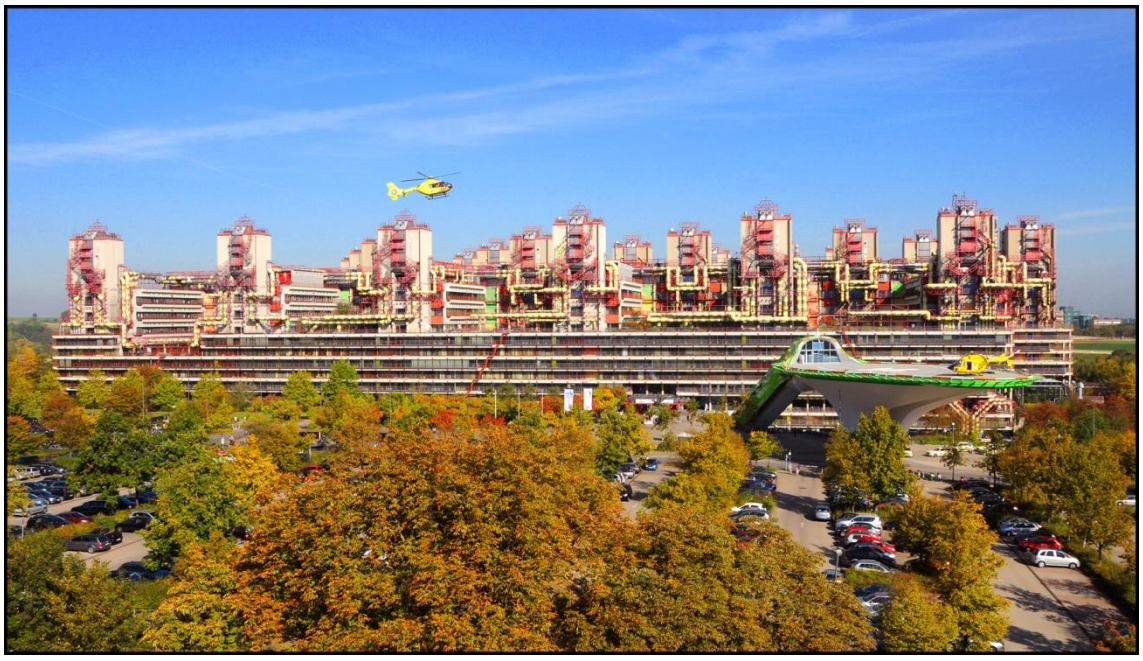
33. Joint Annual Meeting of the German Society for Minerals and Trace Elements (GMS) with Zinc-UK

Aachen, Germany, 28.-30.09.2017

Zinc and other Transition Metals in Health and Disease

Program & Book of Abstracts



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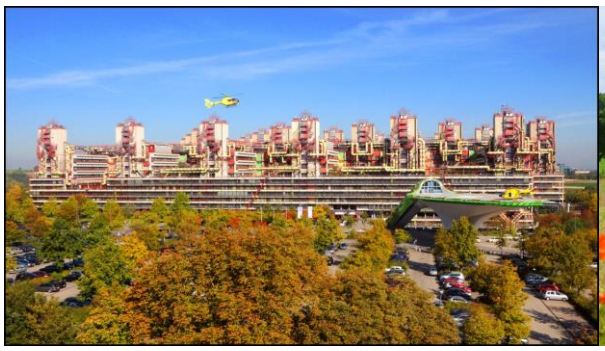
CONTENTS



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PROGRAM

Thursday, September 28th

12:00	Registration (Entrance)
	Coffee (Seminar Room)
13:00-16:00	GMS-Workshops (Lecture Halls 4 & 6)
	Alternatives for Animal Toxicity Testing: Principles, Approaches and Limitations Tanja Schwerdtle (University of Potsdam, Germany) (Lecture Hall 4)
	Terms, Strategies and Quality Control in Element Analysis and Element Speciation Bernhard Michalke (Helmholtz Zentrum München, Germany) (Lecture Hall 6)
16:00-18:00	**GMS Board Meeting (Bibliothek Immunologie; Elevator D4, Level 6, Room 35)**
17:30	Registration (Entrance)
	Coffee (Seminar Room)
18:30	Öffentlicher Abendvortrag (Public Talk in German) (Lecture Hall 4)
	Verborgene Wirkungsweisen von Zink in Diabetes Wolfgang Maret (King´s College, London, UK)
19:30	**Welcome Reception with Finger Food Buffet (Seminar Room)**

Friday, September 29th

8:00	Registration (Entrance)
	Coffee (Seminar Room)
8:45 – 9:00	Opening (Lecture Hall 4)
	INTERACTIONS OF TRACE ELEMENTS WITH THE IMMUNE SYSTEM I (Lecture Hall 4) Chair: Bernhard Michalke (Helmholtz Zentrum München, Germany)
9:00 – 9:30	**T1. Iron and Inflammation** Manfred Nairz (Medical University Innsbruck, Austria)
9:30 – 10:00	**T2. Zinc in Inflammatory Processes** Inga Weßels (RWTH Aachen University Hospital, Germany)
10:00 – 10:30	**T3. Targeting Zinc to Stop Cancer Growth** Kathryn M. Taylor (Cardiff University, UK)
10:30 – 11:00	Coffee Break (Seminar Room)

INFLUENCES OF ZINC ON THE NERVOUS SYSTEM (Lecture Hall 4)
Chair: Tanja Schwerdtle (University of Potsdam, Germany)

11:00 – 11:30	**T4. Effects of Zinc on Cognitive Function** Nicola M. Lowe (University of Central Lancashire, Preston, UK)
11:30 – 12:00	**T5. Impact of Zinc on Behavior** Andreas Grabrucker (University of Limerick, Ireland)
12:00 – 12:30	**T6. Zinc and Eye Diseases** Imre Lengyel (Queen´s University Belfast, UK)
12:30 – 13:00	**Heinz-Zumkley-Prize 2017 Lectures (Lecture Hall 4)** Martina Maywald (RWTH-Aachen University Hospital, Germany) Nikolay Solovyev (St. Petersburg State University, Russian Federation)
13:00 – 14:00	**Lunch (Seminar Room)**

GENERAL HEALTH IMPACT OF TRANSITION METALS I (Lecture Hall 4)
Chair: Dirk Schaumlöffel (Université de Pau et des Pays de l'Adour/CNRS, France)

14:00 – 14:30	**T7. Dietary Copper and Human Health** Muriel Bost (CHU, Lyon, France)
14:30 – 15:00	**T8. Impact of Zinc on Cardiomyocytes** Belma Turan (University of Ankara, Turkey)
15:00 – 15:30	**T9. Zinc Homeostasis and Healthy Ageing** Robertina Giacconi (INRCA, Ancona, Italy)
15:30 – 16:00	**T10. Investigation of Zinc Uptake and Bioavailability in an *in vitro* Model of the Gastrointestinal Tract** Hajo Haase (Technical University Berlin, Germany)
16:00 – 16:30	Coffee Break (Seminar Room)
16:30 – 17:30	Oral Presentation of Selected Poster I (Lecture Hall 4)
17:30 – 18:15	**GMS Business Meeting (Lecture Hall 4)**
17:45	**Poster Viewing (Seminar Room)** with Coffee, Drinks
19:30	**Dinner (Seminar Room)**

Saturday, September 30th

GENERAL HEALTH IMPACT OF TRANSITION METALS II (Lecture Hall 4)
Chair: Anna Kipp (University of Jena, Germany)

9:00 – 9:30	**T11. The Role of Nickel in Bacterial Pathogenicity** Barbara Zambelli (University of Bologna, Italy)

9:30 – 10:00	**T12. Perspectives for Vanadium in Health Issues** Dieter Rehder (University of Hamburg, Germany)
10:00 – 10:30	**T13. Copper Associated Hepatitis in Dogs** Louis C. Penning (University of Utrecht, The Netherlands)
10:30 – 11:00	Coffee Break (Seminar Room)

INTERACTIONS OF TRACE ELEMENTS WITH THE IMMUNE SYSTEM II (Lecture Hall 4)
Chair: Esther Humann-Ziehank (University of Veterinary Medicine Hannover, Germany)

11:00 – 11:30	**T14. Zinc and Autoimmunity** Dirk Reinhold (Otto-von-Guericke University Magdeburg, Germany)
11:30 – 12:00	**T15. Impact of Cobalt (II) on the Immune System** Eeva Moilanen (University of Tampere, Finland)
12:00 – 12:30	**T16. Toxicity of Manganese** Julia Bornhorst (University of Potsdam, Germany)
12:30 – 13:15	Oral Presentation of Selected Poster II **& Poster Prices** (Lecture Hall 4)
13:15 –13:45	Lunch “to go” (Seminar Room)
13:45	Optional City Tour in Aachen (Starts at Seminar Room)

ABSTRACTS

Talks

Public Talk in German
Title: Verborgene Wirkungsweisen von Zink in Diabetes

Author: Wolfgang Maret

Affiliation: King's College London, Metal Metabolism Group, Departments of Biochemistry and Nutritional Sciences, School of Life Course Sciences, Faculty of Life Sciences and Medicine, London, UK. wolfgang.maret@KCL.ac.uk

Overview: Mindestens zwanzig chemische Elemente sind für den Menschen essentiell. Viele zusätzliche Elemente sind in Geweben nachweisbar, und deren Anwesenheit hat weitgehend unbekannte Folgen. Änderungen der Konzentrationen dieser Elemente können die Entstehung und das Fortschreiten von Diabetes Typ 2 hemmen oder fördern. In diesem Vortrag erkläre ich warum gerade Zink eine entscheidende Bedeutung als Mikronährstoff hat. Wir haben etwa soviel Zink wie Eisen in unserem Körper. Zink hat allerdings als Zellbaustein eine größere Anzahl wichtiger Funktionen als Eisen. Was die Regulierung von biochemischen Vorgängen betrifft, so ist die Wirkungsweise von Zink eher mit der des Calciums vergleichbar. In Diabetes ist Zink grundlegend in der Biochemie der Langerhans-Inseln und der Insulin-abhängigen Zellen beteiligt. Zink wird zur Speicherung und Ausschüttung von Insulin aus den Betazellen benötigt und beeinflusst die Glukagonausschüttung von den Alphazellen. In den Insulin-abhängigen Zellen hat Zink aufgrund einer Steuerung des Insulinrezeptors insulinähnliche Effekte. Im Verlauf der Diabetes entsteht durch den Stoffwechselstress ein Zinkmangel, für den es allerdings keinen guten klinischen Test gibt. Während in der Wissenschaft die Zusammenhänge zwischen Diabetes und einer Störung des Mineral- und Spurenelementstoffwechsels verhältnismäßig gut beschrieben sind, bleiben für die Praxis Fragen unbeantwortet: zum Beispiel wie man Störungen des Zinkstoffwechsels beim Diabetiker bestimmen kann und wie man Zink oder andere Substanzen, die den Zinkstoffwechsel beeinflussen, in der Vorbeugung und Behandlung von Typ 2 Diabetes einsetzen kann.

Conclusion: Als verborgen bezeichne ich die Wirkungsweisen von Zink weil die wissenschaftlichen Erkenntnisse im krassen Gegensatz zu der medizinischen Praxis stehen und weil es bisher nicht gelungen ist sie zu Gesundheitszwecken mit erwiesenem und durchschlagendem Erfolg in der Prophylaxe und Therapie anzuwenden.

Abstract: Hidden Actions of Zinc in Diabetes

Several essential and numerous non-essential elements affect the development and progression of diabetes. A case in point with the widest implications is the micronutrient zinc, which in addition to its multiple housekeeping functions in cells is critically involved in the biochemistry of pancreatic islets and insulin-sensitive target cells. Genetic susceptibility and environmental factors affect the control and utilization of zinc. It will be discussed how perturbations of zinc metabolism contribute to the development and progression of diabetes and what the present challenges are in translating research findings into clinical practice.

T1
Title: Iron and Inflammation

Authors: Manfred Nairz*, Andrea Schroll, David Haschka, Piotr Tymoszuk, Igor Theurl, Günter Weiss

Affiliation: Department of Internal Medicine II, Infectious Diseases, Immunology, Rheumatology, Pneumology, Medical University of Innsbruck, Austria, Email: manfred.nairz@i-med.ac.at

Overview: The acute-phase response is triggered by the presence of infectious agents and other kinds of danger to the integrity of the mammalian body. One central feature of this response is the removal of trace elements such as iron and zinc from the circulation. The sequestration of these essential nutrients into storage compartments limits their availability for pathogens, a host defense strategy known as 'nutritional immunity'.
Iron metabolism and the immune response are intimately linked. In bacterial infections, the availability of iron affects both the efficacy of antimicrobial immune pathways and pathogen proliferation. However, mechanisms of microbial iron withholding vary largely dependent on the localization of pathogens: In infections with extracellular microbes, the mechanisms to reduce serum iron levels culminate in the hypoferremia of the acute phase response. The iron-regulatory hormone hepcidin is a major executor of this pathway because it tags the iron-exporter ferroportin for internalization from iron-absorbing enterocytes and iron-recycling macrophages which disrupts both routes of iron delivery to the circulation. When intracellular microbes are present, other strategies of microbial iron withdrawal are needed.
We found that in infections with Salmonella Typhimurium, which invades macrophages and replicates in their phagolysosomes, ferroportin was up-regulated to stimulate iron efflux from infected cells. The underlying mechanism could be traced back to a nitric oxide-induced transcriptional induction. This also led to reduced iron-incorporation into the iron storage protein ferritin. When the cellular control of macrophage iron homeostasis was disrupted in macrophages lacking both iron-regulatory proteins 1 and -2, iron became more available to Salmonella because the bacterium may have access to ferritin-associated iron.
Hfe-associated hemochromatosis is a genetic iron-overload disorder that affects parenchymal cells such as hepatocytes and cardiomyocytes while sparing the mononuclear phagocyte system. Notably, Hfe-deficient mice were protected from infections with Salmonella Typhimurium because of the scarcity of iron in myeloid cells. Therefore, the resistant phenotype was also present in mice lacking Hfe exclusively in monocytes and macrophages. In contrast, mice lacking Hfe in hepatocytes succumbed earlier to Salmonella infection because interferon-gamma and nitric oxide production were not properly induced.
In the same in vitro model, we found that macrophages infected with Salmonella Typhimurium had elevated intracellular zinc levels. While macrophages responded to infection by enhanced expression of zinc scavenging methallothioneins-1 and -2, free intracellular zinc inhibited the activation of nuclear factor-kappa B thus impairing the transcription of nitric oxide synthase and NADPH oxidase and pathogen elimination, consequently.

Conclusion: Together, our study shows that trace elements such as iron and zinc exert major impact on macrophage effector functions in the setting of Salmonella Typhimurium infection. A more detailed understanding of the interactions of trace elements and the immune system may provide the rational basis to develop immune-modulatory therapies for infectious diseases that act independent of established antibiotics.

