key stakeholders in helping to achieve people's preferences and responding to their needs, also puts enormous pressure on informal caregivers. These have been recognized as a cornerstone of patient care when it takes place in the community, providing long hours of care and carrying out a range of tasks.[28] Being prepared for caregiving, such as providing physical care, emotional support, organizing the services needed, dealing with the burden of caregiving, is only one part. With the longer but not necessarily healthier life that many of us will live, comes the need to act preventively but also, at the same time, respond to any acute health and social care crisis that can arise at any point in a multimorbidity trajectory. An integrated approach to care, built on patient-centeredness, cannot sustain without including interventions that improve informal caregiver's understanding of health and disease and support them in their caregiving role. Health literacy and support for caregiving is therefore a priority for any aging society.[29]

26.4.3 Dementia

Dementia designates a group of heterogeneous disorders affecting the brain and manifesting with a sustained impairment in cognitive function, which goes beyond what would be expected from normal aging and is severe enough to affect the capacity of the individual to perform the usual activities in daily life. Worldwide, there were approximately 50 million people with dementia in 2020 and it is estimated this number will almost double every 20 years, to 82 million in 2030 and 152 million in 2050.[30] Dementia is also the disease group that will see the largest rise in the global burden of serious health-related suffering until 2060, as described above.[15]

There is no known cure for dementia and the impact of the unmet needs frequently reported by people with dementia is dramatic. Their main problems are the loss of individuality due to the memory and capacity decline, the pain and discomfort experienced, and the behavioral and psychological symptoms of dementia. This high level of complexity in need translates into a disinvestment by the health professionals and a lack of person-centered care, which further burdens the quality of life and the human dignity of those affected. Dementia is therefore a major challenge for health systems around the globe, considering the quickly growing number of aged individuals with dementia and the prolonged dwindling disease trajectory they will experience.[31] The prevalence of dementia increases exponentially with age, from 5% in people with ≥ 65 years to up to 30% in those with ≥ 80 years. The median survival is 4.5 years, but this is highly variable, ranging from 10.7 years in those aged 65–69 years to 3.8 years in those with ≥ 90 years.[32]

While the number of dementia cases is projected to rise due to ongoing demographic changes, recent epidemiologic studies revealed an unexpected decrease in the incidence and prevalence of dementia as a result of more healthy lifestyles including diet, physical exercise, smoking eviction, prevention of vascular risk factors and cultural enrichment. A report published by the Lancet Commission on Dementia in 2020 warns that 40% of dementia cases are potentially preventable and presents a new life-course model showing 12 modifiable risk factors from early to later life, including education, smoking, depression and social isolation, among other factors.[33]

While much can be done to prevent dementia, the Lancet Commission Report also reminds us that 60% of the risk remains unknown;[33] hence, the importance of providing the best available care to people with dementia, their family and carers, all the way from diagnosis to after death. Many national dementia plans recognize the importance of better care integration. Dementia models of integrated assessment and management are still in development but must involve primary and specialist care and emphasize on improving care coordination, interdisciplinary work, personalized care and care continuity across settings.[34] Integrated dementia care must consider

family and carers. It must include end-of-life care. It requires a transformation to societies where people with dementia feel integrated.

26.4.4 Palliative, end life, and bereavement care

Palliative care is an active and holistic care approach that improves the quality of life of persons living with a life-threatening illness, including their family. It prevents and relieves suffering through the early identification, correct assessment and treatment of symptoms and concerns, whether physical, psychosocial, or spiritual, to help people live as actively as possible until death.[35] It can be provided alongside any curative treatment and it should be provided regardless of diagnosis to whoever can benefit from a palliative care approach (e.g., people with cancer, cardiovascular diseases, respiratory diseases, organ failure, neurodegenerative diseases, HIV/AIDS and many others conditions).[36] Palliative care uses an interdisciplinary team approach to support the patient, their family and caregivers throughout the disease trajectory and, in the case of family and caregivers, into bereavement.[35]

Although recognized under the human right to health,[37] palliative and end-of-life care is one of the most neglected areas in health systems worldwide,[14] with 47 countries (out of 198; 24%) with no known palliative care activity, 13 countries (7%) showing first initiatives and 65 (33%) countries with isolated provision within national healthcare systems.[38] Integrated models of palliative care for older people are especially scarce due to two reasons. First, palliative care has been traditionally involved in the care of people with cancer, but people aged 70 years and above are more likely to die from a disease other than cancer.[39] The development of integrated palliative care models in heart disease, chronic obstructive pulmonary disease, and dementia, for example, is much more limited than in cancer.[40] Secondly, multimorbidity and frailty related to older age increase uncertainty as to when someone is nearing end-of-life and could benefit from palliative care.[39]

It is due to the complexity of their health situation that older people are often not able to reside and be cared for at their preferred place. Transfers may be needed between different places of care, including emergency and hospital admissions in the very last weeks of life.[41] Integrated palliative care should therefore aim at improving quality of life and preventing unnecessary institutionalized care, especially if this is not in line with the person's preferences. Large surveys show that hospitals and care homes are people's least preferred places of death, while most would prefer to remain at home.[42] Palliative and end-of-life care providers in the community are usually generalists, that is primary care professionals (e.g., family doctors, primary care nurses) and care homes with the help of specialist teams when available (health professionals with advanced training in palliative care). Both generalist and specialist palliative care providers, when working in the community, are known to increase people's chances of

dying at home.[25,26] A meta-ethnography[43] showed home palliative care teams, with their presence and competence, contribute to allowing the patient and their family to feel secure in continuing to live their life at home while preparing for death.

Integrated care should result in the continuity of care across all levels (primary to tertiary, generalist to specialist care) and settings. However, the continuity of care is only as good as the care that all health and social care teams involved deliver. Evidence on the effectiveness of palliative care started to emerge in 2003 and has showed a favorable impact of specialized palliative care teams on quality of life, symptom burden, physical and psychological functioning, satisfaction with care, and place of death.[25,26,44–49]

Traditionally, palliative care is introduced towards the end of life, when it becomes clear life-prolonging or potentially curative treatments offer little or no benefit. Better-integrated palliative care aims to intervene in earlier stages of the disease, with a view to derive more benefits and accommodate different trajectories (e.g., cancer, organ failure, dementia, frailty, among others).[45] For some common function-related symptoms like sarcopenia, early recognition and response are crucial as there are limited interventions to reduce weight and muscle loss, especially when already advanced. Exercise-based interventions are one of the most effective available options[50] and the sooner they are implemented the more positive the impact will be. Early palliative care in the older person's life is also known to lower the risk of inappropriate use of aggressive treatments that have little to no benefit, but markedly compromise quality of life.[51] As an example, in Portugal, this is a pressing issue, as a nationwide study of the aggressiveness of cancer care in the last month of life showed a prevalence of over 70% in care aggressiveness at the end of life.[52] For timely identification, avoiding unnecessary treatment burden and a well-managed care, primary care is thought to play a crucial role.

The last stages of a life trajectory and death initiate grief, which is a natural response to the loss experienced by the family members. Bereavement care is an essential part of palliative care that aims to provide ongoing support to family members and other informal caregivers in their pre-death grief and grief and mourning after death. It is a shared responsibility between communities and healthcare services in that family and friends usually provide the majority of the support. However, around 40% of people who cared for their relatives are at moderate to high risk of developing more complex needs for support, including developing a prolonged grief disorder.[53] This warrants a higher level of support that goes beyond the capacity of the family members to provide.[54] Besides the scarce evidence on the effectiveness of the existing models of bereavement care and specific interventions, matching the loss-related needs of spouses of older people with the right support is especially challenging. A recent report showed that older bereaved are less likely to seek help than younger people and they also have less chances to be referred for bereavement support than younger people.[55]

A recent systematic review identified two service delivery models of integrated care for older people.[40] An integrated geriatric care model aims towards improvements in the functional decline, while an integrated palliative care model focuses on symptom severity and the psychosocial/spiritual aspects of the older person's life. Both models build on the integration of administrative, organizational and clinical aspects of care. However, in the challenging and changing world we live in, innovative models that go beyond the state of the art are needed. Holistic approaches, aligning different stakeholders, including science and technological developments, citizen empowerment, alongside the training of new generations of health professionals and informal carers can bring new care management solutions that will ensure delivery of the right care at the right time, and one that will meet people's care preferences and goals.

26.5 Key messages

- The challenges that health systems face today carry with them the opportunity to transform the now fragmented care into better integrated care for older citizens, avoiding waste in spending and serious health-related suffering of millions of people.
- Building integrated care is a major endeavor that means joining different care levels, aligning stakeholders, involving both formal and informal carers and mobilizing societies as a whole. The effectiveness and continuity of care depends on it.
- For such health system transformation to occur, particular attention should be given to four priority areas that can serve as examples for future care modeling: healthy aging and disease prevention, patient-centeredness and multimorbidity, dementia, and palliative, end-of-life, and bereavement care.
- Innovative models that develop bottom-up and go beyond the state of the art are needed to shape the future of integrated care in aged individuals.

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CHAPTER 27

Moving from reactive to preventive medicine

Lina Badimon[1,2,3], Teresa Padro[1,2] and Gemma Vilahur[1,2]
[1]Cardiovascular Program ICCC - Research Institute Hospital de la Santa Creu i Sant Pau, IIB-Sant Pau, Barcelona, Spain
[2]Centro de Investigación Biomédica en Red Cardiovascular (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain
[3]Autonomous University of Barcelona (UAB), Barcelona, Spain

27.1 Introduction

The best doctor prevents illness, an average doctor visits when the illness is imminent, and the unskilled doctor treats your present illness.

(Chinese Proverb)

Reactive medicine is the medical care activation and initiation in response to an adverse disease case presentation, injury, an acute condition, or symptom. The physician and hospitals are used to responding to reactive medicine. Also, the patients are accustomed to calling the doctor or visiting a hospital emergency room when some pain is triggered. On the other hand, preventive medicine tries to implement medical practices that are designed to reduce, revert or even avoid the causes of disease. Preventive medicine takes a proactive approach to patient care, minimizing the possibilities of adverse case presentation. Preventive care, as a prophylaxis, is intended to take measures to avoid the presentation of the disease.

Disease and disability are affected by environmental factors, genetic predisposition, disease agents, and lifestyle choices, and they are dynamic processes that begin before an open disease episode is declared. Preventive medicine focuses on the health of individuals and communities. The goal of preventive medicine is to promote health and well-being and to prevent disease, disability, and anticipated death. The purpose of preventive medicine is healthy, successful aging and longevity without disease.

As we grow older, we are more likely to be diagnosed with one or more chronic ailments. These ailments include life-threatening diseases such as cardiovascular disease (CVD), diabetes, cancer, and debilitating conditions like arthritis, fatigue, and frailty. These ailments reduce our quality of life. The question is: How does the aging process affect the disease process and susceptibility, and vice versa?

Over the years, researchers studying the basic science of aging have sought to answer these questions. Still, their work was confined primarily to investigations of the specific activities and mechanisms that contribute to the aging process and not as

Aging
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much on the effects of the aging process on various diseases. While aging itself is not a disease, the aging process represents a major risk factor for several chronic diseases and conditions, including frailty and lack of resilience.

Preventive medicine has evolved during the last few years, mainly in an areas that have a chronic character and are the major causes of death and disability in the community, such as CVDs, where the control of risk factors and implementing a healthy lifestyle has shown significant effects in reducing morbidity and mortality. Yet, preventive medicine has also progressed in other areas of human pathophysiology as well.

Understanding genetic, molecular, and cellular mechanisms of aging-associated diseases will also be a driver for improved preventive medicine.

27.2 Aging and major chronic diseases

Aging is a complex pathophysiological phenomenon characterized by the dynamic remodeling of numerous biological pathways, frequently developing into a progressive decline of physiological functions, representing the driving force for all age-related diseases. Indeed, aging is often associated with a higher incidence and prevalence of chronic pathologies, such as CVDs, metabolic diseases, arthritis, musculoskeletal disorders, cancer, neurodegenerative diseases, and dementia. Although several features of premature aging are found in young adults with these chronic pathological conditions, age-related diseases are frequently presented with multimorbidity in the elderly, affecting more than half of the aged subjects,[1] with severe consequences including disability and functional decline, poor quality of life, and high healthcare costs for the patients, their families, and society as a whole.[2]

Preventive medicine aims to develop strategies to increase the duration of a healthy life and lessen the incidence of age-related disease; something that has been continuously increasing over the last decades because of the longer lifespan of the population. Studies aimed at improving our understanding of the etiopathogenic relationship between biological aging and age-related chronic diseases have mostly focused on this area's research in recent years. In addition, the effects of environmental factors or pharmaceutical treatment are extensively investigated.[3–6]

27.3 The mechanistic interplay between aging and age-related diseases. In the search of evidence for preventive medicine

Accumulating evidence, based on the study of many different types of organisms, suggest aging as a continuous pathophysiological process in which several "hallmarks" coincide.[7] These include processes such as adaptation to stress, stem cell exhaustion, metabolism imbalance, proteostasis, and macromolecular damage, in addition to genomic instability and epigenetic alterations which may converge, leading to a chronic

low-grade inflammatory condition, referred as "inflammaging" which in turn can lead to age-related chronic diseases (Fig. 27.1).[8,9] It is now well established that low-level chronic inflammation mediates several pathologies including cancer, arthritis, obesity, diabetes, CVDs, and neurological diseases.[10] Thus, inflammaging is increasingly recognized as the main process connecting aging to age-related diseases and therefore suggestive as a potential target for preventing age-associated pathologies in presymptomatic stages.[11] However, these diseases also present many common features that do not relate to age. Thus, most of these age-related diseases begin the pathological processes at younger ages, and all of them need one or more specific triggers inducing inflammation and/or inflammation-related changes directly affecting the disease target tissues and organs, as it is the case of high circulating levels of low-density lipoproteins for atherosclerosis development in the arterial vessel wall.[12,13] Accordingly, the development and progression of age-related diseases would occur

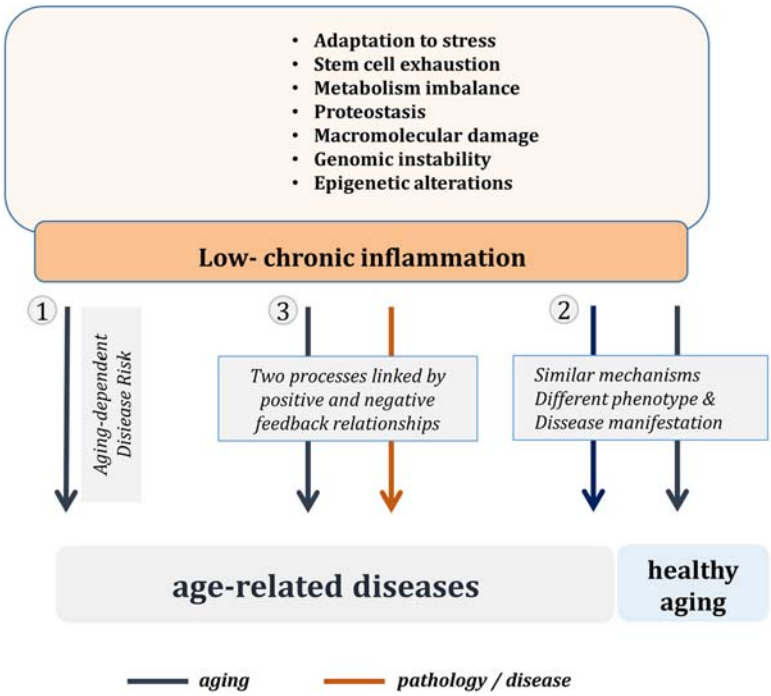

Figure 27.1 Mechanisms that contribute to healthy and pathological aging. Schematization of the three different concepts relating aging with age-related diseases. (1) Aging as a major risk factor for the age-related diseases (geroscience). (2) Aging and age-related diseases as two processes linked by positive and negative feedback relationships which influence their evolution. (3) Aging and age-related diseases as processes shearing similar underlying molecular mechanisms, but with different phenotypic expression and disease manifestation depending on the genetic background and epigenetic regulation.

independently of age. However, molecular and cellular changes occurring during aging, including inflammaging, may contribute to these diseases and favor clinical manifestation in the elderly.[7]

27.3.1 Immunosenescence and age-related chronic diseases

Weakness of immune receptors and senescence of the immune system is considered the main cause of increased levels of inflammatory markers found in cells and tissues in older organisms,[14] in the absence of evident signs of infection. Although chronic inflammation has many features of acute inflammation, it results in responses that lead to tissue degeneration, differing from transient immune responses to harmful conditions such as traumatic tissue injury or invading pathogens which facilitate repair and turnover.[15]

Several sources of low-grade chronic inflammation in aging (inflammaging) have been suggested, including (1) damaged macromolecules and cell- and organelle-debris products that function as endogenous "damage"-associated molecular patterns (DAMPs) initiating immune responses and becoming maladaptive when they occur chronically; and (2) harmful products produced by the microbial constituents of the human body, such as oral or gut microbiota which can translocate into the blood circulation triggering thereafter immune responses and inflammatory processes affecting organ homeostasis and promoting pathologies.

In addition, inflammaging might be due to the accumulation of senescent cells that release senescence-messaging secretomes, referred to as senescence-associated secretory phenotype (SASP), including proinflammatory cytokines.[14] SASP modifies the tissue microenvironment, and disrupts its architecture and function.[16] The evidence that senescent cells are not just correlated but causally involved in age-related diseases come from experimental studies. In mice, the selective elimination of senescent cells attenuates various features of age-related pathological conditions such as osteoarthritis, atherosclerosis, and neurodegeneration, without exerting overt adverse side effects.[17,18]

While considered separate systems, coagulation and inflammation are highly integrated with an extensive cross-talk between them. Dysregulation or activation of one of both systems affects and activates the other resulting in a wide range of illnesses characterized by excess inflammation and thrombosis. In recent years, several studies have focused on analyzing coagulation and inflammation pathways among older adults. Interestingly, Cubedo et al.[19,20] evidenced a coordinated change in the circulating pattern of several plasma proteins related to inflammation and hemostasis in unhealthy octogenarians with a previous episode of vascular disease, suggesting a potential implication of this protein-network in the development of CVD and cognitive impairment in the elderly.

27.3.2 Epigenetic drift and age-related chronic diseases

Accumulation of genetic damage resulting in genome instability is considered one major hallmark of aging and has been the focused interest of many studies. Over the years, however, the role of epigenetics in aging has gained increasing attention, due in part to epigenetics' high plasticity and adaptability opening new avenues based on prevention and therapeutic strategies for age-related diseases.

Common epigenetic regulation refers to DNA methylation, histone post-transcriptional modification and transcriptional control by noncoding RNAs. Global epigenetic patterns tend to be stable during lifespan. In contrast, aging associates with gradual deregulation of epigenomic markers ("epigenetic drift"). Hence, the aged epigenome fails to respond to environmental factors leading to the loss of active epigenetic markers directly affecting the regenerative capacity of stem cells among other relevant biological processes affecting somatic cells. Thus, the stability of epigenetic mechanisms is critical for a suitable molecular activity, limiting the risk of developing various diseases and delaying the aging process.

27.4 Age-related diseases—prevention initiatives are in order

Non-communicable diseases refer to a range of chronic pathological conditions, including cancer, CVD, diabetes, hypertension, that in addition to cognitive and neurogenerative diseases, are the most common age-related human diseases. Furthermore, sensory failures such as auditory and macular degeneration increase significantly in the aged population, and conditions affecting the skeletal system, particularly osteoporosis and osteoarthritis, are common in the elderly.[21]

Aging has a significant pathophysiological effect on the arterial system and the heart, being CVD (ischemic disease, congestive heart failure, and arrhythmia), are major causes of death in older adults. Age-related alterations in arteries range from the large elastic arteries to the conduit and small resistance arteries, down to the microcirculation.[22] Yet, a main underlying cause of age-related CVD is atherosclerosis that manifests as coronary heart disease, stroke, or peripheral arterial disease,[23] which also causes cognitive impairment and other organ damage. To note, the incidence of heart failure, a complex clinical syndrome derived from the progressive decline of the structure and function of the heart, progressively increases with age and represents the main cause of hospitalization in patients older than 65 years in Western countries.[24] Preventive strategies have been developed against cardiovascular-related diseases.

Inflammaging and immunosenescence are increasingly recognized as the most important factors implicated in neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. It is now considered that the aging of the immune system may accelerate cognitive decline and memory loss.[25] It is well accepted that the excessive

and continued release of proinflammatory cytokines in the central nervous system is associated with oxidative stress, cytotoxicity, apoptosis, and neurogenesis, processes implicated in cognitive decline.[26]

Age is also a major risk for solid cancers, cancer being the second leading cause of death in the elderly until the 9th decade of life.[27,28] Accumulation of senescent cells, inflammaging, and immunosenescence represent the main factors involved in the increase in carcinogenesis with age.[29] In this respect, aging, by compromising innate and adaptative immune responses, contributes to cancer progression. Cancer patients have many characteristics of premature cellular aging, which relates to poor clinical outcomes. Yet, a causal relationship still needs to be established.[23] In this area, we need further developments to have promising strategies for prevention.[25]

27.5 Do we have preventive strategies for ameliorating age-related diseases?

Even though many questions in the pathophysiology of age-related diseases remain unanswered, anti-aging strategies aiming to prevent or lessen age-related diseases have been the focus of attention in the last decades. An important and yet challenging area refers to identifying non-pharmacological interventions and pharmacological treatments to reverse cellular and molecular changes that occur as hallmarks of aging, limiting, therefore, cellular damage and extending health span. To date, different strategies have been proposed, albeit with few results mainly because of the insufficient clinical data available thus far despite the several achievements in experimental animal models.[3]

27.5.1 Non-pharmacological approaches

Lifestyle and environmental factors have been suggested to impact age-related biological pathways, including the decline of the immune system. In recent years it has become clear that strategies such as caloric restriction (to 30% of ad libitum values) and exercise are associated with a delayed onset of age-related diseases in animal models, including those in primates.[30] Interestingly, both caloric restriction (without malnutrition) and exercise have been shown to reduce levels of proinflammatory cytokines and ameliorate age-associated immune-cell proliferative defects.[3,4]

Regular aerobic exercise elicits a wide range of functional and structural positive adaptations in all organs and tissues of the body, maintaining optimal oxygen and nutritional supply during increased demand, culminating in a reduced risk for noncommunicable diseases. A major issue in the elderly is the sharp decline in physical activity duration and intensity. Importantly, results from a large longitudinal prospective study among older adults with an average age of 71 years at baseline evidenced that leisure-time activity and walking are prospectively associated with specific patterns of more favorable indices of autonomic function (circadian fluctuations and less erratic

sinoatrial firing), this being a favorable pattern especially evident in older adults who increased their walking pace or distance over five years of follow up. In addition, exercise in older adults has been associated with lower levels of proinflammatory cytokines and direct effects on the immune system.[31]

In addition, different studies have evidenced that caloric restriction is associated with reduced insulin levels, glucose, and insulin-growth-factor-1, which have been suggested to relate to longer lifespan in rodents and humans.[32,33] Several studies have reported that caloric restriction has a positive effect on DNA repair and telomerase mechanisms;[34] in mammals, caloric restriction, induces the expression of sirtuins which are directly involved in the aging process.[35] Furthermore, human clinical trials have demonstrated that continuous caloric restriction, without malnutrition, reduces oxidative damage to tissues and organs[36] and induces significant suppression of inflammation.[37]

27.5.2 Pharmacological interventions

The benefits of caloric restriction improving health in old age have led to considerations of different food-derived bioactive substances acting as caloric restriction mimetics in the elderly, but with fewer side effects (i.e., malnutrition) associated with caloric restriction. This is the case of polyphenolic compounds such as resveratrol and curcumin, among others, that are suggested to attenuate aging-derived epigenetic alterations and, as a consequence, to attenuate the effects of various age-related diseases.[38,39] Several biochemical pathways which have been shown to be affected by dietary restriction are relevant for aging. The mTOR pathway is associated with longevity in different animal species[40] and has been a key molecular target in several clinical studies in aging, to date. Thus, rapamycin, used in the clinic as an immunosuppressant in transplant patients, inhibits the mTOR pathway and increases lifespan in mice.[40,41] In addition, the non-immunosupressive dose of its analog, everolimus, improves the immunological response to influenza vaccination in healthy older subjects after six weeks of treatment.[42] Other drugs such as metformin, widely used as an antidiabetic, have been found to target molecular mechanisms of aging through activation of AMP kinases.[43] Clinical data have revealed that treatment with metformin improves lifespan in diabetic patients[44] and epidemiological studies have suggested that metformin may reduce the incidence of cancers and neurodegenerative diseases.[45]

More recent interventions focus on "senolytic" therapies aimed to selectively eliminate senescent cells or to more specifically target the senescent cells-bioactive secretome, referred to as the senescence-associated secretory phenotype (SASP). SASP has been shown to drive tissue degeneration and age-associated pathologies/diseases such as cancer and atherosclerosis.[46,47] These are probably the most effective interventions currently. However, this therapeutic approach focuses on the symptoms rather than on the causes of aging, which differs from emerging strategies based on rejuvenation

approaches consisting of reprogramming cells toward a pluripotent embryonic-like state. Pioneering examples of tissue regeneration were found in heterochronic parabiosis studies. Exposing aged tissue to a youthful systemic environment restored injury-induced satellite cell activation by upregulation of Notch signaling.[48,49] New strategies of regenerative medicine are based on pluripotent stem cells, including induced pluripotent stem (iPS) cells obtained by reprogramming of differentiated somatic cells.[50] Thus, a cell-reprogramming clinical strategy will consist of the production of differentiated cell from iPSCs to regenerate or replace cells in damaged tissue (i.e. heart).[51,52] However, although progressing very rapidly, these therapeutic approaches are still in their infancy. In addition, the identification of molecular and cellular pathways for tissue improvement or repair during aging, opens the door to a wide range of therapeutic solutions to intervene during aging and to improve the health span.

27.6 Cardiovascular disease: the success of prevention

The WHO estimated that 17.9 million people died from CVDs in 2016, representing 31% of all global deaths. Of these deaths, 85% were due to coronary artery disease (mainly myocardial infarction (MI)) and cerebral vascular accidents, both clinical manifestations of atherosclerosis. Risk factors for atherosclerosis have been classified as non-modifiable (age, family history, sex, race) or modifiable (hypertension, sedentary lifestyle, obesity, hypercholesterolemia, smoking, and diabetes) according to their nature. In most populations, the risk of CVD rises steeply with age, and, at most ages, the risk for CVDs is higher in men than in women, although this difference declines with increasing age and is higher for coronary heart disease than for stroke. The INTERHEART study assessed the association between various modifiable risk factors and MI in 52 countries. In 2004, the study reported in 2004 that abnormal lipids, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, consumption of fruits, vegetables, and alcohol, and regular physical activity accounted for 90% of the risk of MI worldwide in both sexes and at all ages in all regions. This landmark study emphasized that modifying lifestyle and habits approaches would prevent the vast majority of CVD.[53] Indeed, improvements in cardiovascular risk factors (CVRF) is associated with reduced mortality, particularly in people with established CVD.[54] Furthermore, the earliest possible prevention of CVD from childhood onwards will prevent an increased total CVD risk in adult ages.

27.6.1 Hypertension: the most prevalent cardiovascular risk factor

Hypertension, defined as systolic BP ≥ 140 mmHg and/or diastolic BP ≥ 90 mmHg, is considered the most prevalent risk factor for CVD worldwide.[55] In 2010, 31.1% of the global adult population had hypertension, and its prevalence has only grown since then. Prospective observational studies have repeatedly demonstrated a strong,

continuous positive relationship between BP and CVD, including stroke, MI, sudden death, heart failure, and peripheral arterial disease as well chronic kidney disease and cognitive impairment.[56,57] CVD events in hypertensive patients manifest around five years earlier than in individuals with a lower level of BP and is independent of other CVRF.[58] This is noted for both sexes, at all ages and in all ethnic groups. Yet, in adults who are middle-aged or older (≥35 years), systolic BP is a more important predictor of CVD risk than diastolic BP[59] and, in the elderly, increased pulse pressure (as a result of arterial stiffness) also has an important prognostic role regardless of the BP.[60] The prevalence of hypertension also shows a steep increase with aging, in both men and women.[55,61] Men have higher blood pressure at younger ages than women, yet, at around 60 years, women have a higher prevalence of hypertension than men. The association between BP and CVD risk underscores the need to treat hypertension, especially when severe, and implement preventive strategies to reduce BP-related CVD risk in those who have a normal to high BP level to at least slow the age-related tendency for individuals to develop hypertension.[62]

Randomized clinical trials have demonstrated that lowering BP with commonly used regimens (Fig. 27.2) reduces CVD risk and all-cause mortality.[63] However, among all hypertensive-treated patients, roughly one-third are controlled. The main reasons for this lack of control include: (1) low adherence; (2) inadequate medication dosage; (3) intolerance to medication, and (4) resistance to treatment (refractory

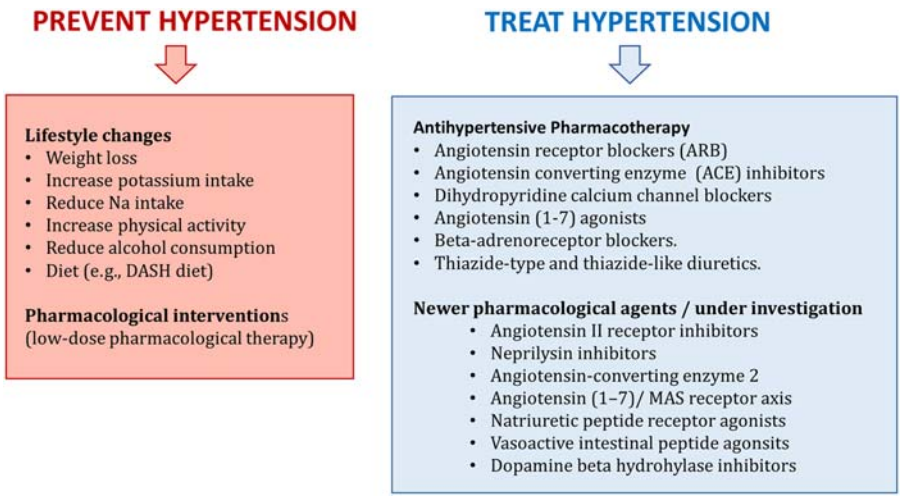

Figure 27.2 Different strategies addressed to prevent or treat hypertension. Hypertension is one of the most prevalent cardiovascular risk factors worldwide. This picture depicts different pharmacological compounds (currently under clinical use or underdevelopment) and lifestyle changes that most effectively lower and prevent blood pressure raise. *DASH diet*, Dietary Approaches to Stopping Hypertension

hypertension). Hence, there is still an unmet need for those who cannot tolerate conventional therapy or are refractory to multiple medications. Moreover, synergisms occur when hypertension is combined with other CVRF leading to a higher total cardiovascular risk than the presence of each CVRF alone. On the other hand, several modifiable risk factors are associated with increased risk of hypertension,[64] and accordingly, lifestyle changes are the most effective strategy for lowering BP and preventing hypertension (Fig. 27.2). It is, nevertheless, important to note that the prevalence of hypertension tracks from childhood to adulthood and is associated with harmful cardiac and vascular effects associated with premature CVD in adulthood.[65] Hence, the early identification and effective treatment of hypertension in children and adolescents are key in the primordial and primary prevention of CVD, particularly for at-risk individuals, such as those with obesity and diabetes. On the other hand, in adults with a normal BP, randomized controlled trials have shown that low-dose pharmacological therapy with an angiotensin receptor blocker[66] and angiotensin-converting enzyme inhibitor,[67] or chlorthalidone/amiloride combination[68] are effective in lowering BP and preventing hypertension.[66]

27.7 Low-density lipoproteins-cholesterol lowering: the lower, the better

Cholesterol circulates through the blood in micelle-like particles called lipoproteins. There are three major classes of lipoproteins carrying cholesterol. Low-density lipoproteins (LDLs), high-density lipoproteins (HDLs), and very-low-density lipoproteins, which typically constitute 60%–70%, 20%–30%, and 10%–15% of the total cholesterol, respectively. While high levels of LDL-cholesterol (LDL-c) can lead to atherosclerosis, HDL-c seems to reduce the risk of CVD in primary prevention due to its major role in reverse cholesterol transport, in addition to other multiple vasculoprotective effects.[69,70] Yet, Mendelian randomization studies and clinical trials aimed at raising HDL-cholesterol levels in secondary prevention have not demonstrated the expected HDL-related benefits.[71,72] It has become increasingly evident that HDL function is more important than HDL-c levels.[70,73–76]

LDL-c plays a key role in the pathogenesis of atherosclerosis.[76] Atherosclerosis is a chronic, inflammatory disease in the intima (with secondary involvement of the media and adventitia) of large- and medium-sized arteries that begins with the development of fatty streaks (early lesions) in childhood and adolescents and progresses with aging.[11] The continuous exposure to CVRF damages the endothelium, which becomes activated and eventually dysfunctional. Endothelial dysfunction is the first step in atherosclerotic lesion formation, entailing compromised endothelial barrier integrity and the generation of proinflammatory cytokines and chemokines that favor leukocyte recruitment, adhesion, and subendothelial transmigration.[69,76] Hence, the dysfunctional

endothelial cells allow LDL particles to infiltrate towards the intima, where they suffer multiple modifications including oxidation, aggregation, and glycosylation. Once modified, LDL particles promote the formation of foam cells derived from smooth muscle cells and macrophages, thereby increasing atherosclerotic plaque vulnerability.[69] The atherosclerotic process advances silently through lipid core expansion and macrophage accumulation at the plaque edges, leading to fibrous cap rupture.[10] Based on the role of LDL in inflammation and atherosclerosis, lowering LDL-c levels has become the main therapy to reduce cardiovascular risk.[69] Statins reduce the synthesis of cholesterol in the liver by competitive inhibition of 3-hydroxy-methylglutaryl coenzyme e A reductase (HMG-Co-AR), the rate-limiting enzyme in cholesterol biosynthesis. The reduction in intracellular cholesterol promotes increased LDL receptor expression at the surface of the hepatocytes, which in turn results in increased uptake of LDL from the blood and the subsequent decrease in plasma concentrations of LDL- and other ApoB-containing lipoproteins. Statins are the first-line therapy based on their high safety and efficacy to reduce LDL-c.[77] A meta-analysis of statin trials by the Cholesterol Treatment Trialists' Collaboration revealed a 22% relative reduction in major vascular events per mmol/L reduction in LDL-c.[77] In addition to LDL-c levels, LDL phenotype also determines CV risk since small dense LDLs have shown a longer circulation time and a more atherogenic profile than large LDLs. This was supported by the ARIC (Atherosclerosis Risk In Communities) [78] and the multi-ethnic study of atherosclerosis studies which demonstrated that small dense LDL-c was associated with a higher risk of coronary heart disease.[79] Guidelines for the management of dyslipidemia were updated in late 2019 in light of recent intervention trials involving innovative lipid-lowering agents combined with statins.[80] The new guidelines advocate for achieving very low LDL-c levels in individuals at the highest risk within the "lower is better" paradigm.[81] In this regard, combination therapy using ezetimibe (a lipid-lowering compound that inhibits intestinal cholesterol and phytosterol absorption) and/or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (monoclonal antibodies against PCSK9 that prevent internalization and lysosomal degradation of LDL receptors) in addition to statins, have allowed for the achievement of extremely low LDL-c.[81,82] Fairly recent clinical trials have shown that profound LDL-c lowering leads to a further reduction in cardiovascular events compared with more moderate lipid-lowering, with no associated safety concerns.[81] However, because of the lifetime exposure of this major CVRF to the vessel, LDL lowering should be started earlier in life to prevent the accumulation of LDL-c and its consequences of atherosclerotic CVD.[83,84] In this regard, the best way to lower LDL-c levels is to rely on implementing a healthy diet (e.g., DASH diet), becoming more physically active, and losing weight. Weight loss of as little as 5% to 10% can help improve cholesterol numbers.[85]

27.7.1 The need to control the obesity pandemic

Obesity is most commonly defined by determining a body mass index (BMI) ≥ 30 kg/m^2.[86] Although BMI does not provide discriminatory information concerning an individual's body fat percentage or distribution, obesity has been subdivided into the following classes: Class I: BMI = 30.0−34.9, Class II: BMI = 35.0−39.9, and Class III: BMI ≥ 40.0 to further stratify the risk. Overweight and obesity are strong risk factors for the development of CVD and have shown to be strongly linked to mortality and multiple comorbidities.[87] Although the exact mechanisms connecting obesity and the development of these conditions are not completely understood, adipose tissue can enlarge and secrete proinflammatory cytokines and chemokines that can directly induce atherosclerosis progression, may affect the susceptibility to thrombosis, and may impair myocardial function.[88] Further, obesity usually coincides with a cluster of CVRF, including hypertension, dyslipidemia, insulin resistance, and diabetes. Accordingly, obese individuals are at an increased risk for developing CVD, particularly coronary heart disease, hypertension, atrial fibrillation and heart failure (HF). Yet, despite the prevalence of obesity in patients with HF seems to be as high as 15% or more,[89] within the last decade, multiple observational studies and meta-analyses have suggested that patients with HF who present overweight and mild-to-moderate obesity tend to have better outcomes compared to patients with normal weight (18.5−24.9 kg/m^2), particularly in the setting of reduced CVRF.[90] This phenomenon has been termed the obesity paradox and has also been shown to occur in the elderly.[91–93] The mechanisms behind this paradox have been difficult to ascertain, yet, numerous theories have been proposed, including the inadequacy of BMI as an obesity classifier, the presence of confounding factors, methodological limitations, and others (Fig. 27.3). In fact, a recently published meta-analysis has concluded that obesity does not have an independent protective effect in patients with chronic HF, casting doubts about the real relevance of this phenomenon.[94] Nevertheless, despite the obesity paradox, weight loss, especially when associated with increases in physical activity and exercise training, is linked with considerable benefits in patients with CVD. Furthermore, if obesity would have been prevented, patients would have not developed that specific CVD in the first place.[95]

27.7.2 Primordial prevention: the sooner, the better

Clinical practice in asymptomatic populations has focused on primary prevention by modifying already present CVRF to prevent an initial cardiovascular event.[96,97] Yet, population studies have revealed the importance of promoting healthy life habits at younger ages to prevent the development of risk factors starting in children (particularly efficacious from 3 to 8 years) and adolescents, a termed labeled primordial prevention.[98] As stated above, particularly worrying trends show increased levels of LDL-c and obesity at younger ages.[99,100] These trends underline the need to implement and acquire healthy

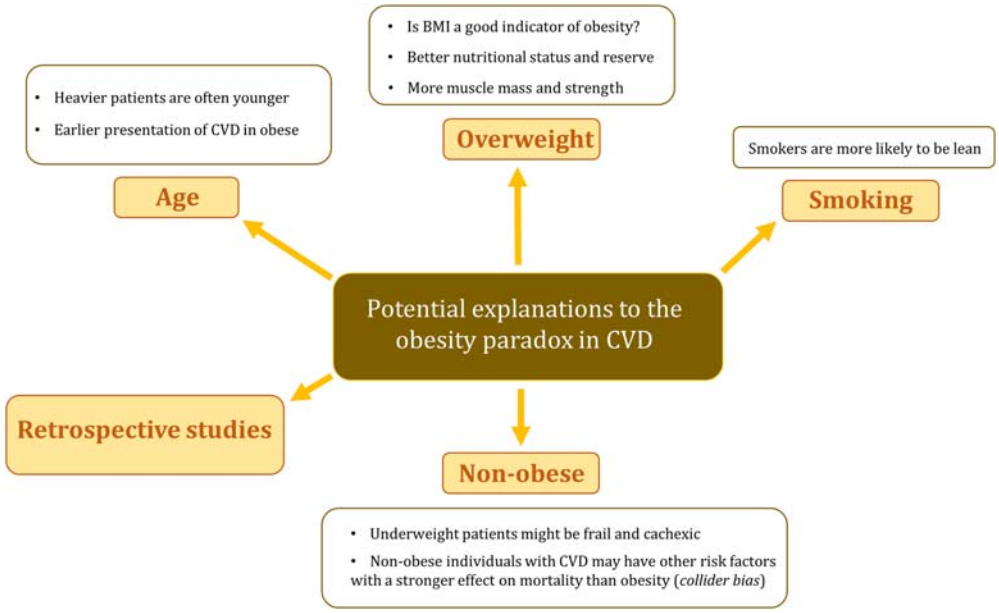

Figure 27.3 Potential explanations to the obesity paradox in cardiovascular disease. Theories that have been proposed to explain the obesity paradox in which patients who present overweight and mild-to-moderate obesity tend to have better outcome compared to patients with normal weigh under certain pathological circumstances (e.g., heart failure).

habits (diet and physical activity components) early in life since LDL-c levels in early adults are associated with accelerated subclinical atherosclerosis and an excess of atherosclerotic cardiovascular events later in life.[101,102] The randomized prospective special turku coronary risk factor intervention project (STRIP) investigators demonstrated that ongoing dietary intervention resulted in significantly lower total- and LDL-c at 14 years of age.[103] The results of this study are consistent with those of the Dietary Intervention Study in Children (DISC), a shorter-term study done in school-age children in the United States.[104,105] It is important to note that the atherosclerotic process dates back to fetal age.[104] Several studies have reported that the sustained exposure of high LDL-c in the vasculature of the fetus may lead to premature CHD. Furthermore, pathological studies have demonstrated the presence of fatty streaks in the aorta and coronary arteries in childhood, whose extension is, in turn, associated with LDL-c levels.[106,107] Accordingly, early identification of children with severe hypercholesterolemia (either primary or secondary hypercholesterolemia) is important to prevent early development of atherosclerosis, when an enhanced benefit can still be obtained via lifestyle modifications and, if needed, therapy.[84,108] In this regard, the CURE ATHERO (Curing Early Atherosclerosis) trial aims to determine whether intensive LDL-c lowering with treatment for 3 years is able to regress early atherosclerosis.[109] Entering adulthood without risk factors is decisive for the future, so it is crucial to intervene at earlier stages.[110]

27.8 Concluding remarks

Preventive medicine, including a healthy lifestyle, is the solution for a longer and healthier life in the population. Giving "life to years" in addition to "years to life" is the objective of medicine nowadays. However, in many pathophysiological areas, the present situation is still the reactive treatment or "response to injury" approach. In many instances, this is just too late to reach the objective of living without disease. We need more scientific knowledge of chronic and age-related diseases at a genomic, molecular, and cellular level to design the appropriate strategies. This generated understanding will be of high value to develop new advances in medicine that will be the application of precision medicine to each characterized group of patients with similar traits and personalized medicine to singular patients with specific requirements.

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CHAPTER 28

Personalized medicine: will it work for decreasing age-related morbidities?

Christopher R. Martens[1], Devin Wahl[2] and Thomas J. LaRocca[2]
[1]Department of Kinesiology & Applied Physiology, University of Delaware, Newark, DE, United States
[2]Department of Health & Exercise Science, Colorado State University, Fort Collins, CO, United States

28.1 Introduction

Major advances over the past several decades have identified many of the underlying molecular mechanisms of aging. These discoveries have led to new methods for determining one's "biological age," as well as interventions aimed at slowing down the process of aging, with the ultimate goal of preventing or delaying chronic age-related diseases. Despite the clinical promise of these cutting-edge therapies, they will likely be associated with heterogeneity in individual responsiveness and a narrow risk-to-benefit ratio, given that these interventions will presumably be administered to otherwise healthy individuals for the purpose of primary prevention. Accordingly, the most effective strategies for improving human healthspan may come from the convergence of two rapidly emerging fields of study: (1) GeroScience—a field devoted to understanding the molecular mechanisms of aging in order to slow its progression, and (2) Personalized Medicine—a field devoted to creating tailored treatments based on individual patient characteristics. This chapter will focus on the potential of personalized medicine for creating individualized treatments to prevent or reverse the biological mechanisms of aging. We will provide a brief background on the field of GeroScience and its important role in facilitating the application of personalized medicine to anti-aging interventions. We will then discuss several unique challenges that must be overcome in order for a "personalized aging" approach to successfully attenuate age-related morbidities.

28.2 Historical perspective

Over the past century, most Western societies have experienced significant increases in life expectancy. Initially, these outcomes were primarily driven by large public health initiatives, including improved food preparation and safety, cleaner drinking water, and advances in housing and sanitation that led to reductions in infant mortality and premature death among young and middle-aged adults. More recently, the

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development of vaccines, antibiotics, and disease-specific therapies have contributed to a steep increase in life-expectancy, mostly by extending the survival time of older adults living with one or more chronic diseases.[1] A combination of these factors likely still contributes to increases in life expectancy today, and it has been argued that the limit of human life expectancy has not yet been reached.[2] Due to the overwhelming success of these public health and modern medical advances, it is estimated that by 2050, the number of people over the age of 65 in many Western countries will nearly double (e.g., in the United states, from ~49 million in 2016 to 83.7 million in 2050).