T2
Title: Zinc in Inflammatory Processes

Authors: Inga Weßels

Affiliation: Inga Weßels, Institute of Immunology, University Hospital RWTH Aachen, Germany; iwessels@ukaaachen.de

Overview: During acute phase response, zinc is transiently transferred from serum to the liver via zinc transporter Zip14, causing serum hypozincemia. Systemic and chronic inflammatory diseases such as sepsis and rheumatoid arthritis are distinguished by augmented serum hypozincemia with persisting, significantly lower serum zinc concentrations. Hallmarks of disease include increased leukocyte numbers and hyper-inflammation, causing severe organ damage and death. Interestingly, our recent research could directly associate aforementioned symptoms to long-term hypozincemia. Low extracellular zinc levels reversibly augmented monopoiesis in human myeloid cells *in vitro*, as published for dendritic cell development and suggested for granulopoiesis. Additionally, long-term zinc deprivation increased basal and activation-induced expression of various pro-inflammatory mediators in human cell-lines *in vitro*. Here, epigenetic mechanisms and changes in reactive-oxygen-species formation were amongst underlying mechanisms. Zinc-replenishment normalized gene expression.

In line with this, our *in vivo* studies revealed beneficial effects of zinc supplementation prior to disease induction, including ameliorated inflammatory responses, lower serum leukocyte numbers and decreased organ damage. Surprisingly, also bacterial load was lower underlining more efficient pathogen clearance after zinc supplementation.

Conclusion: Recent years strongly improved our understanding of the association of altered zinc homeostasis with the transition from physiological acute phase reaction to detrimental inflammatory diseases. Extending existing data, especially to the human system, could path the way for developing alternative zinc-based therapeutic options.

T3
Title: Targeting Zinc to Stop Cancer Growth

Authors: Kathryn M Taylor*, Olivia Ogle and Silvia Ziliotto.

Affiliation: School of Pharmacy and Pharmaceutical Sciences, College of Biomedical and Life Sciences, Cardiff University, Redwood Building, King Edward VIIth Avenue, Cardiff, CF10 3NB, UK Email: TaylorKM@cardiff.ac.uk

Overview: Zinc has been known to be essential for cell division for over 50 years, yet the mechanism explaining its exact involvement has been elusive. Investigation of the ZIP family of zinc transporters (SLC39A) at the molecular level has enabled the discovery of a key role for zinc transporters in driving the growth of cancers. We have demonstrated that release of zinc from intracellular stores requires activation of zinc transporter ZIP7 by phosphorylation. Having generated an antibody that only recognizes the active form of ZIP7 we have been able to develop a new immunohistochemical test to be used on clinical material. Our analysis has confirmed the association of activated ZIP7 with aggressive disease states on clinical material, providing ZIP7 as a new marker of cancer and a potential new target for preventing cancer growth. Additional investigations of two other zinc transporters have discovered a mechanism for zinc influx triggering the onset of mitosis. We have shown that a heteromer forms from two distinct zinc transporters on the plasma membrane and that zinc influx through this heteromer is essential to trigger cell division. The subsequent pathway involves STAT3 binding in a complex which leads to activation of the usual mitosis cascades. Having generated antibodies to the extracellular domain of these two ZIP transporters we demonstrate how antibody binding can block the zinc influx and thus also inhibit the onset of mitosis, even in the presence of mitotic causing agents such as nocodazole. This concept has been demonstrated in multiple cancer cell lines providing these antibodies as novel clinical agents in the prevention of cell division in hyperproliferative diseases such as cancer.

Conclusion: Our functional investigations of the ZIP family of zinc transporters has now provided a new cancer biomarker which should be useful at assessing aggressive breast cancer growth and development. Furthermore, we have developed new antibody tools to inhibit the growth of multiple cancer, including breast, colon, lung and prostate cancer as well as melanoma.

T4
Title: Effects of Zinc on Cognitive Function

Authors: Nicola M Lowe[1]*, Pamela Qualter[2], Mukhtiar Zaman[3]

Affiliation: [1] International Institute of Nutritional Sciences and Food Safety Studies, Faculty of Health and Wellbeing, University of Central Lancashire. [2] School of Psychology, University of Central Lancashire. [3] Lady Reading Hospital, Peshawar, Pakistan.

Overview: It has been estimated that 20% of the world population are zinc (Zn) deficient. Zn deficiency occurs in individuals and populations whose diets are low in sources of readily bioavailable Zn (such as red meat and seafood) and high in unrefined cereals (rich in phytate and dietary fibre). These dietary patterns are characteristics that are common in many developing countries where Zn deficiency has a major impact on child development. The precise role of Zn in cognitive function is still unclear, however, Zn is present in relatively high concentrations in the hippocampal and neocortical regions of the brain. Much of the evidence for the role of Zn in the function of the central nervous system has come from animal studies, which have shown that Zn deficiency results in reduced activity, poor memory and attention, also in offspring rats, Zn deficiency during the last trimester of pregnancy and during lactation impaired spatial learning and memory and had a negative effect on motor activity (Boroujeni et al., Biol Trace Elem Res 2009; Golub et a., AJCN 1994). A systematic review and meta-analysis of studies conducted in children failed to show a significant effect of Zn supplementation on cognitive functioning in children though, taken as a whole, there were some small indicators of improvement on aspects of executive function and motor development following Zn supplementation (Warthon-Medina et al., EJCN 2015). In collaboration with the Abaseen Foundation (AF), a non-government organization that supports healthcare and education in North West Pakistan, we are engaged in a programme of research, based in the brick kiln region on the outskirts of Peshawar. Anthropometric data from the local health centre indicates that malnutrition and associated growth stunting affects over 60 % of the under 5 year olds in this community. AF have initiated a school lunch programme for children attending two primary schools in this area. We have recently completed a study examining the impact of providing the school lunch, with and without additional multiple micronutrients, on cognitive function in children attending these two schools. A third local government school, where no lunch was provided, served as the control. Cognitive function was assessed using Raven's Coloured Progressive Matrices (RCPM) test, at three time points; Time 1 (baseline, before the initiation of the school lunch programme), Time 2 and Time 3 (6 and 12 months respectively after the introduction of the school lunch). Between Times 2 and 3, half of the children receiving school lunch also received an additional multiple micronutrients in the form of Sprinkles (DSM Nutritional Products, Singapore) added to the school lunch immediately prior to serving. ANCOVA with follow-up post-hoc comparisons revealed that, for the children in the lowest quartile of cognitive performance at baseline, all three groups significantly increased their scores on RCPM over time, but that change was greater for pupils in the two intervention groups compared to the control group from Time 1 to Time 2 and Time 2 to Time 3.

Conclusion: Observational studies have suggested a relationship between Zn deficiency and poor cognition, but the evidence from randomised controlled trials during infancy, pregnancy and lactation has shown little effect. In human populations, it is not always possible to examine the impact of single micronutrients in isolation, as in reality, multiple micronutrient deficiencies together. Data from our recent study in malnourished children indicate that fortifying food with multiple micronutrients can improve the cognitive function of children in the lowest quartile of performance.

Acknowledgement: We would like to thank the Abaseen Foundation UK and PK for financial and logistical support for this work, and the children and teachers of the brick kiln community for their participation in this study. Also DSM for donating the Sprinkles.

T5
Title: Impact of Zinc on Behavior

Authors: Andreas M. Grabrucker

Affiliation: Cellular Neurobiology and Neuro-Nanotechnology lab, Dept. of Biological Sciences & Bernal Institute, University of Limerick, Ireland, andreas.grabrucker@ul.ie

Overview: Prenatal and early life zinc deficiency has been associated with the occurrence of autistic behaviors in humans. To understand whether zinc deficiency is causative and to investigate the involved mechanisms, we have recently generated a mouse model for prenatal zinc deficiency. These prenatal zinc deficient mice indeed show autism-like behavioral alterations similar to other mouse models for Autism Spectrum Disorders (ASD) that have been generated by manipulation of ASD candidate genes. In addition, in prenatal zinc deficient mice, we observed synaptic phenotypes that can be linked to alterations in pathways reported dysregulated in mouse models generated by genetic mutations. However, despite the common behavior that leads to the diagnosis ASD in humans (and establishes a mouse as model for ASD), it is widely unknown how the similar synaptic alterations observed after gene mutation or zinc deficiency as environmental factors in ASD act to generate the behavioral abnormalities. To bridge this knowledge gap, we have investigated connectivity and lateralization of specific brain regions in prenatal zinc deficient mice on molecular and behavioral level.

In this talk, I will summarize the behavioral phenotype of prenatal zinc deficient mice and present data from our latest analyses of our ASD mouse model based on prenatal zinc deficiency. Taken together, the results obtained in our recent study propose a novel role for zinc-signaling in the establishment of brain lateralization during development and suggest that prenatal zinc deficient animals display a disturbed lateralization of brain functions, which may lie at the core of ASD-like behavior. Altered brain lateralization has also frequently been reported in human ASD patients.

Conclusion: Metal ion imbalance during pregnancy is an important player in autism. Prenatal zinc deficient mice show ASD like behavior and synaptic alterations. Zinc signaling during brain development has a critical role in the establishment of brain lateralization in mice. Autism-like behavior of prenatal zinc deficient mice may, in part, be caused by abnormal brain lateralization.

T6

Title: Zinc and Eye Diseases

Authors: Imre Lengyel* and Eszter Emri

Affiliation: Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Science, Queen's University Belfast, 97 Lisburn Road, Belfast, BT9 7BL; Email: i.lengyel@qub.ac.uk

Overview: Zinc is a multifaceted trace element which plays a role in many metabolic pathways, either as a signaling molecule or as a component of proteins. It is involved in DNA replication, transcription, protein synthesis and signaling pathways, influencing cell division and differentiation. Zinc is found in unusually high concentrations in ocular tissues highly concentrating within the retinal pigment epithelium and choroid. As decreased zinc levels are associated with eye diseases, zinc supplementation was proposed to slow the progression of diseases, despites that there is no clear understanding of how this might be beneficial at the cellular level. Over a decade ago, Grahn et al. (Grahn, Paterson et al. 2001) reaffirmed the sentiments of Karcioglu (Karcioglu 1982) in 1982 that knowledge on zinc metabolism in the eye is fragmentary and confusing. Despite the widening research on zinc in general and extensive molecular works on zinc transporters only a few seminal works on eye (Chappell and Redenti 2001, Ugarte and Osborne 2001, Redenti and Chappell 2005) have been published since 2001. Therefore the statement of Karcioglu is still in force. Recently zinc research on eye has gained momentum following the publication of a number of major population based studies focusing on the role of nutrition in prevention of Age-related Macular Degeneration (AMD), the leading cause of blindness in western societies. Correlational studies among dietary zinc and clinical eye manifestations were not a new concept however the findings of AREDS group (Anderson, Ozaki et al. 2001), the Blue Mountain Eye Study (Finamore, Massimi et al. 2008), the Beaver Dam Eye study (VandenLangenberg, Mares-Perlman et al. 1998) and the Rotterdam Eye Study (Hofman, Breteler et al. 2007) have captured the interest of the public and clinicians, highlighting a vital need for better understanding of the molecular mechanisms involved. Other factors behind the intensified interest in zinc biology of the eye is our greater knowledge of the role of zinc in the brain and in particular its association with Alzheimer's disease (Sensi, Paoletti et al. 2009) (Singh, Xiang et al. 2009) as well as a better understanding of zinc transporting (Lichten and Cousins 2009) and buffering in cells (Colvin, Holmes et al. 2010). Our extensive animal and cell biology results hopefully partly fill this gap with described effects of zinc supplementation that supports the idea of the importance of appropriate zinc balance in the eye.

Conclusion: Epidemiological studies and our experimental laboratory results show that appropriate zinc balance is key for normal vision. The relevance of zinc in the eye still needs more clarification, but through the EU funder Eye-Risk project we now combine genetic, clinical and laboratory data to gain a more precise understanding for zinc biology in the eye, one that may lead to the development of personalized intervention strategies involving changes in zinc levels in the eye.

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T7
Title: Dietary Copper and Human Health

Authors: Muriel Bost

Affiliation: Laboratory of Trace Element and Toxic Metal Analysis; Laboratories of Neurogenetic and Hereditary Diseases of Metabolism, CBPE; National Reference Centre for Wilson Disease – HFME; President of Trace Element – Institute for UNESCO – Lyon, France; Email: muriel.bost@chu-lyon.fr

Overview: Copper (Cu) is an essential trace element (TE) in both humans and animals. It is the third most abundant TE with only 75-100 mg of total amount in the human body, present in almost every tissue and stored chiefly in the liver along with the brain, heart, kidney and muscles. Cu is absorbed in the gut and transported to the liver. In human blood, Cu is principally distributed between the erythrocytes and in the plasma. As a transition metal, it is a cofactor of many redox enzymes, ceruloplasmin (Cp) being the most abundant Cu-dependent ferroxidase enzyme with a Cu-dependent oxidation activity. Beyond its role in iron metabolism, the need for Cu also derives from its involvement in a myriad of biological processes, including antioxidant defense, neuropeptide synthesis and immune function. As a consequence, the wide range of clinical features resulting from perturbations in the activities of cuproenzymes means that although severe Cu deficiency is relatively straightforward to diagnose, identifying marginal deficiency is somewhat problematic. Though an essential micronutrient for man, Cu is toxic at high levels. An overload of this metal easily leads to Fenton-type redox reactions, resulting in oxidative cell damage and cell death. However, Cu toxicity as a result of dietary excess is generally not considered a widespread health concern, probably as a result of the homeostatic mechanisms controlling Cu absorption and excretion. Menkes syndrome and Wilson disease are genetic conditions associated with severe Cu deficiency and severe Cu toxicity, respectively. Effects of milder degrees of Cu deficiency and excess Cu exposure are not well described, mainly due to lack of sensitive and specific indicators; serum Cu concentration and Cp are the most used indicators, but they only detect rather intense changes of Cu status. Uncertainties remain regarding Cu reference values for humans, as illustrated by discrepancies between recommendations issued by different national authorities. Human studies published since 1990 on relationships between intake, Cu balance, biomarkers of Cu status and health pointed out several gaps and unresolved issues which make it difficult to assess Cu requirements. Results from balance studies suggest that daily intakes below 0.8 mg/day lead to net Cu losses, while net gains are consistently observed above 2.4mg/day. However, because of an incomplete collection of losses in all studies, a precise estimation of Cu requirements cannot be derived from available data. Data regarding the relationship between Cu intake and potential biomarkers are either too preliminary or inconclusive because of low specificity or low sensitivity to change in dietary Cu over a wide range of intakes. Results from observation and intervention studies do not support a link between Cu and a risk of cardiovascular disease, cognitive decline, arthritis or cancer for intakes ranging from 0.6 to 3mg/day, and limited evidence exists for impaired immune function in healthy subjects with a very low (0.38mg/day) Cu intake. However, data from observation studies should be regarded with caution because of uncertainties regarding Cu concentration in various foods and water. A recent study reported the Cu-2 hypothesis for causation of the current Alzheimer's disease epidemic together with dietary changes that enhance the epidemic.

Conclusion: At present, the lack of a suitable biomarker for Cu intake greatly limits our capacity to assess the relationship between dietary Cu exposure and health, and highlights the urgent need for high quality randomized controlled trial in healthy subjects evaluating the responsiveness of putative new biomarkers of Cu status over a wide Cu intake range.

T8

Title: Impact of Zinc on Cardiomyocytes

Authors: Belma Turan

Affiliation: Ankara University Faculty of Medicine, Department of Biophysics, Ankara, Turkey

Introduction: Zinc (mostly as free Zn^{2+} ions) is an essential trace element for mammalian life. However, it can be very toxic to most living cells beyond normal physiological levels. Cardiovascular toxicity with severe symptoms has been reported in people exposed to high levels of Zn^{2+} include premature atrial beats, hypertension secondary to intravascular volume, hypovolemic shock and hypertension. At the cellular level, intracellular free Zn^{2+} level is less than 1-nM under physiological conditions in cardiomyocytes while it can be increased severely under pathological conditions such as oxidative stress, hyperglycemia or ischemia-reperfusion. Recent studies have emphasized that similar to increased intracellular free Ca^{2+} as a second messenger, the free Zn^{2+} plays an important role in cellular signaling basically by modulating signal recognition, second messenger metabolisms, increases in protein phosphorylation and the activity of many enzymes.