One of the major concerns with increased life expectancy is that older age is the greatest risk factor for most common chronic diseases, including cardiovascular, metabolic, and kidney diseases, sarcopenia (and related muscle disease), cancers, frailty, and dementia (collectively known as the multimorbidities of aging).[3] It is projected that the increased prevalence of these diseases as a result of the growing older adult population over the ensuing decades will stress the current healthcare system, with an estimated $346 billion going towards healthcare for older adults in 2040 (as opposed to $207 billion in 2020).[4] Therefore, an important current goal of aging research is to develop and identify strategies (e.g., pharmaceutical, nutritional, lifestyle) to increase healthspan (the period of life during which we are healthy and disease-free), improve quality of life in older age, and reduce the financial burden of an aging population on healthcare systems.

28.3 Slowing aging with GeroScience

The conventional approach to addressing chronic disease in Western medicine is to treat specific diseases when they arise. This strategy often results in numerous procedures and prescriptions over the course of an individual's lifespan, and it is common for older adults to be prescribed five or more daily medications for the treatment of different age-related conditions.[5] The frequent application of this "polypharmacy" among older adults is not only potentially harmful to health (i.e., due to adverse interactions among drugs),[6] but fails to address the single risk factor that unifies all age-related conditions, the biological aging process itself. Accordingly, the current approach is not likely to slow aging and reduce the burden of chronic disease. As a result, a new field called "GeroScience" has emerged with the goal of targeting the aging process itself to *prevent* age-associated diseases as a group.

28.3.1 The biological hallmarks of aging

A major goal of GeroScience is to emphasize and understand the role of biological aging mechanisms, and to show that targeting these mechanisms directly may be the most effective way to increase healthspan.[7] This approach focuses on key cellular and molecular mechanisms that underlie the aging process, which are collectively known as the

"hallmarks of aging." These hallmarks include: genomic instability, telomere shortening, epigenetic changes, reduced proteostasis, dysregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication.[8]

Documentation of these aging hallmarks has led to a search for new compounds and intervention strategies that delay aging, increase lifespan and inhibit age-associated diseases (discussed in depth in Gonzalez-Freire et al.[9] and in earlier chapters). For example, the loss of protein homeostasis (proteostasis) may be partially reversed via supplementation with autophagy activators (e.g., spermidine), which enhance degradation and the recycling of damaged cellular components (proteins and organelles). Compounds that target dysregulated nutrient sensing (e.g., rapamycin, metformin, resveratrol) act on proteins involved in energy signaling pathways, including the mechanistic target of rapamycin (mTOR) and silent mating type information regulator 1 (SIRT1).[10] More recently, senolytic compounds have been developed to clear senescent (cell cycle-arrested) cells, which are thought to drive age-related tissue dysfunction and inflammation.[11] There is mounting evidence that these compounds and others like them may effectively target aging and reduce age-associated diseases, especially in preclinical models. Based on this work, several complementary clinical trials have emerged to test the efficacy of similar compounds for delaying age-related morbidities with a current emphasis on those that have already received approval from the US Food and Drug Administration (FDA) for other indications. For example, the targeting aging with metformin (TAME) trial is a large, multi-site clinical trial that will investigate the FDA-approved drug metformin typically prescribed for type 2 diabetes) as a potential candidate for preventing the accumulation of age-related diseases in otherwise healthy older adults.[12] Several other promising drug candidates for inhibiting aging include acarbose (an anti-diabetic drug that reduces cardiovascular disease risk in humans),[13] angiotensin converting enzyme (ACE) inhibitors, and aspirin, which reduce the risk of cardiovascular disease, cancers, and cognitive dysfunction with aging.[14]

In addition to new drug development, there also is interest in non-pharmacological strategies, including dietary supplements and "nutraceutical" compounds, as well as novel lifestyle paradigms (e.g., specific diet and exercise strategies) for targeting the hallmarks of aging. For example, clinical trials have investigated the safety and tolerability of endogenous precursors of nicotinamide adenine dinucleotide (NAD^+) in middle-aged and older adults.[15] NAD^+ is necessary for the activation of energy sensing and DNA repair enzymes, and it declines with advancing age. However, supplementation with exogenous NAD^+ precursors (e.g., nicotinamide riboside) has shown promise for extending healthspan in preclinical models by reversing several hallmarks of aging. Similarly, several promising lifestyle and dietary interventions may inhibit multiple aging pathways in humans; these include caloric restriction (CR, reducing total calorie intake),

intermittent fasting (IF, alternating periods of feeding and fasting) and protein restriction.[16–18] These interventions mainly enhance metabolic pathways that have downstream (protective) effects against the hallmarks of aging. While the efficacy of these interventions in humans is still under study, emerging data suggests that CR and IF may at least benefit vascular health with aging. In fact, one recent study demonstrated that long-term (2-year) 25% application of CR, increased cardiometabolic health in younger, sedentary humans.[16] Studies also suggest that protein restriction may reduce some cancers, and may protect the brain and improve cognition during aging in mice.[19]

28.3.2 A case for personalized aging

While there is mounting evidence that targeting the hallmarks of aging may hold promise for the extension of human healthspan, the underlying mechanisms are controlled by highly conserved and biologically redundant pathways. As such, off-target effects of drugs (and perhaps nutritional interventions) are possible. As an example, rapamycin is an established immunosuppressant that has been shown to extend lifespan in animal models through the inhibition of the mTOR signaling pathway. However, rapamycin treatment may also have adverse side effects including insulin resistance and hyperglycemia in some populations.[20] Similarly, despite promise for extending healthspan in animal models and emerging safety data in humans, it has been suggested that NAD^+ precursors and certain senolytic drugs may fuel specific cancers under certain conditions.[21] Nutritional interventions, like CR and protein restriction, are also a concern in frail older adults, as these dietary patterns may drive sarcopenia, impair wound healing, and suppress the immune system.[22] Thus, more work is needed to determine the optimal dose, duration, and ideal target populations to receive these preventive therapies. Finally, not all humans respond to interventions in the same manner, most likely due to a combination of genetic and environmental factors.[23] Therefore, it has been suggested that rather than prescribing certain compounds or interventions broadly (e.g., an "anti-aging poly-pill"), a more "personalized" approach may be the best path towards healthspan extension.

28.4 Personalized medicine for optimal longevity

Personalized medicine (also known as precision or individualized medicine) refers to the tailoring of interventions to an individual person for the treatment or prevention of a disease. The goal is to deliver the correct intervention at the optimal time and dose to those individuals who stand to benefit the most.[24] Although the concept of personalized medicine has existed for several decades, it has gained more widespread attention following the sequencing of the human genome in 2003. Since then, large Genome-Wide Association Studies (GWAS) have identified common genetic variants associated with

drug responses to the treatment of specific diseases. One of the earliest examples was the identification of a single-nucleotide polymorphism (SNP) in the *IL28B* gene that is highly associated with the response to peginterferon-α, a drug used to treat hepatitis C infection.[25] More recent advances in next-generation sequencing and other "omics" technologies have made it possible to identify and selectively target specific forms of cancer, based on genetic mutations within tumor cells. For example, the chemotherapy drug trastuzumab (Herceptin) was approved for use in breast cancer patients whose tumors overexpress the human epithelial growth factor 2 (HER2) protein.[26] These examples highlight the potential impact of precision medicine on the treatment of specific diseases for which there is a known genetic driver. However, it remains to be determined whether precision medicine will work similarly to delay, treat, or reverse the hallmarks of aging and extend healthspan.

28.5 Emerging predictors of "biological aging"

Against the backdrop of aging research and personalized medicine, strong interest has developed in biomarkers that predict biological age. Conceptually, an accurate "biological aging clock" could both predict chronic disease risk and, perhaps, identify specific intervention targets (e.g., hallmarks of aging that are particularly advanced in an individual patient). Some work in this area has focused on traditional biomarkers like cholesterol and circulating inflammatory cytokines,[27] but there is growing interest in more specific readouts that directly reflect biological mechanisms of aging (e.g., telomere length, which decreases with age and is associated with adverse health outcomes). More recently, several groups have harnessed omics approaches to comprehensively and simultaneously measure molecules of the same type (e.g., RNA expression with RNA-sequencing) in biological samples. Most of these approaches are powerful, but also costly and complex, and they involve numerous considerations like tissue specificity and inter-assay variability. Still, this approach is a particularly promising area of future research on aging and offers a strong potential for personalized medicine approaches for optimal longevity.

28.5.1 Epigenetic clocks

Early aging-targeted omics work focused on the epigenome and, in particular, age-related changes to the methylation status of the cytosine-guanine dinucleotide (CpG) bonds found in human DNA. The methylation of CpG sites along the genome plays an important role in regulating gene expression, usually by inhibiting transcription factor binding around gene promoter regions. However, more recent evidence suggests that methylation of loci within non-coding areas of the genome may also be important for maintaining genomic stability, and thus may have important implications for regulating healthspan. Methylation status is strongly influenced by aging and environmental/genetic factors and may therefore serve as a biomarker of biological age. In this regard, age-related epigenetic

changes at specific genomic loci have been characterized in multiple tissues and have been used to develop a variety of "epigenetic clocks" that predict mortality risk and other relevant aging outcomes,[28,29] and in some cases even incorporate clinical biomarkers for more accurate estimates.[30] These clocks have been used to derive various predictions of "DNA methylation age," and may one day be useful for identifying patients at risk for accelerated aging, and in need of lifestyle or pharmacological intervention. Whether these clocks are amenable to change by lifestyle or pharmacological intervention remains to be determined and is a growing area of research.

28.5.2 Transcriptomics

A limitation of epigenetic clocks is that the mechanistic links between methylation profiles and specific hallmarks of aging are poorly understood. In this context, transcriptomic age predictors that measure global gene expression itself may hold more promise. Indeed, next-generation sequencing has made transcriptomics (RNA-seq) more accessible, and several groups have documented transcriptional signatures of aging in multiple tissues.[31,32] Several of these studies have identified age-related transcriptional changes associated with the hallmarks of aging, including mitochondrial dysfunction and DNA damage. Other groups have used machine learning approaches to predict age based on the transcriptome[33] and have demonstrated that these approaches may be more accurate than epigenetic clocks. Interestingly, emerging evidence also indicates that non-coding and even repetitive RNAs (e.g., transposons, which are often ignored in RNA-seq studies) can be used to predict age, and may even reflect biological age more accurately than the standard (gene) transcriptome.[34]

28.5.3 Metabolomics and proteomics

In addition to genome-focused approaches, metabolome and proteome studies may be useful for predicting biological age, as these "-omes" contain countless analytes. Most metabolomics work has focused on circulating (plasma) markers, and some groups have identified lipids and other metabolites related to key pathways (e.g., glycolysis) that predict age, future mortality, and indicators of healthspan.[35–37] Others have shown that metabolomic signatures are unique in centenarians and/or their offspring,[38–40] and that certain metabolites (e.g., specific amino acids, fatty acids and nucleotide metabolites) are related to physiological declines with aging.[41] These observations underscore the potential for metabolomics to predict biological age per se. However, as current technical limitations make it impossible to profile the entire metabolome, the exact utility of this approach remains to be seen. In fact, the same is true for proteomics (the proteome is particularly large); as a result, some groups have pursued "targeted" proteome analyses like the SOMAscan assay, which captures ~5000 specific proteins with known predictive value. This strategy has been used to

identify specific circulating proteins associated with age and health outcomes,[42,43] as well as predictors of frailty, mortality and even cognitive function in older adults.[44–46]

28.5.4 The gut microbiome

One final, emerging omics approach for studying biological age is to profile the microbiome (bacteria/microbes populating the gastrointestinal tract and other regions). In general, the microbiome becomes less diverse with aging, and this is coupled with a shift in microbial populations. Age-related microbial "dysbiosis" is linked to inflammation and other hallmarks of aging, as well as multiple age-associated diseases.[47] Certain microbial profiles have also been identified in healthy versus unhealthy older adults,[48] but so far there is no consensus on any "clock-like" microbiome assay. Ideally, this would include parallel measurements of the metabolites that may mediate the effects of good/bad microbes,[49] but work in this area is just beginning.

Finally, an important caveat to measurements of biological age is the need to also account for organismal health. Indeed, in some respects, physiological readouts that directly reflect health, function and quality of life may be more informative than molecular measurements. For example, measurements of cognitive function, strength/coordination, body composition, etc., along with standard clinical variables like blood tests and blood pressure can be combined into composite scores that differentiate healthy (biologically young) versus unhealthy (accelerated aging) adults.[50] Approaches like this are time- and labor-intensive, but they may have more "real world" relevance, depending on the goal, than models that simply predict one's chronological age.[51,52] Ideally, such functional measurements would be combined with the molecular/omics measures described above for highly personalized and accurate estimates of biological age (Fig. 28.1), as several groups have reported in "multi-omic" studies.[53–55]

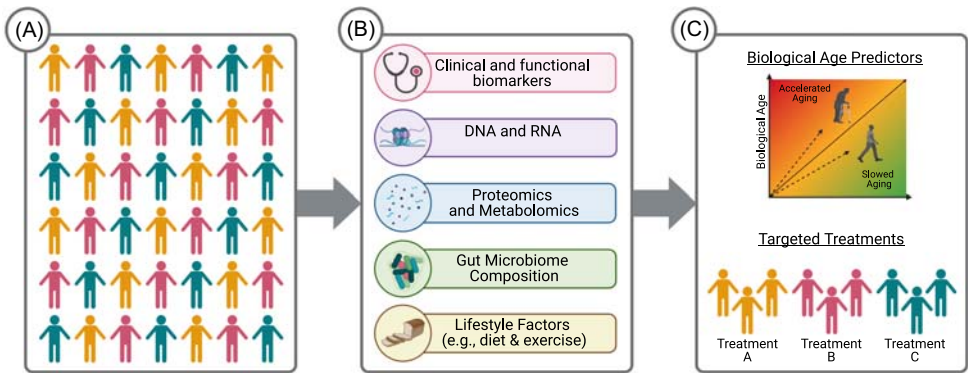

Figure 28.1 Generalized framework for personalized aging. (A) The heterogeneity of human aging requires (B) broad phenotyping of clinical and functional characteristics combined with molecular biomarkers to (C) more accurately determine biological age and predict responsiveness to interventions.

28.6 Applying personalized medicine to GeroScience

As the field of GeroScience continues to grow and develops new therapies for slowing the biological process of aging, there is growing interest in applying principles of personalized medicine to ensure that these interventions reach the correct patients. A "personalized aging" approach may be the safest approach to implementing new therapies and therefore more likely to obtain regulatory approval from government agencies (e.g., the US FDA) that have expressed caution about treating aging as a bona fide disease. Unlike conventional medicines, which currently receive regulatory approval for treating specific diseases, anti-aging drugs will presumably be prescribed to healthy individuals to prevent aging, well before the emergence of age-related symptoms. Therefore, the risk-to-benefit ratio of these new interventions must be considerably low in order to receive regulatory approval. In this regard, a personalized medicine approach has potential to help distinguish among those individuals most likely to receive a benefit while preventing untoward side effects in others.

Initial "proof-of-concept" studies of anti-aging interventions have focused on titrating the dose of repurposed medicines for preventing hallmarks of aging in healthy individuals. For example, Mannick et al. demonstrated that low dose administration of the mTOR inhibitor rapamycin improved immune system function in healthy older adults, with fewer side effects than the high dose that is typically prescribed for immunosuppression in transplant patients.[56] Similar studies have demonstrated differential responses depending on dosage of the anti-diabetic drug metformin, which is now being investigated as a potential therapy for slowing multiple age-related diseases through the TAME trial.[57] These studies and others like them will undoubtedly provide an important basis for determining the optimal dosing regimen for approved drugs that are repurposed as preventive-aging medicines in healthier adults.

Careful end-point selection will be critical for determining success of anti-aging medications, as it will take years, if not decades, before an appreciable increase in a person's healthspan can be observed. One approach is to incorporate multiple intermediate end-points, such as indicators of healthspan and physiological function, into early clinical trials of anti-aging interventions. Such trials will not only help with target end-point selection, but provide greater opportunities for exploring baseline factors that may contribute to drug responsiveness. For example, a short-term pilot study examining the effects of the NAD^+-boosting nutraceutical compound, nicotinamide riboside (NR), found no major improvements in physiological function in a group of healthy middle-aged and older adults. However, when the results were analyzed in subjects with elevated systolic blood pressure at baseline, there was an improvement in blood pressure and arterial stiffness.[15] Larger-scale studies in hypertensive older adults are now underway (NCT03821623, NCT04112043) to confirm this initial observation. Without hard evidence that a drug or intervention slows the progression of aging,

future trials may also consider incorporating surrogate biomarkers of aging (e.g., biological age) as endpoints to determine if a drug has anti-aging properties.

28.6.1 Personalized nutrition

One promising subfield of personalized medicine known as "personalized nutrition" holds immense promise for the field of aging and may carry substantially less risk than repurposed drugs or new chemical entities.[58] Negative lifestyle behaviors, including eating an unhealthy diet and a lack of physical activity, represent major independent risk factors for numerous chronic diseases and compound the underlying effects of aging. Historically, guidelines for nutrition and physical activity have adopted a one-size-fits-all approach. However, like the development of personalized approaches to medicine, efforts are underway to develop more targeted lifestyle- and diet-based interventions. In this regard, personalized nutrition seeks to integrate complex health information such as age, sex, genetic make-up, and metabolism, to develop individualized diet recommendations for optimal health and wellbeing. Like medicine, a diet that works in one person may not be effective at increasing healthspan in another individual due to individual differences in metabolism. Thus, personalized nutrition aims to recommend the right diet to the correct individual at the appropriate time to prevent or treat disease.

The field of personalized nutrition is still in its infancy and will likely require the convergence of multiple disciplines to resolve the complex interactions among food, biology and the environment. However, recent efforts to identify genetic variants that are predictive of one's response to certain foods or diets have begun to unravel the complex interaction between food and physiology, and this has led to a subfield of personalized nutrition known as "nutrigenomics," which may have important implications for aging. In this field, several common SNPs have been identified on genes involved in the metabolism of common nutrients in the human diet. Much of this genetic variation evolved over hundreds of thousands of years as humans populated the earth and encountered variations in climate and food sources that necessitated selection towards differences in metabolism. A well-known example is a common variant in the gene that increases expression of the enzyme lactase, which metabolizes the sugar lactose that is found in milk and other dairy products. Natural selection favored this trait following the domestication of dairy cattle; however, certain segments of the population lack this particular mutation, making them lactose intolerant. Other SNPs have more recently been identified in genes that are involved in the metabolism of fatty acids and have implications for the development of cardiovascular disease and other age-related conditions. For example, results from the Coronary Diet Intervention With Olive Oil and Cardiovascular Prevention (CARDIOPREV) study identified an interaction between a specific SNP (rs12696304) on the telomerase

RNA component (*TERC*) gene and monounsaturated fatty acid metabolism.[59] Individuals carrying this mutation exhibited a slower rate of telomere attrition and reduced inflammation when consuming a Mediterranean diet, suggesting that underlying genetics may interact with diet to control the rate of aging. There is already strong commercial interest in exploiting these associations through direct-to-consumer genome sequencing platforms, which seek to provide personalized diet recommendations to individuals based on their underlying genetics.[60] However, more research is needed on the interaction between specific genetic polymorphisms and markers of biological aging before this strategy can be put into practice to slow the rate of aging.

In addition to genetics, there is growing interest in the interactions among diet and other clinical and molecular biomarkers including the gut microbiome.[61] In this regard, clustering of individuals by their metabolic phenotype (i.e., "metabotyping") has been used to predict postprandial responses to various diets and may be a useful strategy for employing personalized nutrition.[62] In a landmark paper by Zeevi et al., machine-learning was used to predict postprandial glycemic response to complex diets from basic clinical markers and the composition of the gut microbiota.[63] The exact composition of microbes in the human gut varies from person to person and is highly dependent on complex factors including host-bacteria relations and environmental factors including diet. Emerging evidence over the past decade has revealed an important influence of the gut microbiota on the metabolism of nutrients and subsequently on host physiological function and disease risk. Although this is still an emerging field, there is growing potential to exploit inter-individual differences in gut microbiota to make personalized recommendations about food intake.

The interaction between genetics and diet, and their combined effects on health and physiological function, depend largely on exposure time, thus having important implications for aging. By identifying such gene-nutrient interactions, personalized nutrition has the potential to predict those at risk for detrimental effects of certain foods or diets and therefore prevent a lifetime of deleterious exposure that accelerates the aging process. Identification of genetic mutations likely to respond favorably to particular diets could help generate targeted nutrition recommendations for achieving optimal longevity.

28.7 Challenges and barriers to implementing personalized aging

Despite many benefits, there are several unique challenges that must be overcome before personalized aging can be successfully adopted as a strategy for increasing healthspan. The next section will highlight many of these challenges and offer insight into potential strategies for overcoming barriers to success.

28.7.1 Minimizing the risk-benefit ratio

Because the goal of GeroScience is to increase healthspan, the primary target population for personalized aging strategies is mostly healthy individuals looking to prevent, rather than treat, chronic disease and disability. Unlike treating diseases, for which the benefits (e.g., a cure or reduction in morbidity) more clearly outweigh the potential risks, it will be difficult to evaluate the risk-benefit ratio of interventions designed to prevent aging in healthy individuals. This problem is not specific to personalized aging. For example, similar concerns have been raised regarding the use of a nonspecific "polypill" to prevent cardiovascular diseases, which could lead to unnecessary side effects in healthy, asymptomatic individuals.[64] However, personalized aging may ultimately be the safest approach to lowering the risk-benefit ratio of anti-aging medicines by separating those most likely to receive a benefit from those more likely to be at risk. To be successful, clinical trials of future anti-aging interventions should include multi-omic biomarker assessments that can be used for later determination of responders and non-responders for personalized aging. It will also be important to ensure that patients' personal health information is protected.

28.7.2 Increasing diversity in clinical research

A major challenge in clinical research is a lack of diversity among study participants. For example, recent analyses indicate that black, Asian, and Hispanic individuals are consistently underrepresented in studies overseen by the FDA.[65,66] This reduces how generalized the study results are and could lead to unnecessary exposure to ineffective medicines or risk of adverse reactions in certain racial and ethnic groups. Moreover, predictive algorithms used to make personalized drug decisions have historically relied on datasets that are equally lacking in diversity. For example, it has been estimated that 96% of subjects included in all GWAS studies conducted to date are of European descent,[67] undermining the ability to accurately predict drug responses in other racial or ethnic groups. Successful implementation of personalized aging will require larger, more diverse datasets than presently exist to ensure that predictive algorithms reflect the racially and socioeconomically diverse patients they are intended to serve. Fortunately, efforts are underway to address the lack of diversity in biomedical research. For example, the NIH-sponsored "All of Us" trial (allofus.nih.gov) aims to generate the largest and most diverse health database in human history.[68] Coordinated efforts to fund research in personalized nutrition using the All of Us cohort are currently underway and a similar case could be made for future studies focused on personalized aging. These studies should ideally enroll subjects across a wide age range (i.e., not only older adults, but also those in middle/late-middle age) as certain interventions may have differential effects depending on the age of the individual.