Aim: We focused to describe the molecular mechanisms of intracellular free Zn^{2+} in the development of cardiovascular disorders under hyperglycemia.

Methods: For this general aim, we used different approaches by using different techniques at cellular level. We used Zn^{2+} specific fluorescence dye FluoZin-3 for quantification of intracellular free Zn^{2+} level in cardiomyocytes in confocal microscopy while FRET technique is used to monitor subcellular distribution of free Zn^{2+}. To compare its functional action in cardiomyocytes with free Ca^{2+}, we used electrophysiological approaches. Biochemical analysis is also used to validate its functional action in cardiomyocytes at cellular level.

Results: We first demonstrated that free Zn^{2+} concentrations in the cytosol, S(E)R and mitochondria of cardiomyocytes are about 0.1−1 nM, $\geq$ 5 nM and about 0.03-0.07 nM, respectively, while these values can be changed dramatically under both acute and chronic hyperglycemia. In diabetic or high glucose exposed cardiomyocytes, increased cytosolic free Zn^{2+} together with depressed S(E)R free Zn^{2+} are closely associated with depressed electrical and mechanical activity of diabetic heart as well as induction of ER-stress. Additionally, we have demonstrated that mitochondrial free Zn^{2+} is significantly increased under not only hyperglycemia but also in faling heart. Our results showed that free Zn^{2+} in cardiomyocytes has very important roles under physiological condition while any change in its subcellular distribution under pathological condition can be very detrimental for cardiomyocte function.

Conclusion: Overall, we have demonstrated that intracellular free Zn^{2+} has important role during cardiac excitation–contraction cycle via Ca^{2+} and redox dependencies and any change in its muffling between compartments in cardiomyocytes under pathological condition including hyperglycemia can affect the ER-mitochondria coupling. Since functional contribution of endoplasmic reticulum (ER)-mitochondria coupling to normal cellular processes is very important, any change in this axis and/or well-controlled cellular free Zn^{2+} may provide new insights for prevention/therapy of heart-failure under hyperglycemia.

T9

Title: Zinc Homeostasis and Healthy Ageing

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Overview: Marginal Zinc (Zn) deficiency is a common condition in aging and a dysregulation of zinc homeostasis contributes to increased inflammation promoting age-related diseases. Intracellular zinc homeostasis is tightly controlled by zinc transporters (ZnT and Zip families) and metallothioneins (MT) which modulate the uptake, storage, and distribution of zinc. In a large cohort of elderly subjects we found a decline of circulating Zn, associated with increased Cu/Zn ratio that represents a biomarker of systemic inflammation and a predictor of mortality. Genetic variation in genes involved in Zn homeostasis are associated with age-related diseases such as diabetes and cardiovascular diseases (CVD) and can influence an individual's response to Zn intervention. The capacity to induce MT in lymphocytes after *in vitro* Zn treatment is not associated with age, but is reduced in lymphocytes from centenarian offspring as compared to the general population that is suggestive of a tighter Zn homeostasis control in blood cells of long living individuals. Moreover, we observed that Zn responsive genes are modulated during vascular senescence and senescent endothelial cells are more resistant to Zn deficiency than proliferating cells. This might promote the accumulation of senescent cells favouring the development of CVD diseases.

Conclusion: A dysregulation of zinc homeostasis during aging may contribute to chronic inflammation and vascular senescence accelerating cardiovascular diseases. Personalized nutritional or therapeutic interventions in elderly with genetic variants of Zn responsive genes more susceptible to zinc dyshomeostasis and inflammation might help to delay the progression of age-related diseases.

T10

Title: Investigation of Zinc Uptake and Bioavailability in an *in vitro* Model of the Gastrointestinal Tract

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Overview: Zinc deficiency is a widespread nutritional problem, negatively affecting the health and well-being of approximately 1 billion people worldwide. This can result from inadequate zinc content of staple foods consumed by large population groups, but also from the presence of food constituents that limit zinc bioavailability. Consequently, the impact of various substances on the bioavailability of zinc is subject of intense investigations. Performing these studies in human subjects is the gold standard, but the large number of potential influencing factors makes it virtually impossible to perform comprehensive analyses exclusively in humans. Similarly, animal studies are well suited to reflect the in vivo situation, but cannot be performed for every substance of interest. Hence, in vitro studies based on intestinal cell culture models could be applied as tools for screening zinc supplements with different bioavailability, or used for identifying major influencing factors of zinc uptake. They could also be employed to evaluate the bioavailability of zinc from novel food sources, such as insects. Unfortunately, the comparability of existing models to the in vivo situation is not always satisfactory. The present study investigates critical parameters, including the role of digestion for changing the chemical speciation and thereby bioavailability of zinc, the interaction of zinc with the intestinal mucus layer, and techniques for reliable monitoring of zinc uptake and transport in enterocytes.

Conclusion: Enterocyte-based intestinal models are a promising tool to estimate zinc bioavailability in vitro. They were applied to investigate the importance of several influencing factors; showing the impact of digestion on the mobilization of zinc from food, an interference of other metal ions with zinc uptake, and zinc-binding by the intestinal mucus.

T11
Title: The Role of Nickel in Bacterial Pathogenicity

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Overview: Nickel is a toxic metal for cellular life, being responsible of nickel-derived allergies, nickel-induced carcinogenesis as well as infectious diseases caused by human pathogens, which rely on nickel-based enzymes to colonize the hosts [1]. In particular, urease is a key nickel-enzyme for many bacteria [2]. The essentiality of nickel for this enzyme requires the bacteria to ensure the correct intracellular nickel levels by developing systems for nickel sensing and homeostasis. These involve the concomitant action of proteins that effect nickel-dependent gene regulation (NikR), and nickel delivery into urease (UreD, UreF, UreG and UreE).
Nickel binding to NikR influences its interaction with DNA through affecting the protein dynamics [3]. UreE is a metallo-chaperone involved in nickel binding and delivery into the urease active site [4]. UreG is a GTPase that provides energy to the process of nickel site assembly [5]. UreF and UreD form a complex that regulates the GTPase activity of UreG [6]. The present talk focuses on the interaction networks between these proteins, integrating several different techniques, such as calorimetry, light scattering, circular dichroism, SPR, NMR spectroscopy and crystallography.

Conclusion: The results show how metal binding can modulate and affect the interactions and functions of proteins involved in nickel metabolism for urease activation.

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T12

Title: Perspectives of Vanadium in Health Issues

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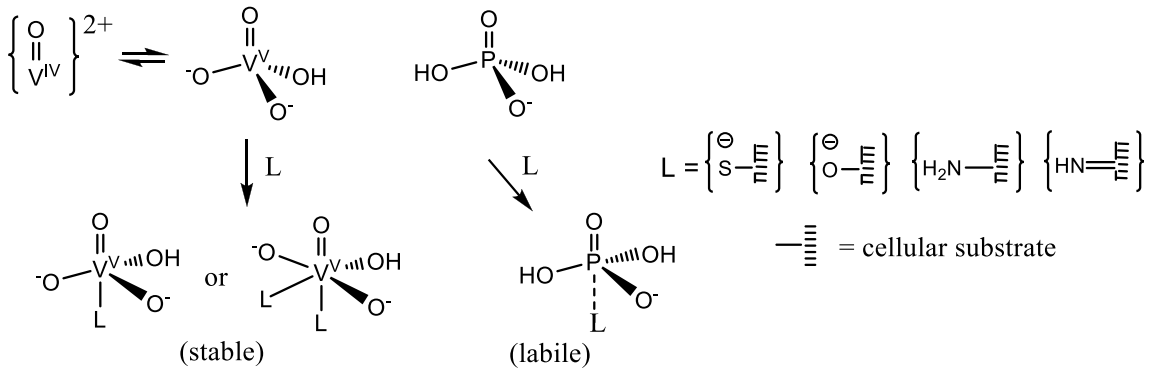
Overview: The structural similarity between vanadate and phosphate accounts for both the potential toxicity *and* the medicinal potential of simple as well as of complex vanadium compounds – including vanadium's possible role as an essential trace element. However, contrasting phosphorus in phosphate, vanadium easily changes between the oxidation states +IV (oxidovanadium VO^{2+}) and +V (vanadate $H_2VO_4^-$), and readily attains stable coordination environments with the coordination numbers 5 and 6.



These similarities as well as differences between vanadate and phosphate enable vanadium to act both as an effective competitor of phosphate (e.g. in phosphatases and kinases) and an efficient binder for functional groups (such as Cys-S^- and His-N) in physiologically relevant (macro)molecules, including cellular membranes. Additionally, vanadium can exert its activity through the production and annihilation of reactive oxygen species (ROS). – A prominent example for the potential use of vanadium compounds in medicinal issues is type 2 diabetes mellitus, where the simple complex $VO(maltol)_2$ has achieved the status of clinical tests IIa. Other investigations towards the medicinal use of vanadium compounds (simple inorganics, coordination compounds, π-sandwiched complexes) have so far been directed towards medicinal applications in vivo (with laboratory animals) and/or in vitro. These investigations include the application of vanadium compounds in (i) cancer therapy, (ii) the treatment of tropical diseases (caused by protozoans) such as leishmaniasis, Chagas disease and amoebiasis, (iii) the treatment of bacterial (tuberculosis) and viral (AIDS) infections and, finally, (iv) coronary heart diseases and (v) flash burns.

Conclusion: Vanadium, in the form of vanadate, is a phosphate analogue/antagonist. The oxidovanadium(IV,V) moiety efficiently binds to protein sites and modifies physiologically relevant functions.

T13
Title: Copper associated Hepatitis in Dogs

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Overview: Aberrant copper levels can lead to diseases; Menkes disease (MD), caused by mutations in the *ATP7A* gene, is a copper deficiency, whereas Wilson disease (WD), caused by mutations in the *ATP7B* gene, is characterized by hepatic copper overload. In certain dog breeds, amongst others Bedlington terrier, Labrador retrievers, Dobermanns, and West Highland White terriers, hepatitis associated with copper accumulation has a relatively high incidence. Around 30% of the idiopathic forms of chronic hepatitis in dogs is likely caused by copper accumulation. A downside of inbreeding, as observed for numerous dogs breeds, is the fact that inherited disorders are highly prevalent in dogs breeds. The population structure of dog breed works as a magnifying glass for geneticists.

In 2002 the genetic cause of copper accumulation in Bedlington terriers was dissected, *viz.* an exon-2 deletion in the COMMD1 gene. Thus far no COMMD1 mutations have been described in humans. This recessively inherited disease, with copper levels above 10,000 ppm, allowed us to create an in-house colony of COMMD1-deficient dogs for the purpose of hepatic stem cell transplantation studies. In Labrador retrievers copper toxicosis is a complex genetic disorder, mutations in both *ATP7A* and *ATP7B* have been found to modulate the rate of copper accumulation. Hepatic copper levels in the Labrador retrievers copper toxicosis are in line with those observed in human WD. The genetic basis for Dobermann hepatitis is not yet solved.

The injured liver is capable to regenerate due to the proliferation of fully matured hepatocytes and cholangiocytes (bile duct cells). Once this regeneration capacity is exhausted or inadequate, liver progenitor cells can become activated. These cells are bipotent and can differentiate into either hepatocytes or cholangiocytes. In order to investigate the functional consequences of various mutant proteins, *in vitro* culture systems of cells with these mutations are crucial. However, hepatocytes are difficult to keep phenotypically stable in culture, this hampers *in vitro* studies on the mechanism of action of the various mutants associated with hepatic copper accumulation. Recently rodent, human, feline, and canine 3D mini-organs, so-called organoids, derived from liver progenitor cells were described. Differentiation of progenitor cells into hepatocyte-like cells is a potent novel in vitro system. Although no fully matured organoid-derived hepatocyte cultures are described, transplantation of liver organoids has been shown to recover liver function in rodent models. As an example of a transplantation with gene-corrected liver organoids into COMMD1-deficient dogs will be discussed.

Conclusion: Hepatic copper accumulation in Bedlington terriers, and Labrador retrievers is caused by different mutations. The specific population structure of dogs breeds allows geneticists to dissect complex genetic problems and the dogs' size and longevity makes them ideal models to study hepatic stem cell transplantation.

T14

Title: Zinc and Autoimmunity

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Overview: Autoimmune diseases, which in humans show a prevalence of 5 – 8%, occur when specific components of the adaptive immune system (i.e. autoantibodies or autoreactive T lymphocytes) are directed against self-antigens. Most frequent autoimmune disorders are autoimmune thyroid diseases, rheumatoid arthritis (RA), celiac disease, type 1 diabetes mellitus (T1D), systemic lupus erythematosus (SLE), autoimmune liver diseases and multiple sclerosis (MS). In addition to a genetic predisposition (MHC genotype) and environmental influences, other factors such as age, hormonal status and especially the condition of the immune system are involved in the pathogenesis of autoimmune diseases.

The trace element zinc is shown to play a regulatory role in the maintenance of immune functions. Thus, changes of zinc homeostasis affect components of both the innate and the adaptive immune system. Therapeutic zinc supplementation can normalize impaired immune function due to zinc deficiency. Interestingly, reduced zinc concentrations have been observed in serum and plasma samples of patients with different autoimmune diseases, like RA, T1D, SLE and MS.

Based on several experimental data, a therapeutic zinc supplementation is under consideration as one possible option to treat T cell-mediated autoimmunity. Multiple Sclerosis, the most frequent demyelinating disease of the central nervous system, is shown to be an inflammatory autoimmune disorder with a clear Th1/Th17-T cell-mediated immune pathogenesis.

Therefore, we decided to study the influence of zinc aspartate (Unizink®), an approved drug to treat zinc deficiency in humans, in the animal model of Multiple Sclerosis, the chronic relapsing Experimental Autoimmune Encephalomyelitis (EAE) of SJL/J mice. We could show that intraperitoneal as well as oral therapeutic application of zinc aspartate significantly reduced clinical and histopathological signs during the relapsing remitting phase of the EAE. Moreover, zinc aspartate increased the therapeutic effect of intravenous immunoglobulin administration (IVIG preparation) in the course of the EAE disease.

In order to study the direct effect of zinc aspartate on T cell function, we investigated the influence of this biometal on the proliferative capacity and activation of human T cells in vitro. Zinc aspartate was capable of suppressing the proliferation and cytokine production (Th1 and Th17 cytokines) of stimulated T cells without influencing resting T cells. The cell viability was not impaired by zinc in the concentrations used.

Conclusion: On the basis of this knowledge and our observations, further preclinical and clinical studies should investigate the effect of controlled immunosuppressive zinc supplementation in T cell-mediated autoimmune diseases like MS.

T15
Title: Impact of Cobalt (II) on the Immune System

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Overview: Cobalt (Co) is vital for cells in trace amounts but in high concentrations it may cause cytotoxicity and inflammation. Cobalt chloride has also been shown to imitate hypoxic conditions in cells by stabilizing the transcription factor hypoxia-inducible factor 1alpha (HIF-1alpha) which enhances inflammatory responses. In recent decades, a significant number of patients have exposed to increased levels of cobalt due to metal-on-metal joint replacements. A part of them have developed an inflammatory response called as “adverse reaction to metal debris” (ARMD) around the prosthesis resulting in severe symptoms and need for revision surgery. The pathogenesis of those metal-induced reactions are not known in detail. In the present study, we addressed the problem by genome-wide expression analysis and further, investigated the immunomodulatory effects of cobalt chloride on macrophage phenotypes *in vitro*.

Pseudosynovial tissue was obtained from revision surgery of Articular Surface Replacement (ASR, DePuy, Warsaw, IN, USA) hip arthroplasties carried out at Coxa Hospital for Joint Replacement, Tampere, Finland. In NGS-based genome-wide expression analysis of the ARMD tissue, 1446 genes were found to be up-regulated and 1881 down-regulated in a statistically significant manner when compared to control synovial tissue obtained in primary hip replacement operations. Among the genes up- or down-regulated with a fold change more than 2.0 (deemed a threshold of biological significance), enriched were genes associated with immune response, macrophage and lymphocyte activation, cell adhesion, skeletal system development and with several leukocyte signaling pathways.

When the transciptome of the pseudosynovial tissue around failed metal-on-metal prostheses was compared to corresponding inflammatory tissue around failed metal-on-plastic prostheses, dozens of differently expressed genes were identified. Many of those were associated with redox reactions, metal ion binding and transport, macrophage activation and apoptosis.

Because cobalt containing prostheses caused a severe inflammatory reaction with features of classical and alternative macrophage activation in a group of patients, we investigated the effects of cobalt chloride on macrophage phenotypes *in vitro*. In resting macrophages, cobalt chloride polarized the cells towards alternative (M2 type) activation. In addition, cobalt chloride was found to remodel the classical (M1 type) macrophage activation by enhancing iNOS/nitric oxide synthesis and decreasing NOX2 and IL-6 synthesis.

Conclusions: Exposure to excessive amounts of cobalt / cobalt chrome due to surgical devices such as metal-on-metal hip replacement implants induces in a part of patients a unique response characterized with enhanced expression of genes associated with inflammation, redox reactions, metal ion binding and transport, macrophage activation and apoptosis. Moreover, cobalt chloride has direct modulatory effects on macrophage phenotypes likely associated with the pathogenesis of the cobalt-induced inflammatory adverse reactions.

T16

Title: Toxicity of Manganese

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Overview: Environmental and/or occupational overexposure to the essential trace element manganese (Mn) may cause an irreversible neurodegenerative disease called manganism. Upon loss of dopaminergic cells, manganism-associated neuropathology results in deficiencies in motor and cognitive development analogous to those inherent to Parkinson's Disease (PD). The underlying cellular and molecular mechanisms by which Mn is inducing neurodegeneration has yet to be conclusively clarified. Cellular and molecular modes of actions of Mn-induced neurotoxicity include oxidative stress, mitochondrial dysfunction, disturbed DNA repair and impaired homeostasis of other trace elements, to name a few. The nematode *Caenorhabditis elegans* (*C. elegans*) constitutes a distinguished model organism for the essential further investigation of *in vivo* mechanisms. Given the conserved oxidative stress and oxidative stress response pathways between the nematode and mammalians, several assays analyzing the contribution of Mn to oxidative stress in this model organism have been established. Multiple endpoints are necessary due to the widely recognized methodological difficulty in quantifying oxidative stress/damage markers *in vivo*. Mn overload resulted in increased oxidative stress markers, including a shift in the GSH/GSSG-ratio towards GSSG, an increase in reactive oxygen/nitrogen species (RONS) and isoprostanes as well as mitochondrial dysfunction. Due to their chemical stability, isoprostanes (non-enzymatic lipid peroxidation products) are suggested to be the most reliable markers for oxidative stress assessment *in vivo*. Since excessive production of RONS can lead to interactions with macromolecules, such as DNA, it has been proposed that DNA damage and defective DNA repair contributes to neurological dysfunction. Since base excision DNA repair (BER) as well as DNA double strand break repair (DSB) is known to occur in *C. elegans*, the worm was used to investigate the connection between DNA repair and Mn-induced neurotoxicity. Following 4 h Mn treatment in L4 larvae, Mn was shown to increase gene expression of the DNA glycosylases *nth-1* (ortholog to human NTH1) and *ung-1* (ortholog to human UNG), as well as the AP endonuclease *exo-3* (ortholog to human APE1), which all act at the beginning of the BER. We further showed that loss of *nth-1* resulted in hypersensitivity towards Mn-induced lethality compared to wildtype worms. Mechanistical studies quantifying Mn-induced DNA lesions and DNA damage related poly(ADP-ribosyl)ation are presently performed in order to identify the respective underlying mechanisms.

Conclusion: These findings establish Mn-induced oxidative stress and impact on various DNA repair pathways all of which likely contribute to the underlying mechanisms of Mn-induced neurodegeneration. Identifying novel targets for understanding the role of DNA repair in the etiology of manganism is warranted.

Heinz-Zumkley-Prize 2017 Lectures

Title: Trace Element Species and Amyotrophic Lateral Sclerosis with Disease associated Genetic Mutations

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Overview: Amyotrophic lateral sclerosis (ALS) is a motor neuron disease with mostly unknown etiology. Certain genetic mutations are associated with the disease; however, the role of environmental factors, such as exposure to metals and organic pollutants is also widely discussed in the literature. ALS, as other neurodegenerative disorders, is related to the brain oxidative stress, so the disturbance of redox homeostasis may be anticipated for such elements as selenium (Se), copper (Cu), iron (Fe), and manganese (Mn). The aim of the study was to evaluate a possible alteration of trace element (Se, Cu, Mn, and Fe) homeostasis in the ALS patients with disease associated gene mutations.

We analyzed cerebrospinal fluid (CSF) samples from 9 patients with ALS-associated mutations (C9ORF72, SOD1, FUS, TARDBP, ATXN2, and TUBA4A) and 42 age- and gender-matched controls. Advanced speciation techniques were used to quantify redox forms of Cu (I/II), Mn (II/III), and Fe (II/III) and Se species (selenoprotein P, glutathione peroxidase, thioredoxin reductase, selenite, selenate, and human serum bound-Se). For the separation of Se species strong anion exchange chromatography (SAX) was used, whereas Cu, Mn, and Fe redox forms were separated by strong cation exchange (SCX). For the species detection, inductively coupled plasma sector field mass spectrometry (ICP-sf-MS), operated at high resolution for Se or medium resolution for Cu, Fe, and Mn was employed. Standard compounds and spikings were used for peak assignment. External calibration vs. matching to the total content of the elements, measured by inductively coupled plasma dynamic reaction cell mass spectrometry, was used for species quantification.

The analytical schemes of species quantification, using SAX-ICP-sf-MS [1] and SCX-ICP-sf-MS [2], have been optimized. The difference in Cu(II) and some Se species were found to be altered in the CSF of the ALS patients with disease-associated mutations. Also, since multi-element speciation had been performed for the same set of CSF samples, some inter-element correlations were observed (between Fe and Se species, Mn and Fe, Mn and Cu).

Conclusion: Despite the limited sample size, we could presume a distortion in trace element metabolism, reflected the altered speciation of Cu and Se in the CSF. However, more insight is required to understand if these findings are an innocent bystander to the pathological changes in the ALS brain or has its own relevant role in the etiopathogenesis of the disease.

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Title: Zinc Supplementation induces Regulatory T cells *in vitro* and *in vivo*

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Overview: Adequate tolerance induction is critical for maintaining immune homeostasis. In this regard, regulatory T cells (Treg) as well as essential trace elements like zinc play a major role. Proper zinc homeostasis is critically important, since its deficiency is associated with severe impairment of the immune response characterized by increased susceptibility to infections, development of allergies, autoimmune diseases, and transplant rejections. Since especially T cell function and differentiation is highly zinc dependent, major signaling pathways and essential transcription factors for proper Treg function might be influenced by zinc homeostasis. Hence, important signaling pathways as TGF-β1 dependent Smad signaling as well as transcription factor expression have been analyzed in this study. Zinc supplementation in physiological doses provoked a significant amelioration of the allogeneic T cell driven immune reaction in an *in vitro* model for graft versus host disease. This is due to specific zinc-dependent signaling pathway modulation and transcription factor manipulation crucial for Treg function and differentiation. In this regard, transcription factors important for Treg function and differentiation as Foxp3 and KLF-10 are induced by zinc supplementation, whereas a negative-regulator, IRF-1, is downregulated due to zinc supplementation. Moreover, the TGF-β1-dependent Smad signaling pathway is boosted by zinc supplementation leading to higher Treg induction and stabilization.
In line with that, zinc supplementation in a rat kidney transplantation model *in vivo* induces Treg in peripheral blood and spleen. Hence, zinc supplementation favors the tolerogenic immunoreaction *in vivo* leading to ameliorated damage score of allografts contributing to better allograft acceptance and prolonged allograft survival.

Conclusion: Taken together, zinc supplementation is capable to ameliorate the allogeneic immunoreaction *in vitro* and *in vivo* by induction of Treg. This is due to modulation of essential molecular targets: Foxp3, KLF-10, and IRF-1. Thus, zinc can be seen as an auspicious tool for inducing tolerance in dysregulated immunoreactions.

Poster

A1
Title: Zn-Status in Ruminants

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Introduction: The effect of marginal Zn intake on growth, reproduction performance, quality of skin and hair, and life expectancy are well known. However, the diagnosis of the trace element status in living wild animals kept in zoos is difficult since, as a rule, only blood, hair, claws and excretions are available for this purpose.
The capacity of indicating the trace element status of several tissues is element-specific. Therefore, not all of them are suited as indicator tissues for the trace element status. Furthermore, it must be taken into consideration that the indicating capacity of organ tissues in the case of deficiency can be different from that in the case of exposure. The investigation of these laws could not be carried out in endangered species of ruminants, but in farm animals.
Aim: For that reason, organ tissues of different species of wild ruminants kept in captivity were obtained at necropsy, analyzed and compared with data of domestic and wild living ruminants as well as with data from wild living omnivores.

Methods: The wild ruminants which were kept in captivity came from the Zoological Society of San Diego and from the Zoo Leipzig. For comparison, organ tissues from wild living and domestic ruminants were obtained from different locations in the states of Germany and Northern California. Wild living opossums and foxes were trapped at the San Diego Zoo (California) and euthanized due to diseases screening. After dry ashing of samples the Zn concentration were analyzed by atomic absorption spectroscopy or optical emission spectroscopy with inductively coupled plasma.

Results: The Zn status of different ruminants depends on species, age, feeding type and - due to the homeostatical regulation - to a lesser extent on Zn intake.
The mean Zn concentration of rib of adult wild ruminants kept in captivity varied between 64 and 122 mg / kg dry matter (dm). All ruminants stored more Zn in their ribs than the limit value for a sufficient Zn supply valid for domestic cows (40 mg / dm) and domestic sheep (50 mg/dm). Furhtermore, adult species of cervinae and hippotraginae accumulated less Zn / kg rib dry matter than adult species of bovinae, antilopinae, caprinae, and tragelaphinae. When the species were classified as morphological ruminant feeding types the Zn concentration of ribs were in same range between the feeding types and between adults and neonatal animals. However, neonatal concentrate selectors significantly stored on average between 2.5 and 3.4 times more Zn in the liver than adult animals. Neonatal grass and roughage eaters accumulated only 1.2 to 1.4 times more Zn in liver dry matter than the adult animals.

Conclusion: The Zn limit values of rib for a sufficient Zn supply in domestic cows (40 mg / kg dm) and in domestic sheep (50 mg / kg dm) may be also valid for the species of wild ruminants.

A2

Title: Molybdenum protein binding in human serum and cerebrospinal fluid

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Introduction: Molybdenum (Mo) is an essential element for mammalian organisms. Three important enzyme families contain molybdenum-based cofactors. At the same time, metabolism of this element has attracted relatively low attention up-to-now. Even the transport of this element in the body was studied scarcely.

Aim: Investigate molybdenum speciation and binding to transport proteins in human serum and cerebrospinal fluid (CSF).

Methods: For Mo speciation size exclusion (SEC), strong cation (SCX) and strong anion exchange (SAX) chromatography were used. Element-specific detection was carried out using inductively coupled plasma quadrupole mass spectrometry (ICP-q-MS) and inductively coupled plasma sector field mass spectrometry (ICP-sf-MS).

Results: SEC studies showed that in both serum and CSF molybdenum was present in a single low molecular weight fraction (<500 Da). Interestingly, a very similar molybdenum speciation pattern was observed in the serum and the CSF with comparable total Mo concentrations. To further characterize the species SAX-ICP-sf-MS was implemented. For both serum and CSF samples, two ^{95}Mo peaks were obtained, one of which (9.5 min) was matched to be related to molybdate (MoO_4^{2-}). Second peak appeared within the void volume in the SAX chromatogramms, so we tried to use the SCX. However, the peak remains non-characterize, since for SCX the compound seemed to elute in the void as well. Thus, further studies, using capillary electrophoresis or reverse phase liquid chromatography, are required. Also, in this project, in vitro model studies with serum and CSF components (human serum albumin, reduced glutathione, and α-globulin) and other metal ions (copper) were conducted. Molydbenum in the form of molybdate, at the physiologically relevant concentrations was found not to bind human serum albumin or other serum major proteins. However, a possible interaction of molybdenum with glutathione was observed. The adding of Mo to the solution of glutathione altered copper distribution towards higher molecular mass compounds, probably, due to the formation of complexes with copper and/or sulfur.

Conclusion: Similar molybdenum content and species distribution in serum and CSF may indicate the presence of a kind of molybdenum transport through the neural barrier and its important role for the brain. Interaction of molybdenum with glutathione and copper may be related to its either toxic or essential functions.

A3

Title: "Effects of Methylcyclopentadienyl Manganese Trycarbonil on dopaminergic neurons in zebrafish"

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Introduction: Heavy metals are environmental factors whose role in the pathogenesis of several neurological disorders is taken increasingly into account. Particularly, alteration of brain manganese (Mn) homeostasis has been linked to neurodegenerative diseases, such as Alzheimer and Parkinson's Disease (PD) [1, 2]. Recently, strong concern was expressed over Methylcyclopentadienyl Manganese Trycarbonil (MMT), an organic Mn-containing gasoline additive, due to health risk from long-term exposure to Mn. Danio rerio, also known as zebrafish, is a useful animal model for studying effects of neurotoxicants on the nervous system, considering homologies of the teleost brain with the mammalian one [3]. Despite evidence of structural and functional damage induced by Mn-based chemicals on dopaminergic (DA) neurons [4, 5, 6, 7], no studies of the effects on neuronal differentiation following chronic exposure to sub-lethal MMT have been performed in vivo.

Aim: The main aim of the study was to investigate transcriptional, morphological and behavioral alterations underlying sub-lethal MMT exposure during neuronal differentiation in zebrafish. In addition, adult cognitive impairments following MMT exposure during embryonic development of the brain was analyzed.

Methods: Zebrafish embryos were exposed to MMT (10-300 µM) for 5-72 hours post fertilization (hpf), critical developmental stages of DA differentiation.