28.7.3 Improving adherence and accessibility

In order to successfully tame the burden of age-related diseases on society, it will be critical for governments, corporations, and healthcare systems to a make these services affordable and accessible to the majority of individuals. Personalized aging may require costly screening procedures (e.g., genomic sequencing) and more frequent follow-up visits that could result in poor adherence, particularly among those without the time or financial means to participate. Similar concerns have been raised regarding the use of personalized medicine for preventing cardiovascular disease with critics arguing that a less-expensive poly-pill could reach a wider population, potentially having a more significant effect on societal health.[69] One potential strategy for making personalized medicine more affordable is to transition away from "fee-for-service" payment models towards "value-based care," paying physicians for positive outcomes instead of per service or procedure. The Centers for Medicare and Medicaid Services, which provides health coverage for over 50 million older adults in the United States, is currently transitioning to this type of system; however, more broad adoption across the entire healthcare enterprise will be needed to have a meaningful effect on the cost of healthcare.[70] By paying physicians to maintain their patients' health instead of billing for individual diagnostic tests or procedures, patients will be better able to reap the benefits of personalized aging at an affordable cost.

28.7.4 Innovation in clinical trial design

The current process for confirming the efficacy of new drugs involves demonstrating a robust treatment effect in a large number of individuals. Even with established disease indications, large group differences are often difficult to come by (leading to failed trials), and when they do occur, they are usually driven by only a subset of trial participants who respond especially well to the treatment. Ultimately, the goal of personalized medicine is not to determine whether a therapy is effective but rather who the therapy will benefit. Therefore, drugs intended to be used within the context of personalized aging must be tested using innovative study designs that allow for the determination and characterization of "responders" and "non-responders" to the intervention. Unlike conventional trials, where success is determined by a significant and often robust change in hard clinical endpoints associated with a specific disease indication, the success of personalized medicine in aging will be benchmarked by small, incremental changes in surrogate markers of aging (e.g., biological age) and, in some cases, no change in a particular variable over time will be evidence that an intervention is working. These trials will need to collect as much information about baseline characteristics as possible, including biological sex, race and ethnicity, genetics and other omic profiles, environmental and socioeconomic factors, and medical and family histories. This information can then be used retrospectively to predict which subset of subjects responded most favorably to the intervention.

Clinical trials are expensive, time-consuming, and often fail to collect all of the key information needed to make effective decisions for personalized medicine, requiring more costly follow-up studies. One potential strategy to overcome these challenges could be so-called "N-of-1" trials.[71] In this scenario, physicians may prescribe a specific drug or intervention to a patient with the intention of slowing the biological hallmarks of aging. Through frequent follow-up testing, the physician can determine whether the intervention is working (by measuring multiple parameters related to healthspan), or if they should experiment with an alternative treatment. One potential strategy for benchmarking success in this scenario could be the long-term tracking of a patient's biological age relative to chronological age. This so-called "age-acceleration" provides feedback regarding whether the patient is biologically younger or older than their chronological age,[72] and thus whether they are in need of more aggressive intervention to slow the rate of biological aging. When tracked longitudinally, models of biological age have been used to identify individuals exhibiting slowed versus accelerated aging with the simplicity of a blood draw.[41] Rather than comparing a person's results to normative values, physicians can establish an individualized baseline for each of their patients and intervene when they observe a significant deviation in a particular biomarker.[71] This strategy can even be expanded to larger-scale studies in which large groups of patients undergo individualized treatments (i.e., multiple N-of-1 trials) and the aggregate results are later analyzed to find common factors among subgroups of patients that predict success (Fig. 28.2).

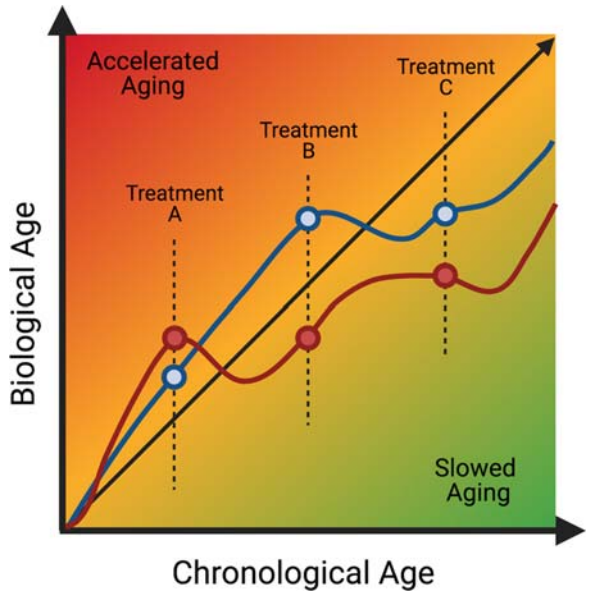

Figure 28.2 N-of-1 Trials for Personalized Aging. Long-term tracking of biological age in two hypothetical patients receiving interventions for slowing the rate of biological aging. The results of such "$N = 1$" trials can be evaluated in aggregate to identify common underlying factors that predict responsiveness to specific interventions.

28.7.5 Ethical and policy concerns

Insurance coverage for genetic screening and other diagnostic tests for personalized aging will require strong evidence that these tests inform clinical decision making and lead to meaningful increases in healthspan. Currently, genetic testing is limited to cases in which there is a clear medical need or benefit and it may be decades before these principles can be implemented for the purpose of slowing human aging. In the meantime, there is already a growing market of direct-to-consumer (DTC) testing of genetic and other biological information and care must be taken to ensure that sensitive health information of patients and their families remains protected.[73] Policies such as the Genetic Information Nondiscrimination Act and the Patient Protection and Affordable Care Act in the United States exist to prevent loss of insurance coverage due to an employer finding out about an employee's underlying genetic risk for disease.[74] These policies will become increasingly important as the amount of individualized patient data used to facilitate personalized medicine continues to expand. Considering the massive amount of data that will be collected over an individual's lifespan for the purpose of personalized aging, it will be especially necessary to implement responsible policies that safeguard patient and consumer data. Consumers and patients should be considered in this decision-making process as primary stakeholders in the generation of these genetic data.[75]

28.7.6 Cross-disciplinary innovation

Scientific progress in artificial intelligence and machine learning is progressing at a rapid rate and there is growing demand for new tools to mine and interpret increasingly large and complex datasets.[76] The adoption of electronic health records and advances in genetic and other omic testing will undoubtedly herald in a new era of computational medicine that will accelerate the development personalized aging. The installation of 5G cellular networks in underserved and rural communities along with new technology for remote patient monitoring and telemedicine will increase patient access and adherence.[77] However, a new generation of scientists will be needed to analyze and interpret these data, and healthcare and insurance programs will need to be reimagined with a focus towards lifelong prevention and away from individual disease management. These challenges can only be solved by cross-disciplinary teams consisting of physicians and allied health professionals, bioinformaticians, statisticians, software developers, genetic counselors, social workers, health coaches, and patient advocacy groups. Only with such investment in new technologies and infrastructure, can personalized aging become an achievable solution to the global crisis of an aging society.

28.8 Conclusion

Ultimately, the question is whether any of the individualized strategies described above will work to ameliorate the hallmarks of aging and collectively reduce the burden of

age-related disease and disability on a large enough scale to have lasting societal impact. The sheer magnitude of work required to make personalized medicine a reality for targeting aging is daunting but not out of reach. Such an endeavor may represent a "moon shot" moment in history with the potential for unprecedented dividends in health and economic prosperity. These efforts represent a critical first step towards the realization of personalized medicine to re-engineer how we age, and they will require the destruction of longstanding silos to foster cross-disciplinary collaboration among scientists, engineers, bioinformaticians, physicians and policy makers. This will, of course, also require buy-in and cooperation from the general population who will be the primary beneficiary of personalized aging and medicine.

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CHAPTER 29

Interventions that target fundamental aging mechanisms: myths and realities

Erin O. Wissler Gerdes[1], Yi Zhu[2], Tamar Tchkonia[2] and James L. Kirkland[2,3]
[1]Robert D. and Patricia E. Kern Center for the Science of Health Care Delivery, Mayo Clinic, Rochester, MN, United States
[2]Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, United States
[3]Division of General Internal Medicine, Department of Medicine, Mayo Clinic, Rochester, MN, United States

29.1 Introduction

Fundamental aging processes appear to be the root cause contributors to the chronic diseases that account for the bulk of morbidity, hospital admissions, mortality, and health costs. They may also contribute to causing aging phenotypes, geriatric syndromes (e.g., frailty, sarcopenia, cognitive impairment, falling, and incontinence), and loss of physical resilience (e.g., delayed or prolonged recovery and increased mortality from injury, infection, or surgery, inadequate response to vaccination, and adverse drug reactions), which is the geroscience hypothesis. In this review, we discuss these fundamental aging processes or "hallmarks of aging," their interconnectedness (the Unitary Theory of Fundamental Aging Mechanisms), and interventions targeting these hallmarks that have been reported, are being tested, or may be developed. Next, we propose ideas about how to intervene at points that would impact different hallmarks of aging simultaneously or sequentially, potentially to achieve additive or synergistic benefits in enhancing health span by delaying, preventing, or alleviating chronic diseases, the geriatric syndromes, aging phenotypes, and/or enhancing physical resilience.

29.2 Pillars of aging

A collaborative effort by the trans-NIH Geroscience Interest Group (GSIG) produced a list of seven pillars of aging, which included adaptation to stress, epigenetics, inflammation, macromolecular damage, metabolism, proteostasis, and stem cell function and regeneration.[1] Using cancer hallmarks developed by Hanahan and Weinberg[2] and the idea that both aging and cancer are related to the accumulation of cellular damage, Lopez-Otin and team devised nine hallmarks of aging: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular

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communication.[3] These hallmarks, rather than being independent processes, appear to be interrelated.[4,5]

29.3 Genomic instability

Genomic instability encompasses mutations or alterations in the genome under specific physiological conditions.[6] Types of alterations include: (1) changes in nucleic acid sequence, (2) mutations of the genetic code, (3) gain or loss of a part of or an entire chromosome, and (4) heritable changes.[6,7] Genomic instability may contribute to the development of cancers and neurodegenerative diseases, among other disorders.[8,9] Organisms are equipped with DNA repair mechanisms to respond to most types of DNA damage.[10] However, the damage that is not repaired can lead to further genomic instability, and this accumulation of DNA damage progresses to cellular dysfunction, cancers, cellular senescence, and/or death. Areas for possible intervention to combat genomic instability include facilitating DNA repair mechanisms or reprogramming by "stemness" factors.[3,11]

29.3.1 Telomere attrition

Telomeres, the complex structures at the end of chromosomes, may act as an aging "clock". They can become dysfunctional or shorten in length with repeated cell replication or other stresses. These telomere alterations have been associated with an increase in many different age-related diseases, including cancers, cardiac diseases, and neurodegenerative disorders.[12] Telomere attrition or dysfunction is exacerbated when telomerase is no longer able to add base pairs to the ends of chromosomes.[3] The initial length of telomeres is mostly hereditary in humans, but can be influenced by other factors, including oxidative stress and reproductive hormones.[12] With each cell cycle, telomeres become shorter or more dysfunctional, and once they reach a critical point, cells stop dividing and enter senescence.

29.3.2 Epigenetic alterations

As epigenetic alterations occur, cells can become more susceptible to dysfunction. The accumulation of these changes may contribute to initiation and progression of many diseases and disorders.[13] Alterations include histone modifications, changes in DNA methylation, and chromatin remodeling.[3] Each of these changes can occur without altering the primary DNA sequence. Coupled with genomic instability, epigenetic changes can contribute to clonal heterogeneity, cancers, and other age-related diseases.[14] Several factors can impact epigenetic alterations, including DNA damage, diet, metabolic processes, physical activity, and oxidative stress.[15–17] Each of these factors presents an opportunity for intervention.

29.3.3 Loss of proteostasis

Proteostasis involves regulating and maintaining the stability of the proteome.[3] Loss of this stability can lead to protein misfolding, unfolding, or aggregation, which may contribute to age-related disorders.[18] Gradual loss of proteostasis is seen during healthy aging.[19] The key components ensuring proteostasis include chaperones, the ubiquitin-proteasome system (UPS), and the lysosome-autophagy system.[18] These components control whether or not a protein is refolded correctly or, if damaged, removed through proteolysis.[20]

29.3.4 Deregulated nutrient sensing

Age-related changes occur in several nutrient sensing pathways that regulate an organism's metabolic state, Among others, these metabolic pathways involve the following four protein groups: insulin-like growth factor (IGF-1) and related proteins/peptides (e.g., humanins), mammalian target of Rapamycin (mTOR), and mTOR-related proteins, sirtuins, and AMP-activated protein kinase (AMPK) and related proteins,[3] as well as changes in glycation by reducing sugars, prompting formation of advanced glycation end-products (AGEs),[21] and accumulation of saturated lipids that contribute to cellular dysfunction.[22] IGF-1 helps promote cellular proliferation and is part of the insulin, growth hormone (GH), and insulin-like growth factor (IIS) pathways.[3] Reductions in IIS pathway activity through genetic or pharmacological means (e.g., in mice in which GH, GH receptor, IGF-1 receptor binding protein [IGFBP], pregnancy-associated plasma protein-1 [PAPP-A, which affects IGFBP action]) are mutated or administration of humanins[23,24] or sirtuin agonists, such as Resveratrol[25] have been associated with increases in lifespan and health span.[3,26]

mTOR regulates anabolic metabolism and homeostasis.[27] With aging, mTOR activity increases. Inhibition of mTOR activity by Rapamycin or related substances has been associated with an increase in lifespan in mice.[28] Sirtuins react to lower energy levels by detecting increases in NAD^+.[3] AMPK acts similarly by regulating metabolism and the epigenetic state when energy levels are low.[3] The upregulation of both sirtuins and AMPK is an opportunity for intervention. Some studies have attempted to achieve this through caloric restriction or Metformin or Resveratrol administration.[25,29–31] Overall, targeting deregulated nutrient sensing through these pathways presents options for therapeutics that enhance health span.

29.3.5 Mitochondrial dysfunction

Mitochondria play an important role in all organ systems. The function of mitochondria changes with aging, impacting the immune, neurologic, adipose, and other systems. As an organism ages, there is an increase in the production of reactive oxygen species (ROS).[32] The increase in ROS up to a certain point helps to maintain homeostasis, however in excess, ROS can cause cell damage and death.[3,33] Mediating the

production of ROS is a possible intervention target.[34] Furthermore, as occurs in cancer cells, there can be a shift from lipid to glycolytic metabolism (Warburg shift[35]) in many tissues with aging, leading to inefficient energy generation and cellular accumulation of lipids, especially cytotoxic saturated lipids, contributing to accumulation of "MAD" (mesenchymal adipocyte-like default) senescent-like cells in multiple tissues, including muscle and brain ependyma.[36,37]

29.3.6 Cellular senescence

Cellular senescence contributes to age-related dysfunction and multiple diseases throughout the lifespan. Senescent cell burden is very low in young individuals but increases with aging in several tissues. These include, among others, adipose tissue, skeletal muscle, heart, kidney, brain, bone, liver, spleen, and skin.[38–45] Senescent cells can accumulate at pathogenic sites including lungs, adipose tissue, the pancreas, brain, blood vessels and the heart, joints and bones, kidneys, and the liver. This occurs in multiple major chronic diseases, especially conditions associated with physical dysfunction (frailty) and inflammation, including chronic obstructive pulmonary disease, asthma, idiopathic pulmonary fibrosis (IPF), diabetes/obesity, Alzheimer's disease and other dementias and Parkinson's disease, cardiovascular diseases, cancers, osteoporosis and osteoarthritis, renal diseases, and the liver in cirrhosis in preclinical species and humans.[41,43,45]

Senescence is essentially a cell fate, like differentiation, proliferation, apoptosis, or necrosis. External and internal signals can contribute to driving a cell into senescence. These are generally cell or tissue damage-related, including DNA alterations (dysfunctional telomeres, strand breaks, etc.), metabolic and mitochondrial dysfunction (ROS, high glucose, bioactive lipids, mitochondrial dysfunction), protein alterations (aggregates, misfolding, failed autophagy), inflammatory signals, mechanical/shear stress, pathogen-associated molecular pattern factors (PAMPs; including viruses, bacterial/fungal proteins, lipopolysaccharide [LPS], etc.), damage-associated molecular pattern factors (DAMPs; tissue damage signals (extracellular nucleotides, etc.)), and repeated replication/mitogens (insulin-like growth factor-1, etc.). Once initiated, senescence takes 10 days to 6 weeks to become established through transcription factor cascades that may, but do not always, include $p16^{INK4a}$/ retinoblastoma protein and/or $p53/p21^{CIP1}$, causing extensive changes in gene expression, histone modifications, altered organelle function (e.g., mitochondria, endoplasmic reticulum, nucleolus, nuclear envelope), elevated protein production due to increased mTOR and decreased autophagy, and profound morphological and metabolic changes, including a partial Warburg shift.[41,46] Elimination of senescent cells in aging or diseased tissues is a therapeutic strategy.

29.3.7 Stem cell exhaustion

Stem cells are unique and incredibly valuable due to their ability to transform into almost any kind of cell in the body. As aging progresses, the regenerative capacity and overall activity of stem cells declines, also known as stem cell exhaustion.[47] The reduced capacity of stem cells can lead to many age-related diseases and age-related complications. Stem cell exhaustion is caused by the accumulation of DNA damage, or cellular senescence.[47,48] Additionally, telomere dysfunction and shortening occurs and reduces stem cell function.[47] In order to combat the effects of stem cell exhaustion, progenitor and stem cell rejuvenation has been explored as a possible target for intervention.[11,49]

29.3.8 Altered intercellular communication

Altered intercellular communication, or changes in the signaling between cells, is a direct result of other hallmarks of aging. Changes in intercellular signaling with aging can lead to tissue damage and are linked with many age-related diseases. Sterile, low-grade, chronic inflammation across multiple tissues can increase with aging. Pro-inflammatory cytokines released by senescent cells appear to contribute to this.[3] Due to the interdependent nature of other hallmarks and altered intercellular communication, intervention options should be focused on treating other hallmarks of aging.

29.4 Unitary theory of fundamental aging processes

The nine hallmarks of aging appear to be interconnected and interdependent (Fig. 29.1). Our Unitary Theory of Fundamental Aging Processes hypothesizes that connectedness of fundamental aging processes might mean that targeting any one might impact several or all of the other processes.[50,51] As described above, each hallmark of aging appears to impact the others and all appear to contribute to the causation of multiple age-related diseases and disorders. By intervening in one process, other mechanisms might be impacted, like a domino effect. Instead of a traditional one molecular target-one drug-single outcome approach, this suggests that a multi-mechanism strategy for drug development could be more effective than the traditional drug development paradigm of one target-one drug-one disease.

29.5 Health span versus lifespan

There are two outcomes sometimes used to develop interventions intended to target fundamental aging processes: increasing health span versus increasing lifespan, that is adding life to years versus years to life, respectively. Targeting health span rather than lifespan at all costs is arguably the more appealing approach. If interventions aimed at

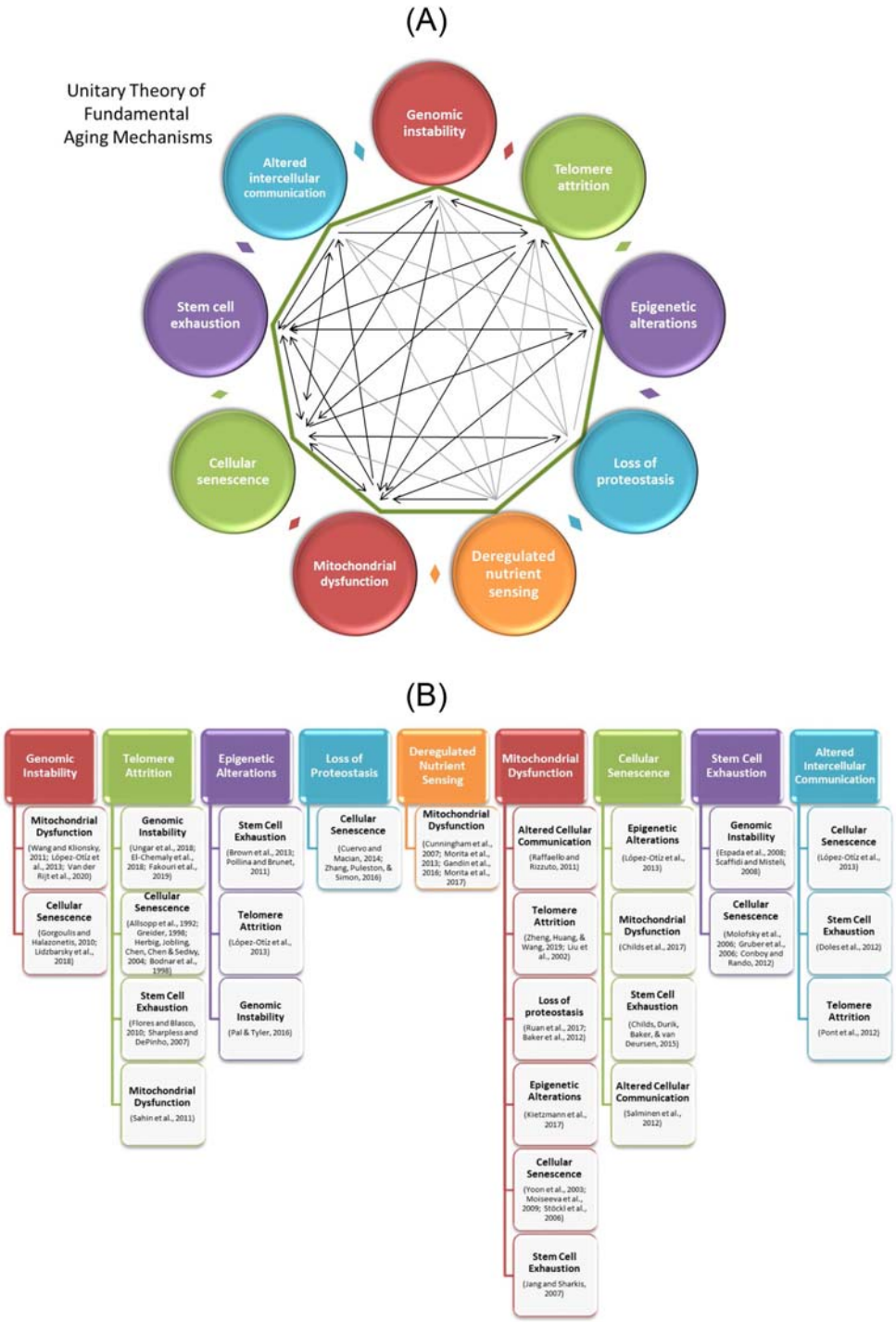

Figure 29.1 ***Unitary theory of the fundamental mechanisms of aging.*** Fig. 29.1 illustrates interactions between the different Hallmarks of aging. While many are known and have been established, there are probable interactions still needing to be investigated. A) indicates the known interactions between the hallmarks of aging, and shows those that have yet to be documented but are presumed. B) highlights the established interactions between the hallmarks of aging and some of the works that have documented these interactions.

the basic mechanisms of aging that lead to the most serious chronic diseases and disabilities can be translated into clinical interventions, this would have a profound impact on quality of life and drastically reduce healthcare costs. By one estimate, a 2% delay in the progression of fundamental aging processes would lead to an increase of 10 million healthy, as opposed to disabled, elderly people in the US by 2060, compared to doing nothing, delaying cancer, or delaying heart disease, with a savings in US health costs of $7.1 trillion over 50 years.[52] While the population steadily grows older, there is an urgent need to focus on increasing health span, rather than attempts to increase lifespan at the potential cost of creating a prolonged period of disability before the end of life.