Results: We performed a transcriptional analysis of genes involved in differentiation of DA neurons following MMT treatment (10-300 µM) during development. Particularly, sub-lethal MMT (100 µM) altered specification (*lmx1b*), intermediate differentiation (*otpa*) and maturation (*th*) of DA neurons. MMT treatment (30-100 µM) was also able to alter the morphology of DA neurons increasing both the number and size of tyrosine hydroxylase-positive (Th^+) cells of specific ventral diencephalic cluster 2 DA neurons, also confirmed in vivo using Tg(*dat*:EGFP) transgenic line. Interestingly, MMT treatment (100 µM) evoked a hyperactive behavior in embryos at 72 hpf. In adult stage, embryos treated with MMT (100 µM) were submitted to the Y-maze test for evaluating their behavior. The analysis revealed changes in cognitive capabilities affecting exploration, orientation and spatial memory.**Conclusion:** Collectively, these findings suggest that chronic exposure to sub-lethal MMT during neuronal differentiation can alter the development (differentiation, number, and shape) of DA neurons and the short- and long-term behavioral traits controlled by the DA system.

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A4

Title: Microelements of female tissues of F_1 rats for the long-term effects of various doses of nano-germanium citrate

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Introduction: Ge compounds are actively investigated in biology, medicine and veterinary medicine, since they have a wide range of biological effects. In Ukraine, by the method of nanotechnology, an environmentally safe citrate Ge is obtained, which has a number of advantages compared not only with the mineral salts of this element, but also with its organic compounds. Experimental study of nano-germanium citrate (NGeCit), which was initiated at the Institute of Animal Biology of NAAS in 2012, indicates its high physiological activity in different doses and a metabolic link with other micro-elements. However, the age, sex and organ-tissue features of the various doses of NGeCit on the content of other trace elements have not been clarified, which was the purpose of these studies.

Aim: To establish the dose-dependent influence of nano-germanium citrate, in the conditions of its prolonged intake into the body of females of rats F_1, on the content of individual microelements in their tissues.

Methods: Physiological, biochemical and statistical methods have been applied with the usage of the atomic absorption spectroscopy of the detection of Zn, Cu, Co and Fe in the liver, kidneys, lungs and muscle of females of F_1 rats, which, in addition to the control group, during growth, puberty and pregnancy, were excised from Water of NGeCit in the following doses: experimental 1 (D1) group - 10; D2 - 20, D3 - 200 μg Ge/kg body weight. Female tissue samples of F_1 rats were obtained during the determination of feto- and embryotoxicity of the applied doses of NGeCit.

Results: The probable lower content of Zn, Cu, Co and Fe in rat liver tissues was found under the action of 10 and 20 μg Ge (groups II and III) and higher ($P < 0.01$) under the action of 200 μg (group IV). In renal tissue, reduction was noted only for Zn - in II and Cu - in the third group. The content of Zn, Cu and Co increased ($P < 0.05$; $P < 0.001$) in lung tissues of animals of groups II and III, in comparison to control, which may indicate the organ-tissue differences in the influence of NGeCit on the metabolism of these elements in the investigated organs. This is confirmed by the less obvious differences in the content of these elements in muscle tissues. In particular, the content of Zn and Fe in the femoral muscle of all experimental groups was not significantly different from the control, but was lower for Cu ($P < 0.01$; D2 group) and Co ($P < 0.001$; D3 group). However, in the muscles of the rats receiving 200 μg of Ge, a higher ($P < 0.05$) Co content was observed, indicating the opposite effect of high and low doses of Ge on the level of Co in the muscles and Zn in the liver tissues, and may be important for the evaluation of the microelemental status of the organism during the period of application of Citrate Ge.

Conclusion: Hence, the prolonged release of low and high doses of NGeCit to females of F_1 rats caused a decrease in the liver tissues of Zn, Cu, Co and Fe in the rats' tissues at 10 and 20 μg Ge, kidneys Zn (10) and Cu - 20 μg, muscle - Cu (10) and Co - 20 μg on the background of a possible increase in Zn, Cu and Co levels in the lungs (10 and 20 μg), Zn - liver (200 μg), Co - muscle (200 μg). The application of a high (200 μg) dose of NGeCit did not cause a significant change in the investigated elements in the tissues of the kidneys and lungs, as well as in Cu, Co, Fe - in the liver; Cu, Fe, Zn - in the muscle.

A5

Title: Zinc distribution in human breast cancer measured by LA-ICPMS, a follow-up study

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Introduction: In cancer cells zinc homeostasis is disturbed leading to abnormal zinc contents in cancerous tissues. Because the distribution of cancer cells in tissue is not homogenous, the histopathologist is differentiating between cancer cell clusters interwoven with normal cells on a microscopic scale. In case zinc will be associated with carcinogenesis, this should be reflected by spatial resolved zinc analysis of the tissue.

Aim: To find out if Zn may be a suitable biomarker for breast cancer from both an analytical and a therapeutic standpoint.

Methods: 28 samples of breast cancer tissue were selected with regard to expression of estrogene- (ER), progesterone- (PR) and human epidermal growth factor 2 (HER2)-receptor, and cryo-cut (-80°C) into slides of 10µm thickness. Frozen sections were microscopically analysed for cancer cells and for determination of zinc content and distribution by mass spectrometric (LA-ICPMS) techniques.

Results: Increasing zinc levels correlate with increasing histological malignancy grade, which supports the results of a former pilot study. Additionally, a correlation between zinc level and profile of receptor-expression is very likely.

Conclusion: Combination of zinc analysis with histopathological and analytical methods raises diagnostic accuracy.

A6

Title: Influence of dietary zinc source on the morphology, integrity, proliferation and biomechanics of the claw and interdigital skin of healthy sheep

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Introduction: Claw diseases threaten sheep health and are major welfare issues. Several studies have shown that zinc supplementation improves claw integrity. However, zinc supplements may differ markedly regarding zinc bioavailability. Zinc bound to single amino acids, such as Availa®Zn zinc amino acid complex (Zinpro Corporation, Eden Prairie, USA) has been shown to be more bioavailable than inorganic zinc sources.

Aim: The aim of this study was to determine the effect of different zinc supplements on the morphology, integrity, proliferation and biomechanics of the ovine claw and interdigital skin of healthy sheep.
Methods: At weaning 30 Merino lambs were randomly allocated to three different feeding groups regarding the pelleted concentrates: 1) no supplemental zinc (**0Zn**); 2) 40 mg/kg Zn as zinc sulphate (**SZn**); 3) 40 mg/kg organic zinc as zinc amino acid complex (**CZn**). Barley straw and concentrate were given ad-libitum. The diets were designed to meet the nutritional requirements for growing lambs and contained 210 g/kg DM crude protein and 13 MJ/kg DM metabolizable energy. After 8 weeks the lambs were slaughtered and the following specimens were collected immediately after death: blood serum, liver, sole and coronary band of the claw, interdigital skin. Copper and zinc were analysed in serum and tissue samples by FL-AAS. Claw and skin samples were fixed in 4% formaldehyde and submitted to pathohistological examination. Ki-67 immunohistochemistry was used to determine keratinocyte proliferation in the basal layer. Samples for electron microscopy were preserved in 2.5% glutaraldehyde with 1% lanthanum nitrate as tracer to assess intercellular permeability. Horn sample of fresh claws were submitted to Shore-C hardness and tensile strength testing. The Franz-Cell-Diffusion System was used to determine the permeability of the interdigital skin. Statistical analysis was conducted using the Shapiro-Wilk-Test, Analysis of variance, the Tukey-Test and the Wilcoxon Rank Sum Test.
Results & Discussion: The concentrations of zinc and copper in serum and liver tissue were not affected by dietary treatment. Claw horn zinc concentration was not affected by dietary treatment, median zinc concentrations were 89.9 mg/kg ww, IQR 76.0-97.1 (0Zn); 97.0 mg/kg ww, IQR 92.2-103.1 (SZn); 84.3 mg/kg ww, IQR 67.2-87.0 (CZn). 0Zn lambs showed higher copper concentrations in claw horn compared to both zinc supplemented groups (mean ± SD: 8.6 ± 1.6, 5.9 ± 2.3, 4.3 ± 1.4 mg/kg wet weight (ww) for 0Zn, SZn, CZn, respectively). Routine pathohistology as well as electron microscopy did not show significant morphological differences between the three dietary treatment groups. Immunohistochemistry results (Ki-67 proliferation index) indicate that keratinocyte proliferation in the interdigital skin was significantly higher in the CZn group compared to the 0Zn and the SZn group. Ki-67 index in the coronary band was significantly higher in the CZn group compared to the 0Zn with SZn being intermediate. Dietary treatment did not affect Shore C hardness significantly. The Franz-Cell-Diffusion-Test resulted in a tendency for decreased permeability of the interdigital skin in both Zn supplemented groups (mean ± SD: 2.015± 0.817; 1.356 ± 0.846; 1.335± 0.937 µg flufenamin acid /ml for 0Zn, SZn, CZn, respectively).

Conclusions: No differences of Zn concentrations in serum, liver and horn were found comparing the feeding groups, which is in agreement with findings from previous studies. The permeability of the interdigital skin of SZn and CZn lambs tended to be lower compared to non-supplemented animals which may indicate improved skin resistance. There were no differences regarding horn hardness between dietary treatment. Keratinocyte proliferation in the coronary band and the interdigital skin was elevated in the CZn group, which might be beneficial for tissue integrity and may help to protect interdigital skin against bacterial penetration.

A7

Title: The balance of zinc and physiogically related elements under nutritional stress

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Introduction: Zinc is one of the most important elements of human body and it is vital for all forms of life. As is well known, the stability of the chemical constitution of the organism is one of the compulsory conditions of its regular operation. But this stability can be infracted under the influence of different factors. Thus, one of the most severe problems in modern nutritiology is consuming of convenience food. High substance of saturated and trans fats, salt, high energy density glycemic index are typical for fast food. Meanwhile, these products contain unbalanced number of vitamins, minerals and reduced level of food fibers. The consuming of these products can cause the disfunction of mineral transput and the development of accessory pathologies, such as progressing of immunodeficient conditions and reducing of organism resistance to infections, the increase of cardiovascular and oncological diseases. The emergence of the diseases of civilization (obesity, diabetes, atherosclerosis).

Aim: The aim of this research is to study the influence of convenience food on the balance of zinc and physiologically related elements in laboratory animals.

Methods: The study was conducted in the two-months-old male Wistar rats. During the reference period animals were divided into two groups (n=20). The first group was control group and it was receiving staple diet and water without restriction. The staple diet was compiled according to the recommendations of the Scientific Research Institute of Nutrition of the Russian Academy of Medical Sciences. The second group was experienced one and it was consuming semi-synthetic ration, consisting of the basic feed mixture (50%), convenience food mixture (50%) and water. Convenience food mixture consisted of noodle, hot-dog and instant porridge in the same proportions. The substance of rations had the same quantitative content of proteins, fats and carbohydrates. The experiment was conducted in compliance with the principles presented by the Directive of the European Parliament and the Council of the European Union 2010/63/ EU. The duration of the experiment was 60 days. Biosubstrates were used as samples of the dry substance of the body tissues while studying the elemental status of the animal organism. Determination of the chemical elements content was carried out by the methods of atomic-emission and mass spectrometry.

Results: The evaluation of the elemental composition of the dry substance of the body tissues showed significant changes in the elemental status of animals in the experimental group. The results of the studies showed that the addition of fast food products to the basic feed had a statistically significant effect on the balance of zinc and related elements. The levels of Zn, Cr and Mn were decreased in the experimental group. Antagonistic interactions were noted, where amidst a deterioration in the level of zinc, an increase in the content of its antagonists, such as Ca, Cu, Fe, and Cd, was observed.

Conclusion: Thus, in spite of the fact that the composition of the rations of both groups was the same in terms of the quantitative content of proteins, fats and carbohydrates, we observed changes in the micronutrient composition of rats in the experimental group. This provides the basis for considering that the consumption of convenience food leads to a disruption of the zinc balance and related elements, which can negatively affect the body.

A8

Title: Tracing of labile zinc in Drosophila melanogaster S2 hemocytes

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Introduction: Numerous studies in recent years provided strong evidence that zinc ions, beside their function as a structural component or as part of the catalytic core of enzymes, control cellular processes by acting as a second messenger. So far quantification of labile $Zn^{2+}$and investigation of the mechanisms involved in the regulation of zinc homeostasis has been mainly performed in various multicellular eukaryotes, including humans, mice, Arabidopsis thaliana plants and the model nematode Caenorhabditis elegans [1-3]. However labile zinc ions were also detectable in primordial unicellular baker`s yeast or even prokaryotic organisms [4,5], suggesting that similar to cell signal transduction by Ca^{2+} supervision by Zn^{2+} remained evolutionary anchored over an almost infinite time. One of the current major topics is the role of zinc in immune-regulatory signaling [1]. So far several signal–regulated kinase pathways were shown to be affected by labile zinc in mammalian cells, but surprisingly none of these evolutionary conserved enzyme cascades was investigated for zinc sensitivity in invertebrates, so far. In particular, the innate immune response is of extraordinary interest, because heavily threatened insects such as honey bees or bumblebees exclusively rely on this path to fight against numerous pathogens.

Aim: The current study was aimed to trace labile zinc in the insect hemocyte cell line Drosophila melanogaster S2 to assess the potential of free Zn^{2+} in insect immune-regulatory signaling.

Methods: Commercially available fluorescent dyes TSQ, Zinquin-AM, Fluozin-3-AM and Zinpyr-1 were applied to establish a sensitive method for labile zinc monitoring in Drosophila melanogaster S2 cells. The distribution of the zinc indicators was monitored in living cells by confocal fluorescence microscopy. Additionally, zinc exposure experiments were performed to analyze the effect of extracellular $ZnSO_4$ application on the quantity of intracellular labile zinc and also the cell viability of the hemocytes.

Results: Only the fluorescein-based sensor Zinpyr-1 was suitable to monitor labile zinc in proliferative Drosophila melanogaster S2 hemocytes. As observed in other cell types Zinpyr-1 fluorescence mainly occurred in vesicular structures inside the cytoplasm. Co-labeling with lysosome- and mitochondria-specific dyes did not allow a clear attribution of the Zinpyr-1 signals to any of these two organelles, suggesting that the zinc pools reside in other cellular storages such as zincosomes. To convert spectroscopically observed fluorescence changes of intracellular Zinpyr-1 signals into quantitative Zn^{2+} concentrations, we applied standard Grynckiewicz equation for non-ratiometric ion probes. Already a treatment with low zinc concentrations led to a clear increase in Zinpyr-1 fluorescence, while maximal intracellular saturation of the probe was achieved after the addition of 400µM $ZnSO_4$ in the presence of the ionophore pyrithione. When Zinpyr-1 loaded cells were treated with the highaffinity membrane-permeant zinc chelator N,N,N',N'-tetrakis-(2-pyridyl-methyl)ethylenediamine we observed a clear fluorescence minimum. Based upon this strategy basal labile zinc pool in Drosophila melanogaster S2 hemocytes was estimated to be around 0.74±0.26nM.

Conclusion: Based upon our results we suppose the existence of a highly dynamic pool of mobile zinc also within invertebrate insect cells. Further mechanistic investigations are urgently needed to assign the function of free Zn^{2+} in innate insect immune response.

A9

Title: Trace elements of bees' tissues after feeding by citrate-based mineral and hydrocarbon complexes.

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Introduction: To date, a wide range of biologically active additives has been developed with compounds of micronutrients made with the help of new technologies. In particular, in animal husbandry, mineral additives of non-toxic and highly bioavailable nanocarboxylates have been tested. The use of nano-aquacitrates, certain micronutrients in the feeding of honey bees increases their viability and physiological activity of products. Known methods of their use for feeding honey bees for the purpose of correction physiological and biochemical processes and increase productivity and resistance. The study of biological effects of microelements on the bees colony biosystem and their interaction with other elements in the application of mineral additives will allow finding optimal and safe methods for stimulating the resistance of the organism of bees and their productivity.