29.6 Myths and realities

Trying to find the fountain of youth, or at least to extend health span or target the chronic diseases for which no satisfactorily effective, mechanism-based interventions currently exist, has been a long and ongoing mission of many scientists, explorers, and lay people dealing with their own mortality. Countless remedies and behaviors have been tested, all hoping to have some impact on the inevitable fate of humanity. Here we discuss treatments or regimens that show promise, and others that appear to be well-intentioned myths. We have modified Koch's postulates (Fig. 29.2) into four criteria that should be satisfied in order to prove that an intervention acts by targeting a fundamental aging process.

29.6.1 Senolytics

Among the promising interventions for targeting fundamental aging mechanisms are senolytics, or drugs that target senescent cells. Senolytics help attenuate senescent cell burden, allowing tissues and organs in the body to function properly. The first of these, Dasatinib + Quercetin, and subsequently, Fisetin, Navitoclax (ABT-263), and others, were discovered using a hypothesis-driven, mechanism-based approach.[50,53–56] This approach takes advantage of the observation that senescent cells kill or damage cells around them because of their pro-apoptotic, proteolytic senescence-associated secretory phenotype (SASP), yet senescent cells themselves do not die. This suggested the existence of protective senescent cell anti-apoptotic pathways (SCAPs) that were indeed discovered in bioinformatics analyses of the transcriptomes and proteomes of senescent versus non-senescent cells.[53] Key nodes needed for survival of those senescent cells that have a SASP were identified by targeting them by RNA interference. Drugs were identified, again using bioinformatics approaches, which target key node(s) across the SCAP network, and these drugs proved to be effective in causing apoptosis of senescent cells expressing a SASP, allowing such cells to "commit suicide."

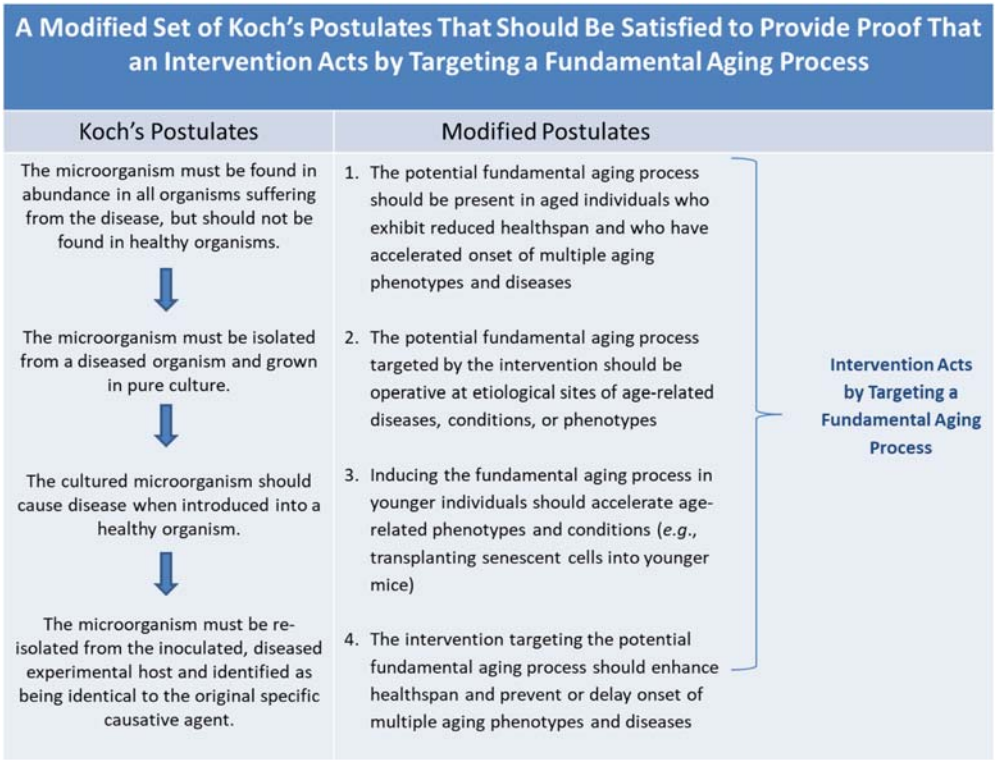

Figure 29.2 ***Modified set of Koch's postulates for proving an intervention acts by targeting a fundamental aging process.*** To prove an intervention targets a fundamental aging process, we propose a modified Koch's postulates comprising four criteria that should each be met.

There are several different types of senolytics that have been tested in preclinical trials so far, including, Dasatinib + Quercetin, Fisetin, Piperlongumine, EF24, goldenrod extract, Navitoclax, ABT-737, cardiac glycosides, and HSP90 inhibitors.[42,54,56–62] In the first report about senolytics, the combination of Dasatinib + Quercetin was shown to reduce senescent cell burden and improve function in mice that were aged, exposed to radiation, or progeroid, and to improve cardiac function in naturally-aged mice.[53] Consistent with expectations of if a fundamental aging process is targeted, since that first report, multiple diseases and disorders have been shown to be alleviated by Dasatinib + Quercetin in mouse models, including dementias, osteoporosis, diabetes, dysfunction caused by transplanting senescent cells or organs from old donors into young mice, frailty, age-, ischemia-, and lipid-induced cardiovascular dysfunction, cancers, pre-eclampsia, fibrotic lung disease, radiation effects, and age- and high fat diet-induced kidney dysfunction (Table 29.1). Also, Dasatinib + Quercetin enhanced median, but not maximum lifespan in mice.[63]

Table 29.1 Preclinical Trials using Dasatinib + Quercetin.

Outcome	Species/disease/disorder
Decreased senescent cell burden	Progeroid mice[53]
Improved cardiac function	Naturally-aged mice[53]
Decreased senescent cell burden	Irradiated mice[53]
Decreased senescent cell burden	Aged mice[53]
Decreased $p16^{Ink4a}$ expression	Dialysis Arteriovenous Fistula (AVF) with Chronic Kidney Disease (CKD) mice[64]
Decrease in toxic effect of PDE on postnatal bone growth in offspring	Prenatal dexamethasone exposure (PDE) in pregnant mice[65]
Decreased senescent cell burden and alleviation of radiotherapy-related bone deterioration	Post-focal radiation treatment (FRT) mice[66]
Decreased liver tumor progression	Diethylnitrosamine (DEN)/Cre mice[67]
Prevents age-related bone loss	C57BL/6 mice[68]
Decreased Aβ plaque-associated senescent OPCs in APP/PS1 AD mice	Alzheimer's disease mice[69]
Reduced aortic calcification and osteogenic signaling	Aged and atherosclerotic mice[70]
Decreased senescent cell burden and enhanced adipogenesis	p16-3Mr mice[71]
Alleviated anxiety-like behavior	Obese mice[37]
Decreased senescent cell burden, alleviated physical dysfunction and increased post-treatment survival (lifespan)	Naturally-aged mice and senescent cell-transplanted young mice[63]
Decreased senescent cell burden, improved glucose homeostasis	DIO mice[72]
Mediate fibrotic lung disease	*INK-ATTAC* mice[73]
Increased p53 expression, reduction of pro-fibrotic miR34a expression	Aged mice[74]

In the first report about senolytics, Dasatinib was demonstrated to target some senescent cell types, such as senescent human adipose progenitors, while Quercetin targeted others, such as senescent human umbilical vein endothelial cells (HUVECs). Their combination was more effective in clearing a range of senescent cell types than either alone. Similarly, while Navitoclax reduced senescence in preclinical models, it targeted some, but not all senescent cell types.[62] For example, Navitoclax is not effective in targeting senescent human adipose progenitors, one of the most abundant senescent cell types in older or diabetic/obese individuals. Most senolytics are not fully selective against senescent versus non-senescent cells. This is especially the case for Navitoclax, which eliminates non-senescent neutrophils and platelets, leading to side effects.[50,56,62]

Some early senolytic clinical studies are showing promising results (Table 29.2). A trial with Dasatinib + Quercetin in patients with chronic kidney disease and diabetes

Table 29.2 Clinical trials using Dasatinib + Quercetin.

Population/disease	Site	Clinical trials info
Senescent cell burden in hematopoietic stem cell transplant survivors	• Mayo clinic	NCT02652052
Senescent Cell Burden and Bone Reabsorption markers in elderly women	• Mayo clinic	NCT04313634
Senescent cell burden and clinical dementia rating scale in patients with Alzheimer's disease	• Wake Forest University Health Sciences • The University of Texas Health Science Center at San Antonio • Mayo Clinic	NCT04685590
Senescent cell burden and MSC functionality in chronic kidney disease in patients with chronic kidney disease	• Mayo Clinic	NCT02848131
Senescent cell burden and frailty markers in patients with idiopathic pulmonary fibrosis	• Wake Forest University Health Sciences • The University of Texas Health Science Center at San Antonio • Mayo Clinic	NCT02874989

demonstrated a decrease in senescent cell abundance, markers of inflammation, and fibrosis in adipose tissue within 11 days of completing a three day course in subjects with diabetes/obesity.[75,76] In another trial in idiopathic pulmonary fibrosis patients, a brief course of Dasatinib + Quercetin suggested clinically significant improvement in frailty measurements.[77] That trial was exploratory and open-label. A larger, placebo-controlled, multicenter trial is about to begin. Currently, trials are underway with Dasatinib + Quercetin and/or Fisetin administered systemically for the following populations and indications: aging adults with frailty and multi-morbidity, adults with chronic kidney disease and diabetes, Alzheimer's disease patients, inpatients, outpatients, and nursing home residents with COVID-19, patients with the accelerated aging-like state that can follow bone marrow transplantation (likely related to the cellular senescence-inducing chemotherapy before bone marrow transplantation), childhood cancer survivors (some of whom also develop frailty and a senescence-associated accelerated aging-like state), age-related osteoporosis, and osteoarthritis. In addition, a dose-escalation trial involving injecting UBX1325, a modified version of Navitoclax, into the eye for retinal disease is underway (NCT04537884). However, clinical trials injecting another possible senolytic, UBX0101 (which appears to be related to Nutlin-3a), into knees of patients with osteoarthritis did

not result in statistically significant improvements in knee pain endpoints (NCT04229225; NCT04129944; NCT03513016). Thus, not all senolytics may translate into effective clinical interventions, perhaps depending on the agent, route of administration (e.g., systemic vs injection), the indication targeted, trial design, and choice of endpoints. Many more trials of a range of different senolytics for a variety of age-, chronic disease-, and senescence-related disorders are needed before these agents enter clinical practice.

Understandably, there has been skepticism and criticism of using these drugs, particularly with Dasatinib, as among other indications, it is used for treating leukemias and lymphomas. However, Dasatinib is not a classical chemotherapeutic antiproliferative, DNA-damaging drug. Rather, it acts by decreasing apoptosis resistance in tumors that secrete pro-apoptotic factors. Therefore, it is used for tumors like CLL and B-lymphomas that secrete apoptotic inducers, such as TNF-α or IL-6. Furthermore, Dasatinib is unlike other tyrosine kinase inhibitors. It inhibits Src kinase and ephrin-related kinases that other "pan"-tyrosine kinase inhibitors, like Imatinib, do not. Imatinib is not senolytic and has a very different side effect profile than Dasatinib yet both are lumped together by some when considering safety profiles. Dasatinib only infrequently causes thrombocytopenia or neutropenia, mainly after long-term continuous use, partly because, unlike conventional chemotherapeutics, it does not act by inhibiting proliferation or damaging DNA directly.

As a senolytic, Dasatinib can be administered intermittently, every two or more weeks, rather than continuously, since senescent cells take 10 days to 6 weeks to reform, at least in culture. Thus, senolytics can be administered in a "hit-and-run" approach: high steady-state levels are not required for effectiveness. Furthering this "hit-and-run" approach and reducing off-target effects, Dasatinib has a short elimination half-life, around 4 h in humans.[78] Also, the safety profile of Dasatinib is quite good: it has been available for any American physician to prescribe since 2006, unlike Navitoclax, which is also used for treating cancers but only for investigational use. As a senolytic, administration of Dasatinib in a "hit-and-run" way, along with its short elimination half-life, and its relatively favorable safety profile, especially if given intermittently, all mean that there may be minimization of off-target effects that depend on continuously occupying a receptor or inhibiting an enzyme, as opposed to selectively inducing apoptosis in slowly reforming senescent cells. Thus, Dasatinib might not be as unsafe as most true chemotherapy drugs or Navitoclax, which can cause severe neutropenia or thrombocytopenia.[79] That said, much work remains to be done to determine if senolytics have unanticipated side effects as a class. Until then, senolytics should not be administered outside the context of carefully controlled clinical trials.

A cytotoxic pro-drug that is activated by senescence-associated beta-galactosidase (SA β-gal) has been proposed as a strategy to selectively target senescent cells.[80] However, not every senescent cell has elevated SA β-gal. High levels occur in non-

senescent cells, such as activated macrophages.[81] Therefore, the pro-drug, which depends on being activated by an enzyme not present in every senescent cell and that is present in many non-senescent cells, might not be more specific for senescent cells or target more senescent cells than other senolytics, such as Dasatinib + Quercetin. Arguably, a single target, such as SA β-gal, may even be less likely to be specific for a cell type than targeting a network, such as the SCAP network. It is rare for any single drug target to be restricted to a unique type of cell. Overall, caution and perspicacity needs to be exercised about early discoveries regarding specificity for senescent cells.

29.6.2 Other pharmacological interventions

There are many other pharmacological interventions being tested for targeting fundamental aging processes in order to try to extend health span. These include Metformin, Rapamycin, Resveratrol, NAD^+/NMN, CD38 inhibitors, 17-α-Estradiol, and ketogenic agents, among others.[82]

29.6.2.1 Metformin

Though primarily used currently to control high blood sugar, Metformin is gaining popularity as a possible intervention to delay progression of aging phenotypes, delay, prevent, or alleviate age-related chronic diseases, and extend health span. Metformin activates AMPK, which results in increased glucose uptake and decreased glucose production.[83] By acting on these metabolic processes and potentially partially reversing the age-related Warburg shift, some studies have shown Metformin increases lifespan in worms and mice.[31,84] Metformin has also been shown to accelerate wound healing when applied locally.[85]

Many aspects of Metformin mechanisms of action are still unknown and require further study. Among other effects, Metformin appears to inhibit the SASP of senescent cells.[86–88] The long-term implications of Metformin use in healthy individuals are still relatively unknown and safety information is needed. The targeting aging with metformin clinical trial that is beginning will address many of these issues.[89]

29.6.2.2 Rapamycin

Rapamycin and related drugs (rapalogs), which target mTOR are antibiotic, antifungal, and immunosuppressive, and are prescribed to treat cancers and prevent rejection of transplanted organs. Rapalogs have been extensively studied in preclinical settings and clinical trials as possible interventions to target the fundamental aging processes. Rapamycin has been shown to extend maximum lifespan in mice, regardless of whether it is administered early or late in life.[28,90–93] Consistent with our Unitary Theory of Fundamental Aging Mechanisms, among other effects, continuous administration of Rapamycin inhibits the SASP[94,95] and reduces senescent cell burden.[96] Using Rapamycin topically has been shown to slow development of skin aging

phenotypes.[96,97] An important study of Rapamycin on health span in dogs is currently underway.[98] Though adverse side effects of Rapamycin appear to be mild, more safety studies are needed in healthy controls.[83]

29.6.2.3 Resveratrol

Resveratrol is a polyphenol that is a sirtuin agonist and antioxidant.[99] It is present in red wine and some foods. Resveratrol and other polyphenols can cross the blood brain barrier and have anti-inflammatory properties.[100] The antioxidant properties of Resveratrol act to combat oxidative stress and the anti-inflammatory properties combat the inflammaging that occurs with natural aging. Studies have shown that Resveratrol attenuates inflammation in aging mice, as well as it improves renal function by activating pathways that alleviate oxidative stress and mitochondrial dysfunction.[101] Other studies have shown that Resveratrol helps to prevent cognitive decline in rats by inhibiting production of inflammatory cytokines.[102] Resveratrol also helped accelerate healing in cutaneous wounds when applied locally.[85] The anti-inflammatory and antioxidant properties of Resveratrol have the potential to protect against arterial aging and restore vascular function.[103,104] While there are many preclinical trials looking at the benefits of Resveratrol, clinical trials, especially with healthy aging populations, are scarce.[105]

29.6.2.4 NAD^+/NMN and inhibitors of CD38

The decrease in oxidized nicotinamide adenine dinucleotide (NAD^+) during aging is associated with an increase in the immune cell ectoenzyme, CD38, and mitochondrial dysfunction.[106,107] CD38 on immune cells breaks down NAD^+ into inactive metabolites. Precursors of NAD^+ include nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN), as well as tryptophan.[106,108] Some researchers have proposed testing NAD replacement therapy with CD38 inhibitors in order to alleviate metabolic dysfunction.[106,107]

The molecule 78c is a powerful CD38 inhibitor, and a recent study showed that 78c partially reverses the effects from age-related decline of NAD^+ in mice.[109,110] Consistent with our Unitary Theory, the SASP of senescent cells causes upregulation of CD38, leading to a decrease in NAD^+.[111] Targeting the decrease in NAD^+ and/or increase in CD38 with specific inhibitors of CD38 and senolytics, holds the potential to be interventions because NAD^+ is essential for DNA repair, signaling, and other enzymatic reactions.[108,109]

29.6.2.5 17-α-Estradiol

17α-estradiol is related to the feminizing hormone, 17β-Estradiol except it is relatively non-feminizing and present in human males as well as females. It modulates nutrient-sensing pathways, including increasing AMPK and reducing mTOR.[112] In male mice,

17α-Estradiol substantially increases lifespan.[112,113] Consistent with our Unity Theory, there is emerging evidence that 17α-Estradiol decreases senescent cells or their secretions.[114] Clinical trials of 17α-Estradiol for geroscience applications are currently being planned.

29.6.2.6 Ketogenic agents

Like Metformin, α-ketoglutarate, β-hydroxybutyrate, and related natural compounds and drugs that are involved in sugar and fat metabolism, as well as ketogenic diets, appear to alleviate age- and disease-related conditions in mice and enhance health span.[82,115–117] α-ketoglutarate and β-hydroxybutyrate are both endogenous metabolites essential to metabolism. α-ketoglutarate plays an essential role in the tricarboxylic acid cycle,[117] and β-hydroxybutyrate inhibits histone deacetylates, which directly impacts glucose metabolism and promotes resistance to oxidative stress.[116] A clinical trial of ketogens for age-related physical dysfunction is being planned, and commercial groups, including Juvenescence, are developing drugs related to α-ketoglutarate.

29.6.3 Behavioral/dietary interventions

Outside of pharmacological interventions, behavioral and dietary interventions, including caloric restriction/fasting/food clocking and exercise, have been tested as possible interventions for age-related dysfunction.

29.6.3.1 Caloric restriction/fasting/food clocking

Short of restricting food intake to the point of malnutrition, caloric restriction impacts several important pathways critical to aging processes. The ideal reduction is about 30% of calories daily in mice and 15% in humans.[118,119] What these calories consist of appears to be important. In rhesus monkeys, high sugar diets with caloric restriction led to an increase in lifespan compared to controls,[30,120] whereas a diet control group with a diet comprising of a balance of vitamins and minerals compared to a CR group fed the same balanced, but reduced diet had no added lifespan benefit.[121]

Caloric restriction reduces insulin, IGF-1, and amino acids, while increasing NAD^+. This impacts several pathways leading to repair of DNA damage, stress resistance, and oxidative metabolism.[47] However, long-term caloric restriction could lead to negative effects, such as infertility.[118] Rather than long-term caloric restriction, short term or intermittent fasting practices ("food clocking") may hold promise. Intermittent fasting can be practiced over different timeframes.[122,123] More studies need to be done of intermittent fasting to test its efficacy as a potential intervention to delay, prevent, or alleviate age-related dysfunction and chronic diseases.

29.6.3.2 Exercise

There are many health benefits from exercise.[124] Specific to aging processes, an exercise routine including a combination of aerobic, resistance, flexibility, and balance

exercises may have beneficial effects.[125,126] Though the impact of exercise at the mechanistic level is still in need of closer examination, exercise appears to reduce mortality, chronic disease, and premature death in older adults.[127,128]

Consistent with our Unitary Theory, exercise alone can reduce markers of cellular senescence and prevent accumulation of senescent cells in mice.[129] Furthermore, the detrimental effects of caloric excess, including expression of pro-senescence markers, were mitigated by exercise.[129] Some other benefits directly associated with aging processes include improved function of mitochondria, decrease in inflammatory markers, and better adaptation to oxidative stress.[127] Exercise activates AMPK, therefore increasing glucose uptake,[83] and increases sirtuins in skeletal muscle.[130] With exercise, epigenetic changes impacting DNA methylation, histone modification, and miRNA expression have been documented.[131] Exercise may also improve DNA repair, increase antioxidant activity, and contribute to skeletal muscle cell regeneration.[132]

29.7 Clinical trials and treating disease

29.7.1 Safety; risk/benefit ratio

While many of the interventions discussed here are promising, safety must be at the forefront of any intervention developed to enhance health span or alleviate age-related disorders. In order to confidently recommend therapeutic interventions, especially pharmaceutical interventions, possible adverse effects need to be extensively explored. There is a potential positive risk to benefit ratio in conducting trials with interventional agents in people who have serious diseases that are predisposed to fundamental aging processes and for which no good treatments are currently available.

29.7.2 Combining or sequencing therapies

By our Unitary Theory of Fundamental Aging Processes, we hypothesize that targeting one hallmark of aging will impact several or all others. Accordingly, if this theory is true, it may be important to consider combining or sequencing interventions that target fundamental aging mechanisms to achieve additive or potentially even synergistic beneficial effects. It may perhaps be best to begin by targeting serious age-related diseases to test such combined interventions, as it could be complicated and time-consuming to study and decipher results in healthy subjects with a lower risk/benefit ratio.