Aim: To establish the dose-dependent influence of nanoaquacitrates mineral-hydrocarbon complexes, on the content of separate trace elements in their tissues.

Methods: Physiological, biochemical and statistical methods using atomic adsorption spectroscopy of determination of Zn, Cu, Co, Fe, Pb and Cd in tissues of the whole organism of honey bees were used in three series of studies.

Results: An analysis of the results of studies feeding bees with sugar syrup and Co and Ni citrates indicate significant intergroup differences in the content of trace elements in their body tissues. The reliably differences in control were observed in the bees of the experimental groups for Co ($p < 0.01$-0.001) and Ni except group III ($p < 0.01$-0.001), as well as Se - IV ($p < 0.05$) and Ge V ($p < 0.001$) groups. Adding 0.2 and 0.5 mg of citrate Ag and Cu to sugar syrup resulted in some corrective action on the content of certain trace elements in the tissues of the honey bees of experimental groups and antagonistic effect on the level of heavy metals such as Pb and Cd. The content of Cu in the tissue homogenate of the bees of the experimental group II decreased on 2.4%, the level of Zn - on 18.8%, compared with control, with an increase in concentrations of Cr and Co, in accordance on 26.7% and 53% ($p < 0.05$) in the III experimental group. Complex feeding of honey bees by citrates of microelements in high dilutions (1:3000; 1:2000; 1:1500 and 1:1000) with sugar syrup caused their corrective effect on mineral metabolism and increase ($p < 0.05$-0.02; $p < 0.01$-0.001) content of Co, Zn, Cu and Ni in homogenized tissues of the whole organism. It was established that in tissues of the organism of bees of the VI-VII experimental groups that received the trace element complex in dilutions 1:700 and 1:500, there were like the directed reliable ($p<0.02$-0.001) differences of the content of Co, Zn, Cu and Ni, however in the homogenized tissues of the whole organism of the bees VI-VII of the experimental group was most deposited Ni, the level of which in tissues increased on 6.69 and 4.01 mg / kg; ($P < 0.001$) and Co (3.70 mg / kg; $p < 0.001$), but only in the homogenized tissues of the bees of the VII-th experimental group compared with the specimens of the tissues of the bees of the control group (I).

Conclusion: The conducted studies have shown that both separate and joint addition of citrates of trace elements to honey bees in the spring period led to even-balanced changes in the content of certain mineral elements in their tissues. These interconnections between mineral elements must be taken into account in the schemes of mineral feeding of bees in the spring period. Activating the physiological capabilities of honey bees by feeding organic salts of trace elements is a safe alternative that can be used to improve the viability and productivity of bee colonies in certain seasonal periods.

A10

Title: Zinc- and copper based welding fumes cause an asymptomatic systemic inflammation

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Introduction: Welding fumes from a metal inert gas brazing (MIG) process with a copper-containing welding wire on zinc coated steel induce a subclinical systemic inflammation 24 hours after exposure in healthy volunteers. Since those welding fumes contain both, zinc and copper, the effects cannot reliably be attributed to one or both metals.

Aim: The aim of the present study was to investigate, whether zinc or copper is responsible for the previously observed effects or whether there are synergistic interactions of both metals.

Methods: 15 healthy male volunteers were exposed to welding fumes over 6 hours. Every test person went through a 3-fold crossover study including exposures to three different welding fumes:

1) welding fume containing zinc (target concentration [tc]: 1.5 mg/m³) and copper (tc: 0.4 mg/m³)
2) welding fume containing zinc (tc: 1.5 mg/m³) and no copper
3) welding fume containing copper (tc: 0.4 mg/m³) and no zinc

For each exposure high sensitive C-reactive protein (hsCRP) serum levels were determined. To attribute the inflammatory response at least partially to toxic effects on the lung, precision-cut lung slices (PCLS) of rats and mice were also exposed to welding fumes containing zinc and copper and toxicological analyses were conducted.

Results: Both, zinc and copper based welding fumes are able to induce increased values of hsCRP 24 hours after a 6-hour exposure indicating a mild and asymptomatic inflammation. A synergistic effect of both metals could not be detected. In PCLS no toxic effects of welding fume particles could be observed for concentrations similar to occupational exposure situations. For higher concentrations severe reductions of mitochondrial activity assessed by WST-1 occurred.

Conclusion: Although asymptomatic, the determined inflammation might represent an increased health risk for welders exposed frequently to zinc- and/or copper-containing welding fumes. Interestingly, the observed inflammatory response is not primarily mediated by toxic effects on lung tissue.

A11

Title: Stable expression of eCalwy-FRET sensors in Caco-2-cells: a practical tool to monitor free zinc in enterocytes

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Introduction: Zinc is an essential trace element and plays an important role for a variety of biological processes in the human body. Zinc homoeostasis is mainly dependent on dietary intake and bioaccessibility from food in the intestine, where zinc is resorbed [1]. Genetically encoded fluorescent sensor proteins are a versatile tool for studying zinc homoeostasis and signaling, and offer several advances compared to low molecular weight sensors [2]. The eCalwy-5 sensor is based on Förster Resonance Energy Transfer (FRET) between two fluorescent domains bound to the metal binding domains WD4 and ATOX1, which are connected by a flexible linker [3]. Knowledge on the amount of labile bound zinc and zinc uptake kinetics in intestinal cell lines is still scarce. Therefore, a stably transfected cell clone Caco-2-eCalwy was produced to monitor free zinc in enterocytes and illuminate sensitive parameters of intestinal zinc resorption. Since future research aims to use Caco-2-eCalwy clones as a cell model representing the intestinal epithelium, it is crucial to reassure enterocyte specific properties.

Aim: The aim was to ensure that characteristic properties of the intestinal cell line Caco-2-WT are still present in the stably transfected Caco-2-eCalwy clone. Moreover, this study examines the effect of sensor expression level on its activity.
Methods: Both cell lines were characterized concerning zinc-induced cytotoxicity by measuring mitochondrial enzyme activity and gene expression of proteins involved in zinc homeostasis by qPCR. Furthermore, live cell imaging and FRET-measurements were performed using laser scanning microscopy (LSM). To investigate some typical and essential features of an intestinal cell line, REM and TEM were used to visualize morphological characteristics for differentiated enterocytes such as microvilli formation and the brush border membrane. Localization of selected tight junction proteins upon differentiation was analyzed using immunofluorescence. Finally, the differentiation marker alkaline phosphatase was analyzed regarding its expression and activity.
Results: Comparison of Caco-2-WT and Caco-2-eCalwy clones revealed only slight differences regarding subcellular localization of the tight junction protein occludin and alkaline phosphatase activity, which did not affect basic integrity of intestinal barrier (measuring transepithelial electrical resistance, TEER). The introduction of eCalwy-5 in Caco-2 cells did not alter gene expression of proteins involved in zinc homeostasis, but increased resistance to higher zinc concentrations. Fluorescence cell imaging showed no organelle-specific distribution of the eCalwy-protein, but some intercellular heterogeneity in expression of the FRET-sensor was visible. However, FRET-measurements confirmed that these differences in expression level have no effect on sensor function and quantification of free zinc.

Conclusion: This study describes a well characterized stable Caco-2 cell clone expressing the eCALWY-5 FRET-sensor, which can be used to investigate intestinal zinc resorption

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A12

Title: Magnesium and Zinc Balance under Cisplatinum-based Radiochemotherapy in Advanced Head and Neck Cancer Patients

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Introduction: Cisplatinum is one of the chemotherapeutic agents most frequently used in radiochemotherapy in a large variety of squamous cell carcinomas, especially advanced head and neck cancers. It causes acute tubular damage with a short reduction of creatinine clearance and often persisting magnesium deficiency.

Aim: The influence of cisplatinum-based chemotherapy on magnesium and zinc homeostasis in combined radiochemotherapy was clinically investigated.

Methods: All patients received a combined radiochemotherapy containing of three cycles of 60 mg/m² body surface cisplatin at day 1 of each chemotherapy cycle. Serum magnesium and creatinine levels as well as the scintigraphic renal clearance in 77 patients receiving combined radiochemotherapy with cisplatin for head and neck cancer were analyzed retrospectively. 20 further patients were investigated prospectively during two subsequent cycles of cisplatin chemotherapy. Zinc, creatinine and magnesium were measured in 24 hour urine and serum before chemotherapy and at day 3 and 6 after application.

Results: In the retrospective analysis, magnesium values fell from 0.77 mg/dl±0.08 to 0.68 mg/dl±0.04 after the last cycle. Serum creatinine rose from 0.9±0.12 mg/dl to 1.0±0.16 mg/dl after the first, 1.1±0.25 mg/dl after the second, and 1.1±0.16 mg/dl after the third cycle. There was no significant difference between patients with low initial Mg (< 0.73 mmol/l) and high initial Mg (> 0.73 mmol/l). The scintigraphic MAG3-clearance showed a decline from 291 ml/min/1.73 m² to 240 ml/min/1.73 m². In the 10 prospectively analyzed patients, creatinine values increased significantly from baseline 0.86±0.07mg/dl to 1.0±0.1 at day 6 returning to normal values at day 1 of the second cycle 0.83±0.1. Creatinine clearance showed a decrease from 98±13ml/min to 29± 5.1 ml/min at day 3 returning to 52.6 ± 14 at day 6, values during the second cycle were comparable. Magnesium values were within normal range at begin of chemotherapy 0.81±0.1 and were unchanged during the first cycle, at baseline of the second cycle magnesium values fell to 0.7±0.05 and remained stable at this value. Fractional excretion of magnesium showed a linear increase from baseline 2.36 % to 3.29 % at day 6 further increasing to 3.4 and 5.3 % during the second cycle respectively. Zinc values increased from 646±82 to 673±107 and 1004±60 ng/ml during the first cycle and increased from 707±109 to 965±103 ng/ml at day 6 of the second cycle. Fractional excretion of zinc was 2.1 to 2.6% and remained constant during the two courses of chemotherapy.

Conclusion: Magnesium values decreased significantly between the two cycles due to increased excretion caused by a persisting tubular damage. Zinc values increased despite constant renal clearance, implicating an increased liberation during radiochemotherapy.

A14

Title: Interspecies differences in Zn content in liver of animals of the Siberian region

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Introduction: The study of heavy metals accumulation in living organisms and environment is of great importance to produce healthy food. It follows that the monitoring of the environment is frequently included in many scientific programs supported by number of countries all over the globe.

Aim: The aim of the research was to determine the zinc content in the liver of cattle Black-and-White, Holstein and Hereford breeds, pigs, yaks, hens and walleye from the Ob reservoir.

Methods: The concentration of zinc was determined by atomic absorption method on the solar series M6. The samples of liver were taken from different species of animals in Siberia. The research was supported by the Russian Science Foundation (project № 15–16–3003).

Results: The reference ranges of zinc levels have been established for the liver of mammals, birds and fish from the Siberian region. Breeds of the cattle do not differ in the accumulation of Zn. Phenotypic variability for Hereford, Black-and-White cattle and chickens was 2 times lower (CV = 12.5-14.5) than in any other species. The interspecies differentiation has been identified in the level of zinc in the liver, indicating the role of hereditary factors in determination of this trait. The highest level of zinc has been detected in the liver of pigs (112.6±5.5 mg/kg), the lowest in laying hens (17.9±0.7 mg/kg).

Conclusion: The levels of zinc in the liver the animal species and breeds presented the ranked series: Pig > Yak > Pike perch > Hereford = Holstein cattle > Black-and-White cattle> Chicken in the following ratio 6.3 : 5.8 : 3.7 : 2.4 : 2.3 : 1.9 : 1. The parameters obtained for zinc content in liver is possible to take into account in ecological, veterinarian and zootechnical research.

A15

Title: *In vivo* characterization of small selenium species in *Caenorhabditis elegans*

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Introduction: The trace element selenium (Se) is required for various physiological body functions and therefore constitutes an essential component of our diet. However, its beneficial range is narrow and an elevated uptake and accumulation may cause toxic effects. Se is taken up in a variety of forms, among them small organic and inorganic compounds like selenomethionine (SeMet) and selenite. These small Se species vary greatly with respect to their toxicity and bioavailability, and their modes of action and metabolic transformations are not fully understood.

Aim: Our study characterizes the Se species selenite, SeMet and Se-methylselenocysteine (MeSeCys) in order to assess their either toxic or protective potential *in vivo*, utilizing the model organism *Caenorhabditis elegans (C. elegans).*

Methods: The toxicity of the Se compounds was assessed by survival and development assays. Total Se was quantified using inductively coupled plasma tandem mass spectrometry (ICP-QQQ-MS), while coupling a high performance liquid chromatography (HPLC) to this system allowed for Se speciation analysis. Reactive oxygen and nitrogen species (RONS) and thioredoxin reductase (TrxR) activity were measured using fluorimetric and colorimetric assays.

Results: After 30 min incubation in the first larval stage (L1), selenite was identified as the most toxic compound (LD_{50} = 12 mM), also causing a developmental delay of worm larvae. In contrast, SeMet and MeSeCys were only slightly toxic, with a decrease in survival rate by 20% at 50 mM. Total Se quantification revealed that all three species were bioavailable, with an inverse relationship between toxicity and bioavailability. Speciation analysis indicated that SeMet and MeSeCys are partially metabolized to other small Se species. After 18 h incubation, protection against RONS induction was observed at a low dose range (1 – 100 µM) for all three Se compounds. This was accompanied by elevated Se levels in the worms. Measuring the Se content in protein precipitates of the corresponding worm lysates pointed to an incorporation of Se into proteins. As this was observed not only in wildtype worms, but also in a deletion mutant strain lacking the only *C. elegans* selenoprotein TrxR-1, the incorporation might occur in an unspecific rather than a genetically encoded manner.

Conclusion: Our findings indicate that SeMet and MeSeCys undergo, at least in part, biotransformation processes and therefore seem to be utilized by *C. elegans*. Moreover, all three Se species shared a protective efficacy against oxidative stress at low exposure concentrations, while the underlying mechanism remains to be elucidated.

A16

Title: The role of zinc in calprotectin expression in human myeloid cells

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Introduction: Elevated levels of zinc binding protein calprotectin have been associated with the severity of inflammatory diseases such as inflammatory bowel diseases, Crohn´s disease and sepsis. Interestingly, maximum levels of calprotectin were correlated with minimal serum zinc concentrations. While a role of zinc in the regulation of other inflammatory mediators such as tumour necrosis factor alpha, interleukin-1 beta and interleukin-6 has been established, the direct interrelation of zinc to calprotectin (S100A8/S100A9 dimer) expression is so far missing.

Aim: The aim of the study was to test the hypothesis that calprotectin might not only affect zinc homeostasis but that zinc itself alters calprotectin´s expression.

Methods: Myeloid cell lines of different maturation stages, Mono Mac 1, THP-1 and U937, were preincubated in zinc deficient medium 24h before LPS stimulation. Intracellular zinc concentrations were measured via flow cytometry using Zinpyr-1 and were verified by atomic absorption spectrometry (AAS). Further mRNA levels of S100A8 and S100A9 were analyzed using RT PCR. Western Blot was used to test if effects noted for mRNA were extended to the protein level.