Some existing examples of combining therapies include a pharmacological agent and behavioral intervention, such as exercise and Resveratrol, though adding supplements might negate some of the positive effects from physical exercise.[133,134] Other groups have looked at combining Metformin and exercise in non-diseased (without type 2 diabetes) patients, and statins with exercise, both providing mixed results.[135–137] The combination of 17α-Estradiol and a calorie-restricted diet in mice produced differing proteostatic

outcomes.[138] Still other groups have tested combining pharmacological agents, such as Rapamycin and Metformin, with results indicating the combination enhances median lifespan in mice.[139] In rats, a combination of a calorie-restricted diet and exercise prevented age-related changes in the corpus cavernosum.[140] Overall, combining therapies is largely unexplored and much research needs to be done in order to target multiple aging processes simultaneously.

29.7.3 Translational geroscience network

In order to determine the efficacy of any intervention, extensive, well-designed, and rapid clinical trials are necessary. This can be a slow process in a competitive environment. Results may be gleaned more quickly if clinical trials were done in parallel by multiple engaged institutions. This is a principle that the translational geroscience network (TGN) is based on.

Innovative solutions are urgently needed to develop the required infrastructure and workforce to speed translational aging research, since targeting fundamental aging mechanisms is a completely new paradigm. To develop strategies to bridge the translational gap, the "valley of death" between bench and bedside, the basic biology of aging and clinical geriatrics communities engaged in a four-year planning process to identify needed resources and to map out strategies through the NIH R24-funded Geroscience Network. During the R24 process, the following roadblocks were identified as contributors to the slow pace of translation: (1) lack of translational strategies, (2) lack of a national infrastructure, (3) lack of trained personnel, and (4) the need to go beyond the traditional drug development approach of "one drug-one target-one disease."

We collaborated to develop a TGN to facilitate and speed the translation by optimizing resource utilization, while avoiding duplication and counter-productive competition. We started with institutions that were a part of our previous R24 grant and that had already made financial commitments to launch programs designed to test the GeroScience Hypothesis, and that also brought complementary expertise and strengths (NIH R33AG 61456: Mayo Clinic, Harvard, Johns Hopkins, Wake Forrest, Universities of Minnesota, Michigan, Connecticut, and Texas together with St. Jude's Hospital and the Steadman Clinic). This initial network will engage in preclinical translational research, completing the appropriate regulatory steps (e.g., acquiring investigational new drug applications (INDs) from the FDA), and assisting in developing several "use case" proof-of-concept Phase II trials. We will commence building this initial network into an inclusive national TGN. By pursuing steps to translate drugs, nutritional supplements, potentially synergistic combinations, and combinations of drugs or natural products plus lifestyle interventions that target fundamental aging mechanisms from bench to bedside concurrently, and by doing so in a coordinated way, the TGN hopes to accelerate development of effective treatments. With

sufficient resources, such interventions might be introduced clinically within the next 5–15 years. Indeed, "use case" human studies based on recent insights from the basic biology of aging have already started at several of our institutions.

29.8 Conclusion

Innovative strategies for conducting proof-of-concept trials were developed during the last 4 years. One such trial has already been reported: administration of a Rapamycin analog to enhance antibody responses to the influenza vaccine in community-dwelling older adults[141]; and other trials are now beginning. Nevertheless, such efforts lack coordination, hindering collaborations and progress. Through the TGN, such trials could: (1) be accelerated, (2) be readily compared to one another, (3) provide biospecimens to a broader group of discovery research laboratories across the basic biology of aging community, and (4) form the basis for ancillary studies. During the R24 phase of this endeavor, we were impressed by the enthusiastic reaction of the biology of aging groups and geriatrics program leaders to forming a TGN. We view targeting fundamental aging mechanisms to delay, prevent, or alleviate chronic age-related diseases, geriatric syndromes, and decreased resilience as a "holy grail" of all biology and medicine.

The TGN has been operating for two years. It was originally intended to assist in conducting 3 early phase clinical trials of interventions that target fundamental aging mechanisms annually, but 17 such trials are already underway, with considerable demand to add more. A central Facility for Geroscience Analysis (FGA) was established to conduct assays related to fundamental aging mechanisms across all TGN trials, and biostatistical and biobanking groups and programs have been established. With the support of multiple organizations and a common goal, we can begin to eliminate barriers and initiate the process of creating the infrastructure and resources required to tackle this urgent need. We will be able to translate interventions targeting fundamental aging mechanisms into interventions that can delay, prevent, or alleviate the age-related decline in health span, major chronic diseases, multi-morbidity, dysfunctional age-related phenotypes, the geriatric syndromes, and age-related loss of homeostasis and physical resilience.

Conflict of interest disclosure

TT and JLK have a financial interest related to this research. Patents on senolytic drugs are held by Mayo Clinic. This research has been reviewed by the Mayo Clinic Conflict of Interest Review Board and was conducted in compliance with Mayo Clinic Conflict of Interest policies. EOWG has no conflicts of interest.

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CHAPTER 30

Being a frail older person at a time of the COVID-19 pandemic

Yaohua Chen[1,2,3], Susanne Röhr[1,4], Berenice Maria Werle[1,5] and Roman Romero-Ortuno[1,6,7]

[1]Global Brain Health Institute, Trinity College Dublin, Dublin, Ireland
[2]University of Lille, Inserm UMR-S1172, Lille Neurosciences & Cognition, Degenerative and Vascular Cognitive Disorders, CHU Lille, LiCEND, Lille, France
[3]Department of Geriatrics, CHU Lille, Lille, France
[4]Institute of Social Medicine, Occupational Health and Public Health (ISAP), Medical Faculty, University of Leipzig, Leipzig, Germany
[5]Moriguchi Institute, Rio Grande do Sul, Brazil
[6]Discipline of Medical Gerontology, School of Medicine, Trinity College Dublin, Dublin, Ireland
[7]Mercer's Institute for Successful Aging, St James's Hospital, Dublin, Ireland

30.1 Introduction

At the time of writing this chapter in December 2020, 9 months after the World Health Organization (WHO) declared a pandemic of coronavirus disease 2019 (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2),[1] we are globally approaching 82 million infections, and almost 1.8 million people have lost their lives.[2]

Notably, across many diverse countries, mortality by COVID-19 has increased exponentially in people over the age of 50 years, the highest being in people aged 80 years and older.[3] Early on, age was identified as the most significant risk factor for severe COVID-19 and adverse health outcomes from the infection. Moreover, older age is associated with a higher prevalence of chronic conditions such as hypertension, obesity or type 2 diabetes, all of which have been shown to exacerbate risk of severe or fatal COVID-19 disease.[4]

30.1.1 A minority of community-dwelling older adults are frail

From a medical perspective, it has been argued that a common underlying factor conferring vulnerability of older individuals to more severe COVID-19 clinical presentations, is frailty. Frailty describes a distinctive health state in relation to the aging processes, in which multiple body systems gradually lose their built in reserves. In other words, frailty is not a medical diagnosis, but an age-related state of vulnerability to stressors due to dysregulation across multiple physiological systems.[5]

Only a minority of older adults are frail. Studies have estimated that among community-dwelling older adults aged 60 years or older, between one in eight and one in four

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(depending on how frailty is measured) can be identified as frail.[6] Importantly, it has been shown that transitions in frailty states, both towards deterioration and improvement, are not only possible but frequent.[7] Research has also shown that multidisciplinary interventions such as physical activity, nutrition optimization, health education, and social engagement can delay, and even reverse, frailty in populations.[8,9] In addition, beyond the physical or medical aspects (e.g., multimorbidity, dementia), other relevant dimensions of frailty have been identified including the psychological and the social,[10] which are essential to understand and effectively address the needs of those who are "medically complex and environmentally challenged,"[11] as seen during the times of COVID-19.

30.1.2 Frailty in the time of a pandemic: the "measured" versus the "lived"

In 2020, there was increasing attention in the biomedical literature to the links between COVID-19 and frailty in older adults. However, frailty has mostly been investigated regarding its associations with hospital mortality or resource allocation strategies, and specific interventions in relation to frailty or its impact on COVID-19 treatments have not been significantly addressed.[12] In addition, there has been little study of the psychosocial dimensions of frailty in the face of COVID-19, with some important reports only starting to appear now. This is despite emerging evidence shedding light on the interplay between older individuals' biomedical status and their social environment, showing that the health and economic impacts of COVID-19 are being disproportionally endured by those who are not only physically frail, but concurrently socially frail.[13] This has prompted controversial debates as to how to best deal with the increased COVID-19 vulnerability in older groups, and especially frail individuals around the dilemma of trying to balance their safety vs. maintaining their autonomy and independence. In all these debates, the voices and personal perspectives of frail older people themselves have not been sufficiently heard.[14] Indeed, there has been a disconnection between the "measured" and the "lived."

One could not understand what it is like to be a frail older adult during the COVID-19 pandemic without paying attention to the contingent and existential aspects of frailty.[15] Undeniably, there is considerable discrepancy between the "frailty" that the biomedical identification tools capture and the extent to which older people self-identify with the "frail" label.[16] It has been argued that those who accept a frail self-identity are more affected by adverse psychosocial states and have less access to resources, which can help frailty become a self-fulfilling prophecy in terms of its association with adverse outcomes.[17]

Against this multifaceted background (Fig. 30.1), in this chapter we aimed to explore: What does it mean to be a frail older person in this COVID-19 pandemic? How is the life of frail older individuals affected? Is it all negative because of COVID-19 or are there even positive aspects? How are frail older adults experiencing the COVID-19 pandemic? Answers to these questions are anything but simple; they are complex, partially resolved and sometimes even delicate. Experiences are rather mixed

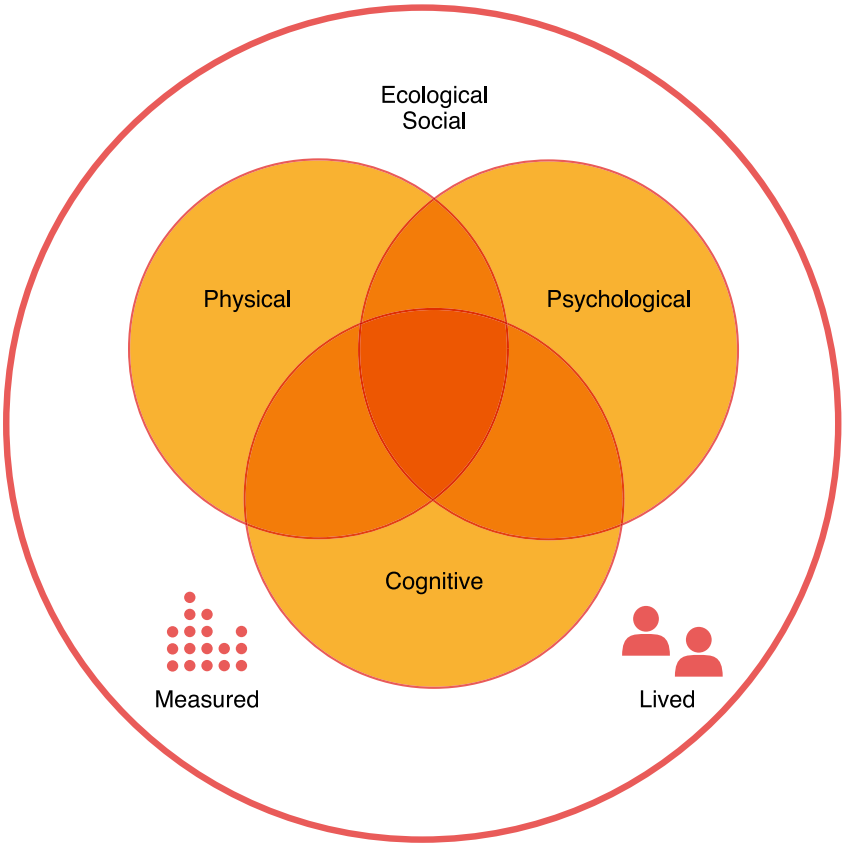

Figure 30.1 Being a frail older person at a time of COVID-19 pandemic: a conceptual framework.

and scientific results are still inconclusive depending on how, where and what one wants to look at. For example, looking at different countries and regions, which are very differently affected by COVID-19 and respond differently to the crisis, or looking at frailty in older individuals in different settings, such as in the community, hospitals or nursing/care homes. While we are still learning in these difficult times, there is a multitude of literature emerging that provides an evolving understanding of what it means to be a frail older individual in the COVID-19 pandemic.

30.2 The community perspective

From the community perspective, the very beginning of the pandemic prompted a discussion on whether additional measures were necessary in order to protect older individuals from contracting COVID-19, and if so, would it be appropriate to do so? Some governments such as Ireland advised their older population to practice "cocooning,"

that is, staying at home as much as possible and avoiding any personal contacts,[18] but other nations decided against specific measures for the older population, prioritizing autonomy and self-responsible decision-making.[19] There are advantages and disadvantages to both approaches. However, In Ireland, for example, cocooning was advised for everyone over 70 years of age, even if "fit and well."[20] Others argued that shielding should be stratified by risk,[21] but frailty was not invoked as part of the risk criteria.

30.2.1 COVID-19-related challenges in community-dwelling frail older people

More than 9 months into the pandemic, infection control measures such as physical distancing and specifically, cocooning/shielding practices were increasingly perceived as competing risks to COVID-19 with regards to their health impact.[22] For older individuals, cocooning poses unprecedented restrictions to their lifestyles and everyday routines with potential detrimental consequences for various aspects of health. For example, after weeks of self-isolation in Italy, Briguglio and colleagues[23] observed a frailty cascade among older adults; the "bed-kitchen-sofa-lifestyle" during lockdown led to low environmental information processing, potentially resulting in spatial disorientation, impaired proprioception, disequilibrium, and incoordination. At-home confinement frequently led to sarcopenia; sarcopenia became osteosarcopenia, and after 2 months, the perceived loss of balance became a fear of falling,[24] leading to a "physical frailty peak."[23] Other studies supported such findings, alerting that the lack of physical activity during cocooning likely contributed to muscle loss, weakness and falls, inducing frailty or worsening prefrail or frail states.[12]

A survey among community-dwelling older individuals in the UK revealed that one third of the respondents had lower levels of energy, one quarter was unable to walk as far as before and one-fifth felt less stable on their feet than before the pandemic.[25] A female participant of the UK survey in the age group 70–74 stated: "Having already had health problems, it's been accelerated and [I] have lethargy, no strength and walking problem. As I was fine before, [I] feel life has been cut short."[25] Other participants typically described that everyday activities, such as walking stairs or washing, suddenly became difficult, and walking aids not needed before were now essential to master small distances.[25] In addition, cocooning may adversely impact diet and nutrition, and in conjunction with less physical exercise, may lead to weight gain and joint pain, further cascading frailty.[26]

Early on, the released a statement on psychosocial considerations during the COVID-19 outbreak, raising awareness about the potential psychological impact of mass quarantine measures, specifically addressing older individuals in isolation and those with cognitive decline and dementia.[27] Typical psychological and behavioral responses may comprise anxiety, stress, agitation, and further social withdrawal. Poor mental health in combination with reduced physical activities have been long

identified as harbingers of frailty and aspects of being frail.[28] However, the COVID-19 pandemic has particularly spotlighted social isolation and loneliness as potential consequences of the stay-at-home-orders and associated restricted possibilities for personal social interactions. Social isolation describes a state of having very limited social resources to be in regular contact with others to discuss private matters or receive support if needed, while loneliness describes a subjective evaluation of an individual's relationships, that is, feeling lonely.[29] Individuals can feel lonely despite being integrated in a large social network and socially isolated individuals may not feel lonely at all; however, both social isolation and loneliness are associated with adverse health outcomes and increased mortality.[30] Older individuals who experience loneliness are at increased risk of becoming physically frail.[31] Moreover, social isolation and loneliness may moderate the effect of frailty on health.[32] It can be understood that social isolation and loneliness create a state of chronic psychological distress due to the lockdown scenario, and the associated lower social support may put heavy demands on older individuals, depleting personal resources.[33] Other frequent adverse situations and events during the pandemic, such as fear of contracting COVID-19, or limited options for personal bereavement, can further compromise mental health.

Social and physical frailty can thus both be risk factors for poor health outcomes, if present already, and poor health outcomes themselves, due to stay-at-home-orders, occurred during this pandemic.[12] They may trigger an increase of physical disease and psychiatric disorders well into postpandemic times.[34] Social frailty is thought to be more strongly associated with mental health, for example, depressive symptoms among community-dwelling older adults.[33] Physical frailty, on the other hand, may be associated with reduced tolerance to acute illness.[35] However, in both scenarios the degree of frailty matters. Importantly, there is an interplay between social and physical frailty, as social frailty has also been associated with both cognitive and physical functions.[36] The picture so far indicates that older individuals are more fragile, malnourished and more ill than in prepandemic times: depleted physical resilience may have worsened age-associated conditions such as high blood pressure, inflammation, immune dysfunction, and glucose intolerance.[23] Specific concerns in this context are cognitive decline and dementia, as frailty is associated with both[37,38]; it has also been suggested that prolonged or repeated COVID-19 lockdowns may exacerbate and accelerate risk trajectories leading to mild cognitive impairment or earlier onset of dementia.

The situation may be even more difficult for people living with dementia, who are among the frailest individuals in the community. Those who still maintained sufficient levels of independence and functionality to manage life in a private home, face the risk of faster deterioration, especially when family support due to cocooning orders are compromised. Being a person living with dementia during the pandemic

might also involve a lack of understanding of the situation and required restrictions, which in turn could complicate compliance behaviors and social relationships. A change of routine in daily life likely causes confusion and induces fear and stress, all of which could easily lead to the tipping point where peoples' reserves of functionality and independence deplete quickly. Moreover, the limited opportunities to engage in beneficial activities that are highly important for people with dementia, such as outdoor exercise, socialization, or rehabilitative and occupational programs, may lead to worsening cognitive, behavioral, and functional impairments.[39] Ultimately, many people living with dementia may no longer be able to master their everyday lives in private homes, requiring them and their families to make difficult decisions around moving to care homes. In these scenarios, the burden for caregivers and pressure on family members also increases enormously. Moreover, caregivers showed reluctance of bringing older patients, with or without dementia, to hospitals when acute medical problems occurred out of worry that they could contract COVID-19. Delayed medical help-seeking can lead to missed chances of treatment and care, worsening clinical outcomes, for example, in the case of heart attack, stroke, and seizure.[40] Taken together, from the community perspective, the COVID-19 pandemic and its restrictions posed unprecedented challenges on frail older people with clear implications for their health.

30.2.2 A glimpse of hope

However, there is also reason for optimism. We can find positive aspects and signs of hope amidst the pandemic and looking beyond. For instance, telemedicine and digital communication have made a skyrocketing entry into the lives of many older individuals, where connections via video calls using tablets and smartphones or utilizing health consultations via online systems have become a means to bridge physical restrictions. The sudden need for these technologies has caused a massive boom in digital literacy among older populations around the world. The utilization of telemedical and digital tools will likely become an established pillar of healthcare and a means of communication in the older population postpandemic. Moreover, the accelerated digitalization in these areas may help to better support and care for underserved populations and may increase outreach to deprived regions.[41] This may improve the healthcare situation particularly in developing countries.

Importantly, the frailty of older individuals with regards to COVID-19 has sparked a global open debate on age and aging, questioning negative and inaccurate stereotypes about "being old." It did not start nicely: during the first COVID-19 wave across countries, discrimination against older people has been evident, for example, through the trending social media hashtag "#BoomerRemover," which suggested the death of older individuals would not be as important as the loss of younger life.[42]

This, rightly prompted hefty objection and an empathetic wave of intergenerational solidarity with the older population, which especially amplified the voices of those who are frail and impaired. As such, the COVID-19 pandemic has torn down walls, opening a new community space for discussion around age and aging. Unsurprisingly, in surveys that investigated the mental health impact of the COVID-19 pandemic, it is the younger generations who responded with much more psychological distress than the older generations. The reason: older individuals have higher mental resilience through having coped with and mastered crisis throughout life.[19]

30.3 The hospital perspective

Using two different approaches for measuring frailty (i.e., the frailty phenotype and the frailty index),[43] a UK Biobank study demonstrated an increased risk of hospitalization and death from COVID-19 among older individuals.[44] Since the first cases of COVID-19 from China, cumulative epidemiological data from different populations worldwide revealed exponential fatality rates with age and/or comorbidities, especially in hospitals.[45] Since then, the medical and scientific communities have been attempting to address still unresolved issues, ranging from a reliable screening tool for frailty in hospitals, to sensitive ethical considerations around the escalation of care and allocation of scarce resources. In this section, we discuss perspectives of being a frail older adult with COVID-19 in an acute hospital. Frailty affects older people with COVID-19 at various levels, from disease onset, through hospitalization to survivorship (Fig. 30.2).

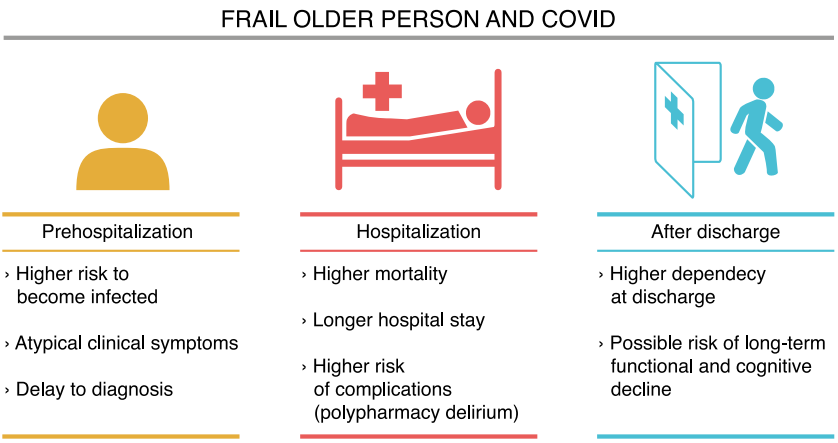

Figure 30.2 The impact of frailty on the course of COVID-19: the hospital perspective.

Table 30.1 Atypical COVID-19 symptoms in frail older patients in different care settings.

	Survey of practitioners[46]	Emergency department[47]	Geriatric inpatient wards[48]	Hospital dedicated to COVID-19[49]
Delirium (%)	28	10	25	35
Gastrointestinal symptoms (%)	20	10	15	10
Asthenia (%)	59	32	63	30
Falls (%)	22	–	–	–
Acute functional decline (%)	–	–	–	69

30.3.1 Atypical presentations

An important clinical lesson that emerged during the pandemic is the fact that frail older adults were more likely to present with less typical symptoms (Table 30.1).[50] The median COVID-19 incubation period for older patients was similar to younger patients; however, whilst the core symptoms in the general population are typically fever, cough, dyspnea, and fatigue, the proportions of core symptoms in frail older people were lower.[51] Among frail older patients, the initial clinical presentation might lack specificity, leading to risk of misdiagnosis, a delay in isolating and treating, and therefore increased likelihood of viral spreading.[52,53] Almost a fifth of frail older patients were not initially suspected as having COVID-19, especially when the initial symptom was falls or delirium.[53] Delirium is defined as an acute disturbance of cognitive function and follows a fluctuating course. A study demonstrated higher prevalence of probable delirium as a COVID-19 symptom in older adults with frailty compared to nonfrail older adults.[54] The subtype of delirium was recorded in some studies.[46,55] The hypoactive form of delirium (which tends to have poorer prognosis) was more frequent, which might also lead to under-detection without proactive clinical screening. Other common clinical features observed in frail older adults were functional decline and hypotension.[49] From a survey among geriatricians, 22% reported falls as the onset symptom of COVID-19 in their patients.[46]

30.3.2 Biological heterogeneity of the population of hospitalized older people

Early reports from Italy and the UK confirmed a higher risk of hospital death in older adults, particularly in those with preexisting diseases.[56,57] The median age of patients who died in hospital from COVID-19 in the UK study was 80 years, and only 11% of those patients did not have a documented major comorbidity.[57] In a relatively more homogeneous frail older cohort admitted to geriatric wards, age was not

associated with in-hospital death.[48] Because of the significant biological heterogeneity of the population of hospitalized older people,[58] the systematic assessment of frailty in the acute hospital was proposed by some as being necessary for better risk-stratification and prognostication.

Indeed, in a large population of patients admitted to the hospital with COVID-19, disease outcomes were better predicted by frailty (as measured by the Clinical Frailty Scale) than either age or comorbidity.[59] The fatal outcome also seemed to worsen with increasing frailty after adjustment for age, comorbidities, and biological abnormalities, in other studies from secondary care settings.[60–62] Using the Frailty Index, frailty was also associated with higher in-hospital mortality or risk of transfer to Intensive Care Unit (ICU).[63] Using the Hospital Frailty Risk Score, a study also demonstrated an association between frailty and higher in-hospital mortality, prolonged hospital stay, the requirement of ICU, and invasive mechanical ventilation.[64]

On the available COVID-19 hospital treatments, many very frail older patients could not receive them because of genuine frailty-related contra-indications. For instance, risk of arrhythmia prevented the use of Hydroxychloroquine in some cases. Remdesivir is contraindicated in patients with creatinine clearance < 30 mL/min and elevated liver function tests. However, the efficacy of these repurposed treatments has been under scrutiny even in the nonfrail. When used in the frail, the efficacy of specific treatments including antiviral therapy, Hydroxychloroquine, and anticytokine or immunomodulatory agents, has not been systematically studied. Most patients (frail and nonfrail) have benefited from symptomatic treatments, including oxygen therapy, antibiotics, proton pump inhibitors, prophylactic anticoagulation, and other symptom control measures.

30.3.3 Ethical considerations

Concerns about potential shortages of healthcare professionals and health supplies in the fight against COVID-19 created focused attention on how these resources are ultimately allocated and used. Some strategies, for example, misguidedly utilized chronological age as an arbitrary criterion in resource allocation decisions, disregarding the wide biological heterogeneity in older adults. This led to justified ethical and age discrimination concerns, as well as renewed debates on ageism in societies.[65] Resource allocation strategies often rely on in-hospital survival and severe comorbidities contributing to short-term mortality.[66] In this regard, the Clinical Frailty Scale (CFS) inadvertently attracted attention as it was suggested by a majority of guidelines to screen for frailty in order to allocate resources.[12] This is partly because most observational studies used the CFS as a screening tool for frailty in hospital settings. One meta-analysis showed that the CFS was associated with an increase in COVID-19 mortality in a linear fashion.[67] A CFS of five points (i.e., moderately frail) was suggested as a cut-off for

no-escalation to critical care as it predicted higher mortality within 30 days after emergency admission.[68] In the UK, National Institute for Health and Care Excellence (NICE) published controversial COVID-19 critical care guidelines suggesting that when the person is significantly frail (e.g., a CFS score of 5 or more), consideration should be given to not provide critical care organ support. Initially, guidance was intended for all adults regardless of age, but this was subsequently backtracked to older adults only after numerous complaints from the public.[69]

Indeed, it has been argued that the CFS (similarly to all frailty identification tools) has limitations, and should be interpreted with caution, together with a holistic and individualized clinical approach when it is used to allocate resources.[70] Moreover, the original CFS creators emphasized that the CFS is meant to complement, and not replace overall person-centered clinical judgement.[71] In the UK, The National Health Service Specialist Clinical Frailty Network quickly recommended that the CFS should not be used in isolation but that clinical discussions must be taken in conjunction with patients' and (where relevant) carers' wishes, and that the guidance might not apply to younger people or those with chronic stable disabilities, in whom the CFS has not been validated.[72] These important societal debates have illustrated some of the dangerous disconnects that exist between the "measured" and the "lived" at the time of the COVID-19 pandemic (Fig. 30.1).

30.3.4 Optimization of the hospital environment and opportunities for in-hospital rehabilitation

Beyond the need for allocating critical care resources in COVID-19, considerations about comfort and quality of life, as well as optimization of the hospital environment, have also taken place. For example, in frail older patients, especially those living with dementia, environmental factors in the hospital can contribute to delirium development during acute COVID-19 infection. The limited interaction with hospital staff may increase the sense of isolation and induce patients' disorientation and reduced awareness. In addition, the use of personal protective equipment (PPE) by staff members has some depersonalizing and possibly frightening effect on those with cognitive impairment. Potentially for all, including frail older adults, hospitalization is a stressful situation often due to sleep deprivation and social isolation, and prolonged hospital stays (as seen in older people with COVID-19 infection) may also increase the risk of delirium. Reduced mobility in the hospital is also a compounding factor for hospital-associated functional decline.[73] In addition, frail older patients can be at higher risk of potentially inappropriate polypharmacy, including the use of sedatives, which may favor delirium and falls.[74]

Nonpharmacological interventions during the hospitalization are essential, especially for frail older patients. In a randomized controlled trial (RCT) designed for older COVID-19 patients (median age 69), a 6-week respiratory rehabilitation program was

reported to improve pulmonary function and quality of life.[75] Multidisciplinary geriatric expertise is fundamental for the rehabilitation of frail older COVID-19 survivors in the hospital.[76] Medical and allied health staff in geriatric wards have vast experience in managing acute disease with underlying frailty, especially when multisystemic failure is triggered by COVID-19. The multidomain interventions, such as nutrition support,[77] pain control, prevention of delirium, early mobilization, polypharmacy, and ethical considerations might improve not only survival and functional outcomes, but also the quality of the end-of-life.

30.3.5 Hospital-associated deconditioning and post-COVID-19 fatigue

Hospital-associated deconditioning (HAD) or posthospital syndrome is a situation of poor functional performance after an acute hospitalization,[78] and in frail older COVID-19 survivors, HAD and post-COVID-19 fatigue have been significant lived experience issues. HAD is a common aftermath of an acute hospitalization, especially in frail older adults. HAD means that a frail older patient may experience incomplete functional recovery and leave the hospital more disabled than before preadmission illness. The overall prevalence of HAD across studies has been estimated to be 30%,[79] but post-COVID-19 could be higher. Indeed, the various mechanisms of HAD are all present during a hospitalization due to COVID-19, such as allostatic overload, prolonged bed rest, maladaptive cognitive perceptions including stress and fear, sleep deprivation, delirium, and altered nutrition, all leading to the exacerbation of preexisting frailty and geriatric syndromes.[80,81] Moreover, many COVID-19 survivors have persistent symptoms long after recovery, even though the virus cannot be detected. Months after the initial infection, reported "long covid" symptoms include dyspnea, fatigue, pain,[82] and various neurocognitive impairments.[83]

In a multicenter European observational cohort study,[84] around a quarter of patients admitted with COVID-19 had increased care needs at discharge, with preadmission frailty being strongly associated with the need for an increased level of care at discharge. However, availability of rehabilitation and support services in the community has been reduced as a result of lockdowns and reductions in outpatient services capacity, posing significant challenges to frail older patients who were discharged from the hospital with HAD. Not surprisingly, in a relatively young cohort (mean age 67), the rehospitalization rate was around 10%.[85] Research on the long-term impact of frailty on quality of life and independent living after a COVID-19 hospitalization is one of the priorities identified by clinicians and researchers worldwide.[86]

30.4 The nursing home perspective

Almost unexpectedly from an initial planning and preparedness perspective, it soon became clear that frail older adults living in nursing homes were the most vulnerable

group of the COVID-19 pandemic. In its 24th April hearing, the WHO noted that more than half of the 110,000 deaths from COVID-19 in Europe were among people living in nursing homes, an "unimaginable human tragedy" that could be avoided in the future with significant improvements in nursing home policies.[87] Likewise, 27% of COVID-19 deaths in the USA occurred in long-term care (LTC) facilities, and nearly half of Canada's COVID-19 deaths were in this setting. Even though the reasons are complex, it was noted that LTC facilities are places with "3Cs" (i.e., crowded, closed spaces with poor ventilation, and conversations taking place at short distance).[88]

Nursing home residents are chronologically very old, have very high levels of physical and cognitive morbidities, and very high levels of physical disability. Indeed, nursing homes support the most vulnerable in societies.[89] Despite that, when nursing home residents are able to self-report, many report that their physical and mental health is fair, good, very good, or even excellent; this provides an important reminder that quality of life is often rated higher by oneself than by proxies, even in the presence of very advanced age and extensive comorbidities and disabilities.[89]

During the pandemic, there have been many difficulties in isolating residents and limiting the spread of COVID-19 in this setting. The combination of a vulnerable population that manifests nonspecific and atypical presentations of COVID-19, staffing shortages due to viral infection, poor availability of rapid, accurate testing and PPE, and lack of effective treatments for COVID-19 among residents, all contributed to create a "perfect storm" in nursing homes.[90] Initially, nursing homes reported severe staffing[91] and PPE shortages,[92] reflecting a prevalent preparedness model that tended to prioritize the acute hospital setting for resource allocation. In nursing homes, lack of personnel, difficulty in transferring patients to hospitals or other facilities, isolating residents with COVID-19, and a higher number of beds in many geographical areas, were the main factors positively associated to the presence of COVID-19 in nursing homes.[93] As high as three quarters of COVID-19 deaths were reported to occur in care homes in some regions.[39] Some studies also suggested that high mortality rates in some nursing homes might have been favored by a lack of medical care management due to limited primary care cover and poor connections with general hospitals.[94] Personal service workers may travel between different facilities providing part-time care to residents in different facilities increasing the likelihood of spread to regular staff and residents. Taken together, both residents and staff were at high risk of infection.

Because visits from relatives were usually banned and social or physical activities were canceled, lack of social engagement, physical exercise, and emotional support may worsen the cognitive, behavioral, and physical condition of the residents.[39] Prevention and control of infection, including COVID-19, in residential care settings depends on a hierarchy of measures encompassing environmental, administrative, and staffing factors, which are at least as important as standard infection prevention and control (IPC) measures. However, IPC training is essential—but often neglected—for protection of staff

and residents, not only during a pandemic but also for routine care. IPC training should be tailored to the educational levels and roles of staff, be nationally consistent to avoid confusion when they move between workplaces, and involve all aged care workers, including personal care, nursing, agency and support staff. In the long term, improved prevention and control of infection in residential aged care facilities should contribute to reducing the impacts of seasonal influenza and antimicrobial resistance, which are important causes of avoidable hospitalization and cost in aged care.[95]

In older adults, social isolation and loneliness increase depression, anxiety, cognitive dysfunction, heart disease, and mortality. When recommending a total moratorium on visiting nursing home residents, policymakers clearly considered the effects, including leaving residents to die with an unfamiliar person holding their hands, rather than their families. Are we benefiting these nursing home residents by putting strict no-contact measures in place and thereby depriving them of human touch?[96] From the lived experience perspective, strictly restrictive visitor policies as a consequence of the severe outbreaks have deeply impacted nursing home residents, their loved ones, and care professionals.[97] This has triggered calls for balancing protection from COVID-19 and the need for "human touch."[96] In 2020, as a result of the COVID-19 pandemic, we have seen renewed interest in telemedicine and remote communication solutions, which are likely to have a lasting impact in this setting.[98] Indeed, a "new era" of person-centered developments has been anticipated thanks to the severe problems that COVID-19 has exposed in this sector.[99] This has highlighted the need for universal adoption of more robust medical and nonmedical standards of care for this very vulnerable population.[100]

The impact of dementia has been very relevant in nursing homes during COVID-19. Many frail older people living with dementia in care homes are often living in close proximity with each other and share common areas (e.g., dining and living rooms) and are therefore at high risk of infection. Moreover, because older people who are infected may present with nonspecific symptoms, their informal or professional caregivers may become infected as they have not been warned in time to take necessary precautions.[39]

30.5 Research on COVID-19 treatments and service development perspectives

During this pandemic, many new and repurposed drugs are being actively studied as therapy or as a prophylaxis against COVID-19.[101] However, a recent systematic review demonstrated that frail older patients are underrepresented in COVID-19 clinical trials.[102] In this review, the mean age of patients was only 56.3 years. An upper age limit was reported in approximatively one third of published and ongoing RCTs. Some trials did not require an age limit however, they excluded functional decline, dementia, liver

disease, severe kidney disease, heart disease or risk of torsade de pointes, or impossibility to obtain informed consent. These are all conditions that are well overrepresented in frail older adults. Moreover, RCTs tend to not report clinical outcomes that are relevant for the geriatric population such as functional and/or cognitive decline or a need for rehabilitation or transfer to LTC facilities. Regarding COVID-19 vaccine development, there have been similar concerns about the absence of advanced age and frailty.[103] It is to be welcomed that the first groups to be vaccinated in late 2020 include frail older adults as well as healthcare staff. However, more research needs to be conducted as to the effectiveness of vaccinations in frail older adults.

Regarding service provision for frail older adults, the COVID-19 pandemic has also uncovered the need to adapt and expand the availability of specialist geriatric services. Although hospitals have rapidly created COVID-19 units, it has been challenging to allocate resources to create new specialized geriatric teams. However, existing teams have rapidly organized worldwide to offer the best possible responses for frail older patients. In France, 24/7 phone support and a mobile team in emergency wards were created to support decision making for these patients. In Brazil, the CO-FRAIL project was designed in a new repurposed COVID-19 hospital in Sao Paulo.[49] Extensive and relevant research was also conducted by the National Front To Strengthen The Long-Term Care Institutions For The Elderly (FN-ILPI) regarding the establishment of the reality of the LTCF in Brazil and what should be done to avoid or diminish the deaths.[104] In Ireland, there were multiple good practice examples of geriatric service responses during the pandemic, including the strengthening and repurposing of the Geriatric Day Hospital model of care.[105] The pandemic is impacting the LTC of frail patients worldwide and is increasing inequalities among different regions. In a coordinated call from Latin American and Caribbean countries, recommendations have been proposed for coordinated dementia care and related action plans.[106]

Given the burdens of COVID-19 for frail older adults, clinicians and healthcare administrators should carefully consider how frailty affects pandemic planning. Although no studies have yet looked at frailty as an outcome marker in COVID-19 infection, a large body of evidence has established that frailty is a good general predictor of post–critical care functional decline and mortality. The COVID-19 pandemic highlights two major opportunities for ethical reflection. The first is that, regardless of resource abundance or scarcity, principles from both clinical and public health ethics encourage clinicians to focus on advance care planning, supporting frail patients to access care in keeping with their values and goals. Additionally, administrators must ensure that there is adequate supportive and palliative care for patients who wish to avoid aggressive interventions. Pandemic planning must not focus exclusively on ventilators or critical care. Patient frailty should carry significant weight, but not the only weight, in clinical decision-making. The healthcare system must support frail older adults who are disproportionately affected by COVID-19 illness and mortality, and

minimize the burdens placed on them. Instead of asking "how do we ration a scarce resource," clinicians and health administrators should ask "how do we best deliver holistic care to the frailest and most vulnerable among us?"[107]

30.6 Conclusions

In many ways, COVID-19 has been an eye opener because it has exposed how fragile our systems are and how quickly our world can turn upside down. But it has also shown us that we are capable of responding to a global crisis, overcome silos and borders, and collaborate to find solutions collectively. This provides hope. From very tough lessons learned at the beginning of the pandemic, we are now seeing instances of "benevolent ageism" including renewed advocacy and recognized higher priority for frail older adults to receive healthcare (triage, COVID-19 testing, COVID-19 vaccine) and obtain better support in the community.[108] We are able to adapt, to cope, to learn and to grow. We may have the most to learn from those who have already learned the most, and those are our older people. The COVID-19 pandemic spotlights a population that is rapidly growing in proportion worldwide, and it provides a unique chance to reimagine our communities, our livelihoods and how we want to live, together across age-groups, in a postpandemic world that has to resolve many more grand challenges lying head of us besides population aging (e.g., climate change, migration, urbanization, and digitalization).

The raising awareness of the importance of protecting the most vulnerable in our societies is an excellent opportunity to advocate for the clinical, societal, and research needs in this population. There is also space for reflections about ethical considerations and an adaptive way to collect consent from people living with noncommunicable diseases. Older frail people and their families should also be invited to be involved in the research, to know their view as priorities.[86] It is vital to acknowledge the current global burden on patients, families, caregivers, and health professionals, dealing with day-to-day activities with the successive waves, and concomitantly the need for research. Collaborative, coordinated, and transdisciplinary work is more than ever necessary, with a perspective to influence health policy.

Competing interest

The authors have no competing interests.

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CHAPTER 31

Aging: an illustrated adventure

João Ramalho-Santos[1,2] **and André Caetano**[2]
[1]Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[2]CNC - Centre for Neuroscience and Cell Biology, CIBB - Centre for Innovative Biomedicine and Biotechnology, University of Coimbra, Coimbra, Portugal