Results: We could depict that zinc deficiency alone enhances mRNA and protein expression of calprotectin in myeloid cells, independently from maturity stage. Moreover, pre-existing zinc deficiency augmented lipopolysaccharide-induced calprotectin expression in $CD14^+$ MM1, but not in $CD14^-$ U937 or $CD14^-$ THP-1. Zinc deficiency and LPS seem therefore to activate different intracellular pathways. Our findings indicate that zinc is indeed a regulator of calprotectin expression in human myeloid cells, suggesting zinc as a new treatment option for diseases with deregulated calprotectin expression.

Conclusion: Regarding induction of calprotectin expression, zinc deficiency and LPS seem to activate different intracellular pathways offering an explanation for the highly elevated expression levels during severe disease, when pathogen load is high and serum hypozincemia is pronounced.

A17

Title: Association of zinc deficiency with gastro-intestinal abnormalities in Autism Spectrum Disorder mouse models

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Introduction: Research in the field of Autism spectrum disorders (ASD) has revealed a variety of associated genetic factors, among them mutations in numerous genes, with particular focus on those encoding proteins located at excitatory synapses of the central nervous system (CNS). However besides genetic factors, environmental factors such as metal imbalances emerged that alone or together with a genetic susceptibility, trigger ASD pathology. Aside from the core symptomology it has been reported that ASD patients suffer frequently from comorbidities surrounding the gastro-intestinal (GI) system, pointing towards GI abnormalities during development as a possible factor in ASD.

Aim: Here, we investigate the impact of developmental zinc deficiency that has been frequently diagnosed in children with ASD and found to lead to ASD like behavior in mouse models, on the gastro-intestinal (GI) system in mice.

Methods: We analyzed gene expression on RNA and protein level, performed fecal microbiome profiling as well as histological analysis of tissue sections.

Results: We show that zinc deficiency during pregnancy causes GI alterations in the offspring thereby possibly affecting intestinal micronutrient uptake as well as inflammatory responses. Besides microbial changes in the offspring similar to those reported in human ASD, maternal zinc deficiency also alters specific inflammatory markers in offspring hinting towards a potential chronic inflammation.

Conclusion: Thus, a model of several environmental ASD risk factors emerges linking prenatal zinc deficiency to GI abnormalities, altered microbiome, inflammation and impaired micronutrient uptake in a common mechanism.

A18

Title: TraceAge – Interactions of essential trace elements in healthy and diseased elderly.

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Introduction: This poster gives an overview about the DFG research unit TraceAge, which has started in August 2017. The homeostasis of trace elements (TE) is affected by nutritional intake, sex, age, and health status. However, interactions of TE under physiological and pathophysiological conditions are poorly characterized.

Aim: Here we aim to characterize the TE status in women and men of different age.

Methods: To this end, we will measure the TE profiles (Se, Cu, Zn, Mn, Fe, I) from serum (e.g. in the Potsdam EPIC Cohort) along with novel and established biomarkers of the TE status. These data will be combined to establish age- and sex-specific TE fingerprints of German elderly, and help to identify genetic and environmental modifiers. In order to test the relevance of such TE fingerprints, patients with cardiovascular disease during hospitalized rehabilitation (TEhab cohort) will be studied as a paradigm of severe disease with strong changes in mobility and nutrition. To get further mechanistic insights we will study TE profiles and their functional interactions in different tissues from young and old male and female mice under TE-replete and TE-poor conditions. Treatments with TE combinations in mice and *C. elegans* will identify TE effects on aging, cellular signaling, neurodegeneration, and immune response as well as interactions with drugs and thyroid hormone metabolism.

Conclusion: Our results will broaden the understanding of the importance of TE in health and disease. This will provide a basis for better care and future intervention studies to improve the TE status of seniors and to better protect them from degeneration and age-related diseases.

A19

Title: Biomonitoring of trace elements and trace minerals in children living in Kazan

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Introduction: Human biomonitoring is a recognized instrument for assessing the effect of the environmental pollutants on the population. Heavy metals are qualified as the priority pollutants of the city of Kazan. Kazan school of hygienists identifies the territory of the Republic of Tatarstan as endemic in zinc deficiency.

Aim: The aim of this research is to study the content of Pb, Cd, Ni, Zn, Mn, Cr, Cu in biological samples (the hair) of the child population living in the districts of the city of Kazan with different levels of pollution with heavy metals.

Methods: The research included analysis of Pb, Cd, Ni, Zn, Mn, Cr, Cu in the hair of 140 children aged 8-12 years. Chemicals in children hair were determined by means of ICP-AES and ICP-MS at ANO "Center for Biotic Medicine". The examined children included the groups of practically healthy children and those with chronic diseases: of the upper respiratory tract, the gastrointestinal tract, the genito-urinary system. The patterns of biomarkers distribution (the sample size (N), %> LOQ, minimum, maximum, the arithmetic mean (AM), confidence intervals (CI) and percentiles (P) were calculated separately for healthy children and those with chronic diseases.

Results: The distribution pattern of certain Me in the group of healthy and ill children was different. Samples of the Cu (copper) content in ill children had a pronounced right-sided asymmetry, and it was normal in the group of healthy children (K-S d=0.13799, p>0.20). The distribution pattern of absolute values of the Zn concentrations follows a normal distribution law (by the Kolmogorov–Smirnov criterion, K-S d=0.10879, p<0.20). The percentage of children with high values of Cu and Zn turned out to be lower in the technogenic zone than in the ecologically favorable one. The health status appears to be a significant factor defining the frequency response of the sample in copper. Comparative analysis of the elementary composition of the hair in these two groups revealed a significantly increased level of toxic elements (Pb, Cr, Ni, Cd and Mn) in the hair of ill children (p<0,01) along with the decreased content of Cu and Zn.

Conclusion: The health status appears to be a significant factor defining the frequency response of the sample in Cu and Zn. Comparative analysis of the hair could be recommended as non-invasive biomonitoring in children living in adverse environmental conditions.

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A20

Title: The effect of vanadium citrate on carbohydrate metabolism and antioxidant protection in pancreas of rats with experimentally induced diabetes

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Introduction: Oxidative free radicals (OFRs) play an important role in the etiology and pathogenesis of diabetes mellitus (DM) (Paolisso et al., 1993). Stable hyperglycemia, during the course of diabetes, causes an increase in the production of oxidative free radicals through autocidation of glucose and also due to the glycation of non-enzymatic proteins (Hunt et al., 1990, Wolff et al., 1987). Intracellular carbohydrate metabolism is impaired under hyperglycemic conditions, which is followed by the overproduction of active oxygen (Kashiwagi, 2001).

Vanadium compounds can be used to mitigate insufficient insulin response in diabetes (Thompson et al., 2006). It inhibits the secretion of protein tyrosine phosphatases, which causes protein tyrosine kinases to be activated and phosphorylate insulin receptor substrates. (Sakurai, 2002, Peters, 2003).

Aim: To find out the effect of vanadium citrate in various concentrations on carbohydrate metabolism and antioxidant defense in pancreas of rats with alloxane induced diabetes.

Methods: The material for the study was determining the activity of superoxide dismutase (SOD: EC 1.1.15.1), glutathione peroxidase (GSH-Px: EC 1.11.1.9), catalase (CAT: EC 1.11.1.6), glucose-6-phosphate dehydrogenase (G6PDH: EC 1.1.1.49) and lactate dehydrogenase (LDH: EC 1.1.1.27) in the homogenates of rat pancreas. The rats received a solution of vanadium citrate in amounts of 0.125, 0.5 and 2.0 μg/ml of water per one month of drinking water. After that, an experimental DM was induced by the injection of 5% solution of alloxane monohydrate in the amount of 150 mg/kg body weight. Experimental diabetes was fixed at glucose levels of 15.14 mmol/L.

Results: In the pancreas of rats with the experimental DM, the activity of enzymes of carbohydrate metabolism and antioxidant protection is reduced, in particular, G6PDH – by 46.13%, LDH – by 32.47%, SOD – by 38.1%, CAT – by 46% and GSH-Px – 19.7%, relative to control. When rats are dosed with a solution of vanadium citrate, the activity of enzymes increases relative to indices in animals with pure diabetes. The activity of enzymes of antioxidant protection reaches the level of control group when giving rats vanadium citrate at a concentration of 0.125 μg/ml of water, and carbohydrate metabolism - at the concentration of the compound 2.0 μg/ml of water.

Conclusion: In general, the results of the conducted studies indicate normalization of carbohydrate metabolism and antioxidant protection in the pancreas of rats with diabetes due to the influence of vanadium citrate.

A21

Title: The content of Zn, Cu, Fe, Co and Mn in tissues of male rats F_2 for long-term effect of various doses of nano-germanium citrate

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Introduction: Ge organic compounds have higher biological activity in comparison with its oxides and mineral acid salts. Was approved that using of Ge citrate (HGeC), obtained by nanotechnology method demonstrates more potent biological activity in 6-8 times lower doses than its other compounds. High metabolic ability of HGeC causes a number of physiological effects, including detection of multidirectional connections with other macro- and microelements, have influence on their accummulation in tissues and organs. However, the peculiarities of HGeC biological activity in animals of different sexes and generations for the Ge long-term body incoming, its dose-depended influence on the content of other vital elements in tissues and organs were not yet studied. Unidentified synergistic and antagonistic Ge connections with other trace elements when it incomes the organism from citrates.

Aim: Identify the influence of Ge dose on Zn, Cu, Fe, Co and Mn content in liver, kidneys, lungs and muscle tissues of male rats F_2 in the conditions of its prolong income with water in the form of citrate.

Methods: Physiological, biochemical and statistic methods of investigation were used were the amount of Zn, Cu, Fe, Co and Mn in liver, kidneys, lungs and muscle tissues of white male rats F_2 determined with the help of atomic adsorption spectrophotometer SP-115 PK. Microelement HGeC was administered daily with water in doses (mcg Ge/kg of body mass) for rat-kids of the experimental groups and female rats-mothers until weaning and also male rats up to 4 months of age: experimental group 1 (G1) — 10, G2 — 20, G3 — 200. Animal slaughter was carried out at the age of 127 days, observing bioethics; liver, kidneys, lungs and muscle tissues samples were taken in which the amount of Zn, Cu, Fe, Co and Mn were determined.

Results: Probable increase of Zn amount (101,4 mg/kg) in male rat kidney tissues was determined that were administered 200 mcg of Ge, and also in muscle — by action of 10 and 200 mcg (23,0 i 21,6 mg/kg). The amount of Cu in liver (4,3 i 4,7 mg/kg), lung (2,1 i 3,4 mg/kg) and muscle (2,1–3,0 mg/kg) were also higher in all experimental groups of rats, but in kidneys — only by action of the dose equal 200 mcg Ge (9,5 mg/kg, $P<0,01$).
The content of Mn is probably higher in kidneys (3,09 i 2,07 mg/kg) by action of 20 and 200 mcg Ge, and also in muscle — for animals of all study groups (0,58–0,82 mg/kg). But Mn concentration in rats` liver in G1 and G2 was lower (1,6-1,7mg/kg), and for G3 group was kept at control level (2,2 mg/kg). The content of Fe in the investigated tissues changed less, the level of which was probably higher only in the muscles of mammals in G1 (12,7 mg/kg) and G2 (9,8 mg/kg) groups, but was lower in kidney tissues of this animal groups. Probable differences in of Zn, Cu, Mn i Fe concentration increase in male rat tissues in study groups comparing with control, and indicate a different synergistic effect of HGeC on absorption, tissue-organ distribution and accummulation of this elements in the body. In addition, probable lower content of Co was investigated in liver tissue of G2 and G3 rats groups, and in kidney and lung tissues for all three experimental groups against of its higher level in the muscles. Determination of the absolute content of trace elements in the internal organs, taking into account their mass retained orientation the estimated differences of their levels of the control and experimental groups.

Conclusion: Thus, the admission to the body of rats of various doses of HGeC causes an unevenly directed effect on the content of other trace elements in the body tissues, which indicates an increase in the level of Zn, Cu, Fe, Co and Mn in muscles and some male organs in experimental groups and decrease the content of Co in liver, kidneys and lungs, also the amount of Fe in kidneys.

A22

Title: Development of a biochemically validated questionnaire and standardization of the biomarker serum zinc for the evaluation of the zinc supply

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Introduction: There is no established method to reliably evaluate the zinc supply in humans. The current standard is the measurement of serum zinc levels which represent only 0.1% of the total body zinc. With this biomarker a manifest zinc deficiency is detectable in the presence of clinical symptoms. In contrast there is an absence of the ability for registration of a latent zinc deficiency in which only diffuse, unspecific symptoms occur. This diagnostic condition is insufficient because also a long-term existing latent zinc deficiency influences the immune and health status badly.

Aim: The aim of our study is the accurate determination of zinc metabolic status.

Methods: From 71 donors blood samples and urine were collected. Within the study group 28 subjects were randomly selected, and fasting blood was drawn before and after drinking 1.5 l of tap water. A food frequency questionnaire was applied to calculate zinc and phytate diet scores over a period of six months. Zinc values in serum and urine were determined by atomic absorption spectometry. A hemogram was performed, especially for the hematocrit (Hct). Cytokine levels were obtained for functional blood cell analysis by ELISA.

Results: Serum zinc and Hct values showed significantly lower levels after water intake (Hct: mean=1.371, $p<0.0001$, $R^2=0.62$; serum zinc: mean=0.065, $p<0.0001$, $R^2=0.52$). A positive correlation was demonstrated between the quotients of serum zinc and Hct before and after hydration ($r=0.70$; $p<0.0001$). For adapting measured serum zinc values via the hematocrit a formula was developed. Moreover a positive correlation was shown between serum and urinary zinc values ($r=0.28$; $p=0.02$). The zinc diet scores correlated positively with serum zinc ($r=0.41$; $p<0.0005$) and urinary zinc levels ($r=0.27$; $p=0.022$). The scores obtained for phytate intake showed a positive correlation with serum zinc ($r=0.27$; $p=0.022$) but no significant correlation with urinary zinc values. The phytate:zinc molar ratios showed no significant correlation with serum zinc levels, but a negative correlation with urine zinc ($r=-0.37$; $p=0.0014$) and a positive correlation with the quotients of serum zinc to urine zinc levels ($r=0.38$; $p=0.001$).

Conclusion: As hydration status largely affects serum zinc levels, the normalization of serum zinc via the hematocrit is warranted. The analysis of the diet-related zinc and phytate consumption combined with the biomarkers serum and urine zinc can reflect the zinc supply in subjects.

B1

Title: Aluminum induced cognitive impairment is mediated through cholinergic system

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Introduction: Aluminum (Al) is known for its neurotoxic effects on cholinergic system; however, the exact mechanism is unknown.

Aim: We studied the neurotoxic mechanisms of Al on cholinergic system using mouse model.

Methods: Mice were treated with AlCl3 (250mg/kg) through oral route for 42 days. Learning and memory, acetylcholine levels, gamma oscillations were assessed in Al-treated animals.

Results: Results showed impaired memory and poor adaptation to new environment in Al-treated animals (n=9) compared to the control. High Al deposition and severe neurodegeneration was observed in Al-treated animals (n=8) compared to control. The acetylcholine levels in cortex and hippocampus (n=4) were significantly reduced in Al-treated vs. the control group. Al treatment caused permanent damage in hippocampal circuit and blocked facilitatory effect of nicotine on gamma oscillation (n=6). These results indicated that neurotoxicity induced by oral exposure of Al caused reduced memory, elevated anxiety and impaired adaptation to a new environment.

Conclusion: This study will help to understand the possible mechanism of cognitive decline induced by Al.