Aging
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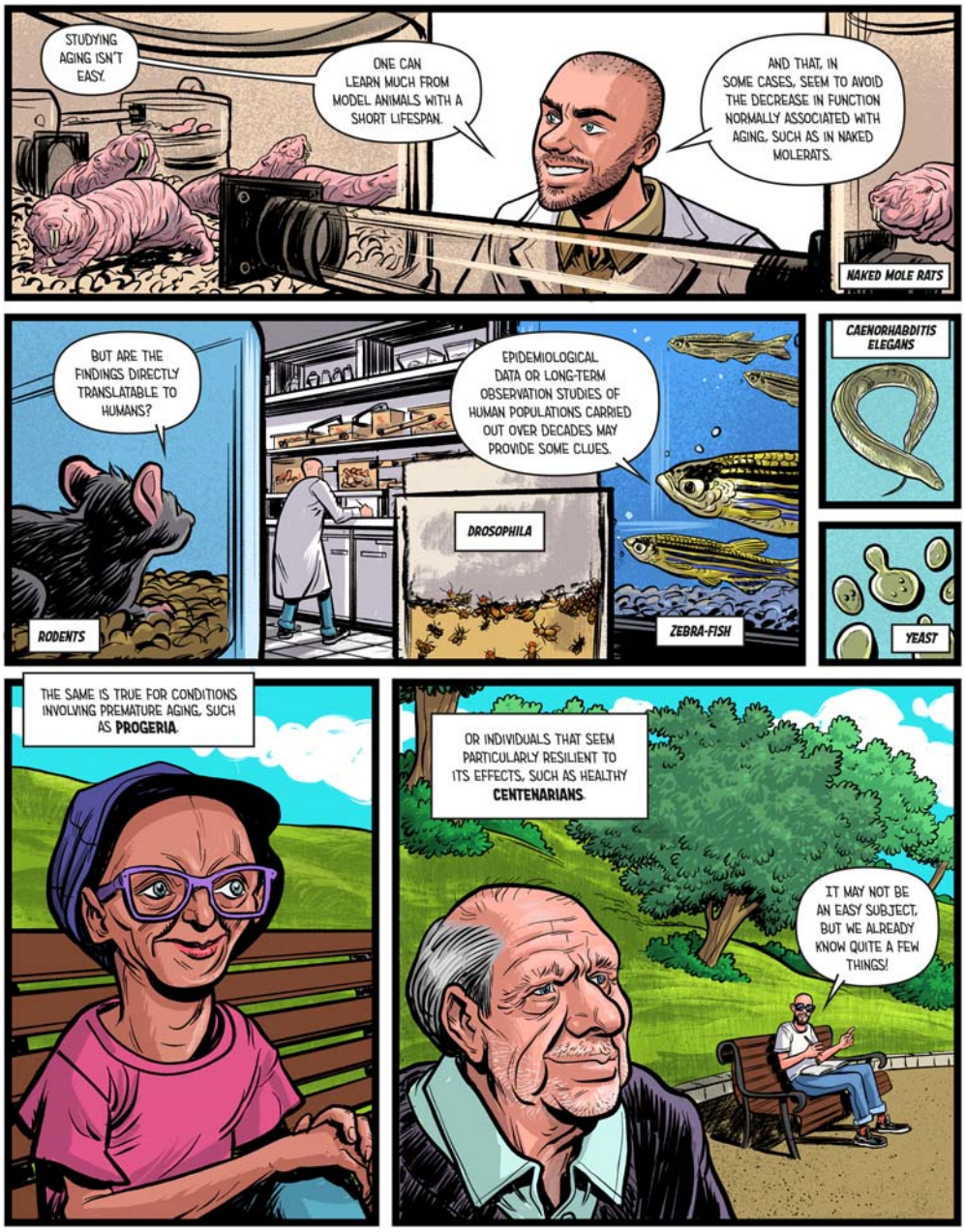
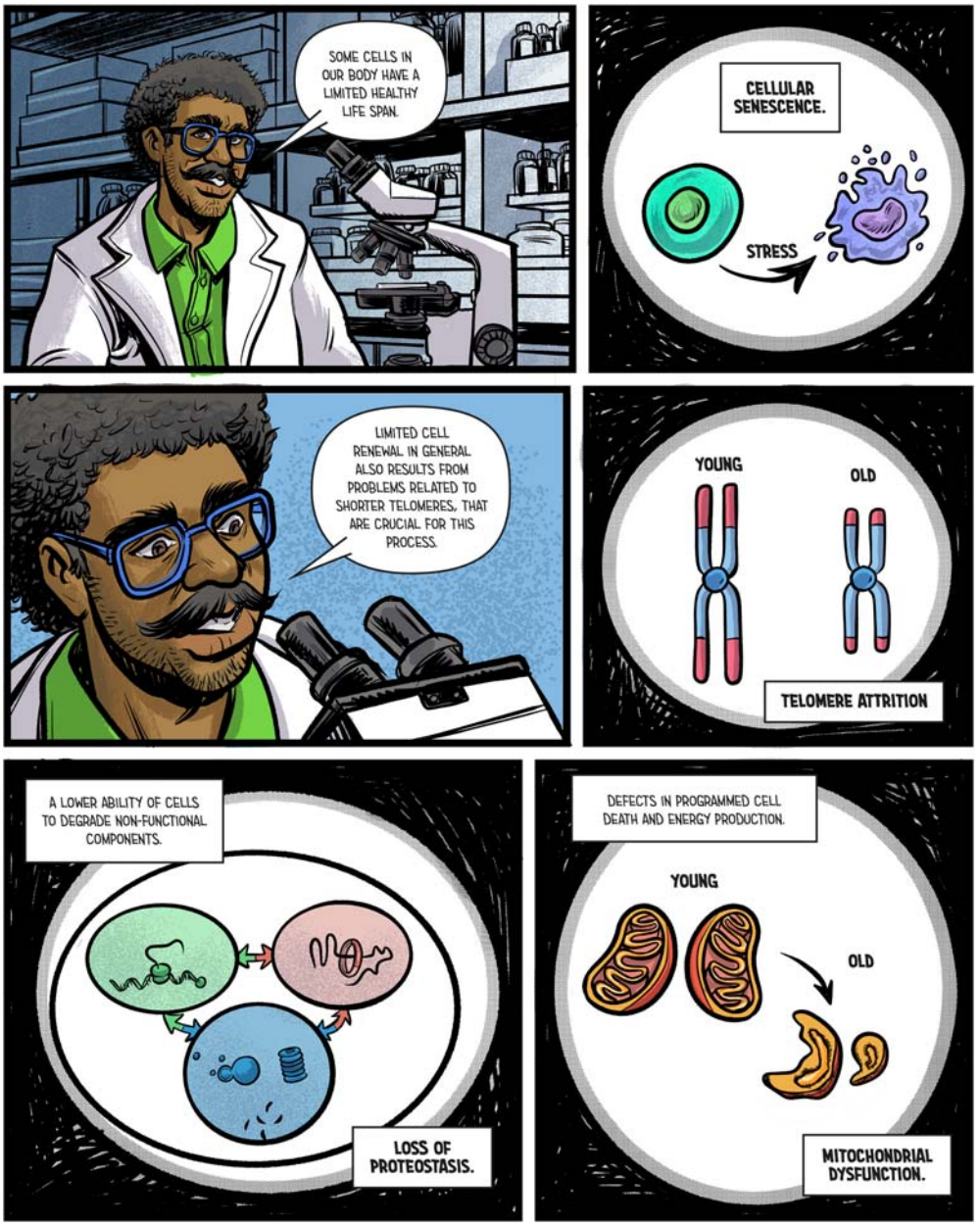
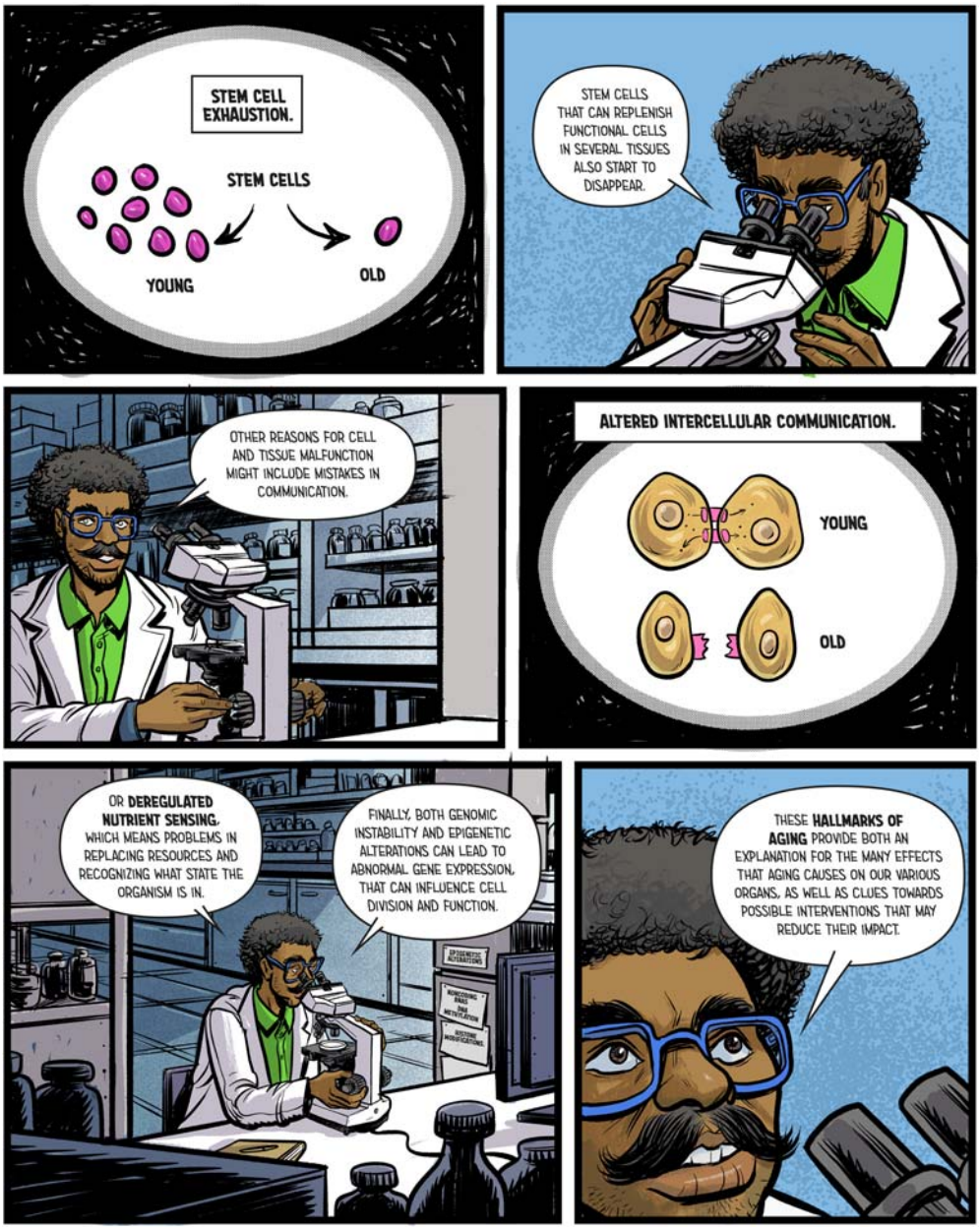
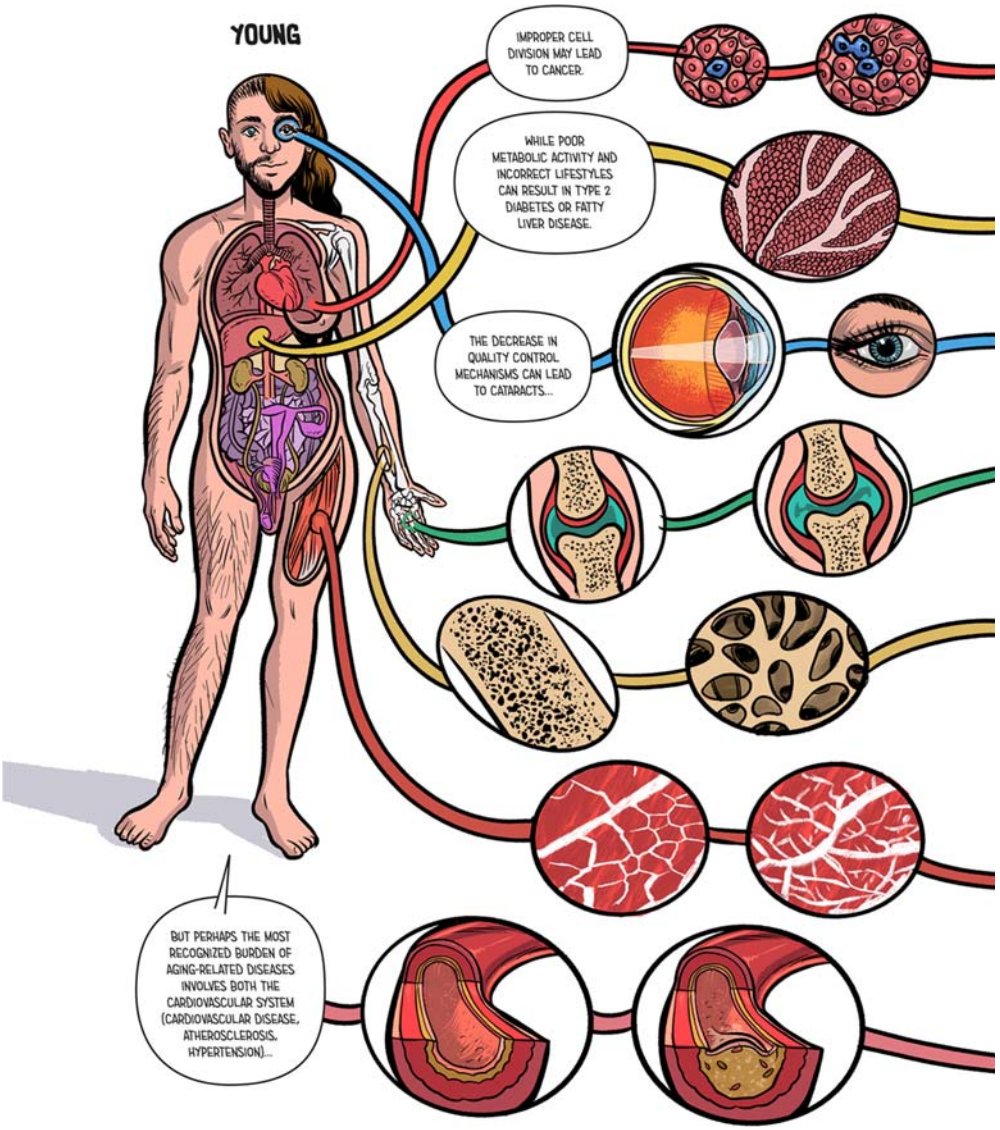
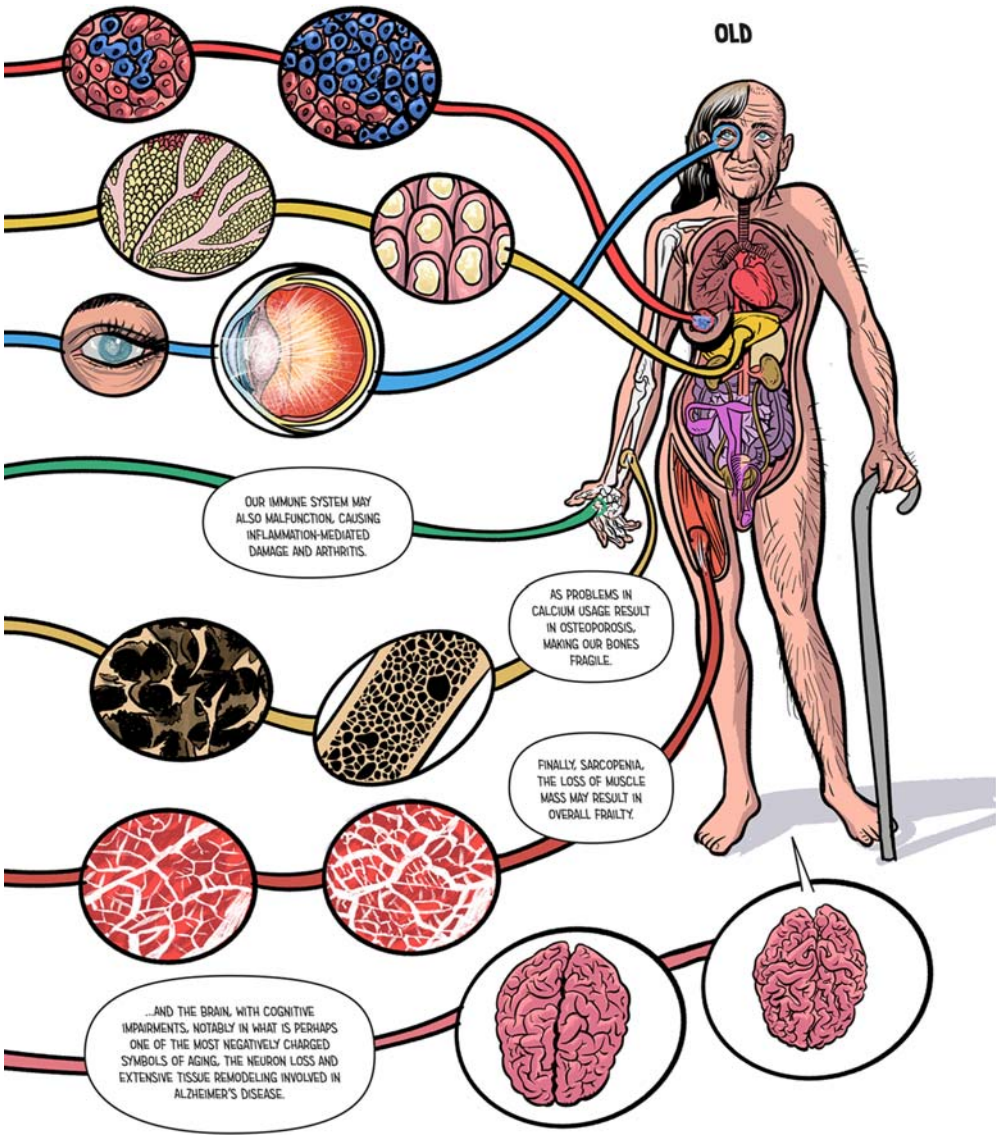
THE FIRST THING WE MUST REALIZE ABOUT AGING, IS THAT IT IS NOT A DISEASE. IT IS A NORMAL PROCESS WE ALL GO THROUGH, ESSENTIALLY SINCE THE DAY WE ARE BORN.
HOWEVER, AGING DOES GREATLY EXACERBATE ALMOST ALL THE DISEASES ONE CAN THINK OF.
GIVEN THE ONGOING DEMOGRAPHIC EXPLOSION, WE WILL FACE MANY CRUCIAL CHALLENGES RELATED WITH AN AGING POPULATION.
COMICS
OLDMAN
KEEPING THE POPULATION AS HEALTHY AND ACTIVE AS POSSIBLE, ESPECIALLY USING PREVENTIVE STRATEGIES, IS THE ULTIMATE SOCIETAL GOAL IN AGING-RELATED RESEARCH...
...TO BOTH INCREASE PRODUCTIVITY AND SELF-WORTH...
AS WELL AS TO REDUCE THE BURDEN ON HEALTHCARE SYSTEMS.


STUDYING AGING ISN'T EASY.
ONE CAN LEARN MUCH FROM MODEL ANIMALS WITH A SHORT LIFESPAN.
AND THAT, IN SOME CASES, SEEM TO AVOID THE DECREASE IN FUNCTION NORMALLY ASSOCIATED WITH AGING, SUCH AS IN NAKED MOLERATS.
NAKED MOLE RATS
BUT ARE THE FINDINGS DIRECTLY TRANSLATABLE TO HUMANS?
RODENTS
EPIDEMIOLOGICAL DATA OR LONG-TERM OBSERVATION STUDIES OF HUMAN POPULATIONS CARRIED OUT OVER DECADES MAY PROVIDE SOME CLUES.
DROSOPHILA
ZEBRA-FISH
CAENORHABDITIS ELEGANS
YEAST
THE SAME IS TRUE FOR CONDITIONS INVOLVING PREMATURE AGING, SUCH AS PROGERIA.
OR INDIVIDUALS THAT SEEM PARTICULARLY RESILIENT TO ITS EFFECTS, SUCH AS HEALTHY CENTENARIANS.
IT MAY NOT BE AN EASY SUBJECT, BUT WE ALREADY KNOW QUITE A FEW THINGS!


SOME CELLS IN OUR BODY HAVE A LIMITED HEALTHY LIFE SPAN.
CELLULAR SENESCENCE.
STRESS
LIMITED CELL RENEWAL IN GENERAL ALSO RESULTS FROM PROBLEMS RELATED TO SHORTER TELOMERES, THAT ARE CRUCIAL FOR THIS PROCESS.
YOUNG
OLD
TELOMERE ATTRITION
A LOWER ABILITY OF CELLS TO DEGRADE NON-FUNCTIONAL COMPONENTS.
LOSS OF PROTEOSTASIS.
DEFECTS IN PROGRAMMED CELL DEATH AND ENERGY PRODUCTION.
YOUNG
OLD
MITOCHONDRIAL DYSFUNCTION.


STEM CELL EXHAUSTION.
STEM CELLS
YOUNG
OLD
STEM CELLS THAT CAN REPLENISH FUNCTIONAL CELLS IN SEVERAL TISSUES ALSO START TO DISAPPEAR.
OTHER REASONS FOR CELL AND TISSUE MALFUNCTION MIGHT INCLUDE MISTAKES IN COMMUNICATION.
ALTERED INTERCELLULAR COMMUNICATION.
YOUNG
OLD
OR DEREGULATED NUTRIENT SENSING, WHICH MEANS PROBLEMS IN REPLACING RESOURCES AND RECOGNIZING WHAT STATE THE ORGANISM IS IN.
FINALLY, BOTH GENOMIC INSTABILITY AND EPIGENETIC ALTERATIONS CAN LEAD TO ABNORMAL GENE EXPRESSION, THAT CAN INFLUENCE CELL DIVISION AND FUNCTION.
THESE HALLMARKS OF AGING PROVIDE BOTH AN EXPLANATION FOR THE MANY EFFECTS THAT AGING CAUSES ON OUR VARIOUS ORGANS, AS WELL AS CLUES TOWARDS POSSIBLE INTERVENTIONS THAT MAY REDUCE THEIR IMPACT.


YOUNG
IMPROPER CELL DIVISION MAY LEAD TO CANCER.
WHILE POOR METABOLIC ACTIVITY AND INCORRECT LIFESTYLES CAN RESULT IN TYPE 2 DIABETES OR FATTY LIVER DISEASE.
THE DECREASE IN QUALITY CONTROL MECHANISMS CAN LEAD TO CATARACTS...
BUT PERHAPS THE MOST RECOGNIZED BURDEN OF AGING-RELATED DISEASES INVOLVES BOTH THE CARDIOVASCULAR SYSTEM (CARDIOVASCULAR DISEASE, ATHEROSCLEROSIS, HYPERTENSION)...


OLD
OUR IMMUNE SYSTEM MAY ALSO MALFUNCTION, CAUSING INFLAMMATION-MEDIATED DAMAGE AND ARTHRITIS.
AS PROBLEMS IN CALCIUM USAGE RESULT IN OSTEOPOROSIS, MAKING OUR BONES FRAGILE.
FINALLY, SARCOPENIA, THE LOSS OF MUSCLE MASS MAY RESULT IN OVERALL FRAILTY.
...AND THE BRAIN, WITH COGNITIVE IMPAIRMENTS, NOTABLY IN WHAT IS PERHAPS ONE OF THE MOST NEGATIVELY CHARGED SYMBOLS OF AGING, THE NEURON LOSS AND EXTENSIVE TISSUE REMODELING INVOLVED IN ALZHEIMER'S DISEASE.


AND THEY ALSO OFFER WINDOWS OF OPPORTUNITY TO DEVELOP NOVEL STRATEGIES AND COMPOUNDS THAT CAN MINIMIZE, IF NOT REVERT, THE EFFECTS OF AGING.
FOR EXAMPLE: SPECIFICALLY TARGETING MITOCHONDRIA, THE CELL'S POWERHOUSE, TO AVOID THEIR DYSFUNCTION.
BOOSTING THE POOLS OF STEM CELLS TO ACCELERATE CELL RENEWAL IN DAMAGED TISSUES.
ALTHOUGH WE CANNOT INFLUENCE WHAT HAPPENED BEFORE WE WERE BORN, WE CAN TAKE AS MUCH RESPONSIBILITY AS WE CAN FOR OUR LIVES AND THOSE OF OUR CHILDREN.
A HEALTHY BALANCED DIET AND REGULAR EXERCISE, BOTH PHYSICAL AND MENTAL, ARE KNOWN TO DECREASE THE EFFECTS OF MOST AGING-RELATED DISORDERS.
AS ARE APPROPRIATE LIVING FACILITIES, ACCESS TO INFORMATION, AND SOCIAL INTERACTIONS, AND A CULTURE OF INCLUSION.


REVERTING GENETIC AND EPIGENETIC CHANGES.
PREVENTING TELOMERE EROSION.
BOOSTING QUALITY CONTROL AND REMOVAL OF POORLY FUNCTIONAL PROTEINS AND ORGANELLES IN OUR CELLS.
CONTROLLING TOXIC INFLAMMATION-RELATED RESPONSES.
THIS IS A FIELD OF GROWING IMPORTANCE, AND IN WHICH MANY INNOVATIVE PROJECTS CAN BE DESIGNED, NEEDING DIVERSE CONTRIBUTIONS.
IN FACT, IT IS HARD TO ENVISION A MORE MULTIDISCIPLINARY FIELD OF STUDY THAN AGING.
AGING MAY BE INEVITABLE BUT HEALTH-RELATED CONSEQUENCES ARE NOT.

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Index

Note: Page numbers followed by "*f*" and "*t*" refer to figures and tables, respectively.

B

C

D

E

F

G

H

I

M

N

Q

R

S

T

U

AGING

From Fundamental Biology to Societal Impact

Edited by Paulo J. Oliveira and João O. Malva

Aging: From Fundamental Biology to Societal Impact examines the interconnection of the cellular and molecular basis of aging and societal-based challenges and innovative interventions. The book consists of four sections: Section 1 takes a societal-based angle on aging, describing several flagship initiatives for healthy living and active aging in different regions; Section 2 covers the biology of aging which includes the hallmarks of aging; Section 3 explains the pathophysiology of aging, describing different comorbidities associated to aging and possible interventions to decrease the impact of aging; and Section 4 envisions the future and innovative measures to tackle aging-related morbidities.

Contributions from an interdisciplinary panel of experts cover topics such as the biology of aging to physical activity, nutrition, psychology, pharmacology, health care, social care, and urban planning. This book is an excellent resource for public health and aging researchers, gerontologists, cellular and molecular biologists, and health and social care providers.

Key Features

- Provides a cross-disciplinary approach to aging at both the biological and societal level
- Highlights frontline scientific knowledge in biology of aging and its translation into societal interventions
- Offers insight on the value of aging research and its future impact not only from a fundamental and translation point of view, but also from the innovation and organizational angle

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