B2

Title: The influence of zinc on granulocyte-macrophage-colony stimulating factor-induced signaling in U937 cells

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Introduction: Zinc has already been shown to be essential for various processes of the human body, especially for the development and function of the immune system. Likewise, zinc homeostasis strongly affects hematopoiesis. For example, monopoiesis is altered during zinc deficiency. The most important growth factor for monopoiesis is the granulocyte-macrophage-colony stimulating factor (GM-CSF). As zinc is known to be involved in intracellular signaling, an association of altered zinc homeostasis with GM-CSF-induced signaling is highly probable.

Aim: In this project, the association between zinc homeostasis and GM-CSF-induced signaling was investigated.

Methods: Pro-myeloid U937 cells were pre-incubated with zinc [100µM] and sodium pyrithione [50µM] for 30 minutes and subsequently stimulated with GM-CSF [50U/ml] for another 30 minutes. Phosphorylation of STAT5 and Raf338 was analyzed using Western Blot. Also, the effect of GM-CSF on intracellular free zinc concentrations was measured via FluoZin3-AM [1µM] using flow cytometry. Finally, the GM-CSF-effect on total cellular zinc was investigated using atomic absorption spectrometry.

Results: Pre-incubation with zinc and pyrithione blocks the GM-CSF-induced STAT5-phosphorylation. Furthermore, the basal and GM-CSF-induced phosphorylation of cRaf is decreased by addition of zinc and pyrithione. GM-CSF-stimulation seems to affect intracellular zinc homeostasis.

Conclusion: Zinc is involved in the regulation of GM-CSF-induced signaling, which might explain changes in monopoiesis during zinc-deficient conditions. Results could form the basis to optimize the use of GM-CSF in therapy of hematopoietic disturbances.

B3

Title: Adaptation of human fungal pathogens to zinc flux

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Introduction: The mammalian immune system has evolved sophisticated mechanisms to withhold micronutrients such as zinc from potential invaders in a process known as nutritional immunity. In spite of this defence mechanism, pathogens still thrive and cause disease. Therefore, pathogenic microbes have, in turn, evolved mechanisms to circumvent nutritional immunity. Despite the fundamental importance of this host-pathogen "tug-of-war", its underlying mechanisms, and how they can be exploited to prevent disease remain poorly understood. We have previously shown how the major human fungal pathogen *Candida albicans* sequesters zinc from its environment. For example, this fungus secretes a metal-binding protein (a "zincophore") to scavenge zinc from host tissue.

Aim: Here, we sought to define the molecular basis of *C. albicans* intracellular zinc compartmentalisation and its impact on fungal pathogenicity.

Methods: We have used a combination of molecular biology, intracellular zinc fluorophores and infection models. Deletion mutants for all nine predicted zinc transporters (four ZIP and five ZnTs) in *C. albicans* have been generated. Zinpyr-1, FluoZin-3 and Zinquin probes have been established to monitor vacuolar and zincosomal zinc flux. *Galleria* infection have been established to examine the impact of zinc storage and mobilisation on fungal virulence.

Results: *C. albicans* adapts to potential zinc toxicity via rapid ZnT transporter-mediated zincosomal zinc compartmentalisation. Simultaneously, zinc is stockpiled in the fungal vacuole via a novel ZnT transporter. This vacuolar zinc reservoir can be subsequently mobilized via ZIP transporter-mediated efflux. Interestingly, these intracellular stores are sufficient for prolific growth in the absence of extracellular zinc - "zinc-loaded" fungal cells can undergo seven generations. That is over 100 daughter cells produced per original mother cell. Importantly, *C. albicans* cells with high vacuolar zinc reserves are significantly more virulent, and this is abrogated upon genetic deletion of the vacuolar export transporter.

Conclusion: The major human fungal pathogen *C. albicans* can stockpile zinc and use this intracellular reserve to circumvent host nutritional immunity.

B4

Title: Influence of Zinc on Antigen Processing and Presentation via MHC class II Molecules

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Introduction: Antigen presenting cells are capable of digesting and presenting peptides via MHC class II molecules in order to activate the adaptive immune system. How zinc influences this process has not yet been fully elucidated. Endolysosomal proteases, namely cysteine cathepsins, are involved in the antigen processing and maturation of MHC class II molecules. Proteins that are endocytosed into the cells are processed inside endolysosomal compartments by cathepsins in order to liberate peptides for loading onto MHC class II molecules. Additionally, cathepsins are involved in the processing of the invariant chain (Ii) that is bound to the MHC class II's peptide-binding groove. The Ii protects the MHC class II molecules from binding to peptides prematurely before they reach the endosomal compartment, where they are loaded with peptides after the Ii is cleaved by cathepsins. Studying the effect of zinc on cathepsins can shed some light on how metal regulation in cellular compartments can influence some crucial mechanisms such as those mentioned above.

Aim: Elucidating the influence of zinc on antigen processing and presentation via MHC class II molecules by measuring the inhibitory effect zinc has on endolysosomal enzymes and how antigen presenting cells process and present antigens during zinc deficiency or zinc sufficiency.

Methods: The inhibitory effect of zinc on endolysosomal enzymes is investigated using enzyme activity assays to determine the IC50 of zinc in a cell-free setting. EαGFP:YAe system is being utilized to monitor antigen uptake and presentation in peritoneal macrophages obtained from C57BL/6 mice using flow cytometry analysis and fluorescence imaging.

Results: Zinc effectively inhibits endolysosomal enzymes, cathepsin B, L and S, with IC50 values of ~6 nM. Furthermore, zinc supplementation is able to augment endocytosis of EαGFP molecules and presentation of Eα fragment via MHC class II.

Conclusion: Zinc homeostasis within cells is finely regulated and directly influences how efficiently antigen presenting cells process antigens and present them on MHC class II molecules, where they can interact with T helper cells and initiate an immune response. Zinc plays a crucial role in regulating the functions of endolysosomal enzymes, which are crucial for processing antigens and for the maturation of MHC class II molecules.

B5

Title: Zinc Homeostasis and Healthy Ageing

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Overview: Marginal Zinc (Zn) deficiency is a common condition in aging and a dysregulation of zinc homeostasis contributes to increased inflammation promoting age-related diseases. Intracellular zinc homeostasis is tightly controlled by zinc transporters (ZnT and Zip families) and metallothioneins (MT) which modulate the uptake, storage, and distribution of zinc. In a large cohort of elderly subjects we found a decline of circulating Zn, associated with increased Cu/Zn ratio that represents a biomarker of systemic inflammation and a predictor of mortality. Genetic variations in genes involved in Zn homeostasis are associated with age-related diseases such as diabetes and cardiovascular diseases (CVD) and can influence an individual's response to Zn intervention. The capacity to induce MT in lymphocytes after *in vitro* Zn treatment is not associated with age, but is reduced in lymphocytes from centenarian offspring as compared to the general population that is suggestive of a tighter Zn homeostasis control in blood cells of long living individuals. Moreover, we observed that Zn responsive genes are modulated during vascular senescence and senescent endothelial cells are more resistant to Zn deficiency than proliferating cells. This might promote the accumulation of senescent cells favouring the development of CVD diseases.

Conclusion: A dysregulation of zinc homeostasis during aging may contribute to chronic inflammation and vascular senescence accelerating cardiovascular diseases. Personalized nutritional or therapeutic interventions in elderly with genetic variants of Zn responsive genes more susceptible to zinc dyshomeostasis and inflammation might help to delay the progression of age-related diseases.

B6

Title: A novel role of S100B in neuronal trace metal homeostasis associated with dysregulation of autism-associated signaling pathways

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Introduction: Several studies reporting elevated levels of the cytokine S100B in autism spectrum disorders (ASD) patients show an association between symptom severity and S100B levels. In addition, the gene encoding for S100B has been recently identified as an autism risk gene. However, the precise role of S100B in the development of ASD is still unknown. Structural analyses of S100B have shown that the protein harbors calcium and zinc binding sites. Thus, we hypothesize a role in regulating neuronal trace metals. Intriguingly, alterations in neuronal trace metals homeostasis have been repeatedly associated with ASD and might provide a possible mechanism how S100B is implicated in the development of ASD.

Aim: Here, we aimed to identify the role of S100B in neuronal trace metal homeostasis and in the development of autism.

Methods: In this study primary hippocampal neurons have been used as *in vitro* system and were analyzed by immunocytochemistry, metal ion fluorophore stainings, Western blot and qRT-PCR analyses.

Results: In this study, we investigated the possible effect of S100B accumulation on neuronal metal ion homeostasis. Primary hippocampal neurons were exposed to different concentration of S100B and possible toxic effects were assessed by immunocytochemistry. No significant increase in neuronal cell death could be observed within the tested concentration range. Similar results have been observed for glial cells. Further we could show that S100B is taken by neurons and therefore might act both intra- and extracellular. A decrease of intracellular zinc levels after S100B exposure could be detected whereas intracellular calcium levels remained unchanged. However, intracellular calcium levels only decrease after saturation of S100B with zinc. To investigate the zinc-binding property of S100B, mutations were introduced at the predicted zinc binding sites by site-directed mutagenesis leading to the loss of zinc binding. The reduced zinc binding capacity of S100B was confirmed by a (2-pyridylazo)resorcinol (PAR) -based assay. Further, S100B mutant protein was applied to primary neurons and we could observe that the S100B mutant influences neither the intracellular zinc nor intracellular calcium levels. Additionally, zinc-saturated S100B does not longer affect intracellular zinc levels. the decrease of intracellular zinc levels was abolished. *In vitro* zinc binding of S100B was confirmed by immunocytochemistry where a clear co-localization of S100B and Zinpyr1 positive puncta could be observed.

Conclusion: Therefore, we conclude that indeed, accumulation of S100B may affect zinc signaling in the brain.

B7

Title: Zinc uptake and storage, an interplay of the ZIP transporter ZupT and the zinc repository in the bacterium *Cupriavidus metallidurans*

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Introduction: Nearly all cells, from bacteria to human, need zinc ions as essential trace elements. Although essential, zinc ions and other transition metal ions are also toxic when present at high concentrations. An adapted and controlled interplay of efflux, uptake and storage systems are required to maintenance the cellular metal homeostasis. As a model organism for investigation of metal fluxes, the β-Proteobakterium *Cupriavidus metallidurans* CH34 possess of a large number of metal efflux systems and an entire repertoire of secondary and primary systems for uptake of divalent transition metal cations such as zinc. In nearly all-living organisms, zinc is an essential metal cofactor as part of the metallomes and in the most prokaryotes the second most abundant transition metal after iron.

Aim: Characterization of the cellular components and their regulation of the bacterial zinc homeostasis as an interplay of intake, efflux, distribution and storage as well as buffering under changing environmental conditions.

Methods: Physiolocical approaches (determination of minimal inhibitory concentrations, growth rates), Regulation (reporter gen assays, LacZ / GFP and microarray analysis), analytical approaches (ICP-MS, determination of cellular metal content) and proteomic (quantitative bottom-up, Nano-LC separation und HD-MS^E).

Results: The zinc-dependent, Zur-regulated ZIP (ZRT/IRT) transporter ZupT of *C. metallidurans* plays a central role in the zinc homeostasis. Studies with Δ*zupT* mutants led to the identification of different cellular "zinc pools". A quantitative bottom-up proteomics approach explore that the overall number of predicted zinc-binding proteins is higher than the zinc content of a cell. Thus, one zinc pool in the cytoplasm of *C. metallidurans* is probably composed of zinc bound to these binding sites, which in their totality form the “zinc repository”. Here we describe how a bacterial cell in case of *Cupriavidus metallidurans* is able to adjust the correct number of zinc ions per cell, how zinc ions are discriminated and finally allocated to zinc-requiring proteins. The newly identified key element here is the zinc repository, a huge number of empty zinc binding sites in cellular proteins, which accepts incoming zinc and other metal ions, stores, discriminates and allocates them, or interacts with export systems to release surplus ions again. The important role of the Zur-regulated ZIP transporter and other compounds of the Zur regulon are shown and ZupT has been identified as a major zinc uptake system in *Cupriavidus metallidurans*.

Conclusion: The transport systems and the zinc repository are major components of the zinc homeostasis and their complex interplay makes this organism to a survivalist in zinc-contaminated environments, as well as in times of scarcity.

B8

Title: Influence of Zinc Citrate on Oxidative and Nitrative Stress in rats' organism with experimentally induced diabetes mellitus

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Introduction: Zinc (Zn) plays an important role in the synthesis, storage, and secretion of insulin as well as conformational integrity of insulin in the hexameric form (Abirami N, 2015). The presence of zinc contributes to the absorption of glucose by insulin-sensitive tissues. The content of zinc in secretory vesicles is adequate to complex storage of insulin as the 2-zinc-containing hexamer of insulin. Zinc demonstrates important physiological and pharmacological properties due to its insulin-mimetic activity. Diabetes mellitus (DM), a leading non-infectious disease with multiple etiologies, is considered as one of the five leading causes of death in the world. More clinical data should be needed to prove that zinc has an insulin-mimetic effect and protects against oxidative stress associated with DM with an increased deficiency of zinc (Yoshikawa Y, 2012).
Aim: determination of zinc citrate effect on oxidative and nitrative stress in rats with streptosotocin-induced diabetes.

Methods: atomic-adsorption method for zinc determination, spectrophotometric methods for determining the activity of NO-synthases, superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase, content of reduced glutathione, lipid hydroperoxides, TBK-active products.

Results: : Under the conditions of DM, the decrease of zinc content was detected in tissues of rats, in particular in the liver – 2.3 times, and in muscles – 1.6 times. Evidently, diabetes leads to hyperzincuria and impaired intestinal absorption of zinc (Rafique S, 2010). Under the conditions of the DM, the decreased activity of antioxidant defense system enzymes and increased lipid hydroperoxides content in rats' blood were found. In addition, hyperglycemia is accompanied by a threefold increase in the activity of inducible NOS, which obviously increases the content of nitrite anion. It should be noted that the NO component, which is synthesized by iNOS, interacts with the superoxide radical, which leads to the formation of peroxynitrite, which causes dysfunction of different systems of the body, nitrates the cytoplasmic proteins, and activates the processes of lipoperoxidation. The increased activity of iNOS is also associated with increased H2O2 formation and the effect of proinflammatory cytokines that activate mRNA expression of iNOS (Van den Oever I, 2010). Adding zinc citrate in doses of 20 and 50 mg Zn/kg of body weight to the diet of rats with experimentally induced diabetes, we observed growth of zinc content in tissues, increased activity of investigated enzymes and decreased lipid hydroperoxides content in blood, and the increase of reduced glutathione content in erythrocytes, that may indicate the activation of antioxidant processes and normalization of the physiological and biochemical state of rats' organism under the action of zinc citrate.

Conclusion: The introduction of oral zinc citrate in doses of 20-50 mg / kg of body weight causes normalization of functioning of antioxidant and NO-synthase systems in rats' organisms with experimentally induced diabetes mellitus.

B9

Title: Increased *CREMα* (cAMP responsive element modulator α) transcription factor expression during zinc deficiency leads to decreased interleukin-2-production

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Introduction: Zinc is an important trace element and essential in the function of a well working immune system. Thus, zinc deficiency causes dysfunction of immunological functions, going along with reduced interleukin (IL)-2-production of T cells. Dysfunction of the IL-2-production is accompanied by immunological defects, such as systemic lupus erythematodes (SLE).

Aim: The aim of this study was to reveal underlying molecular mechanisms, causing decreased IL-2-production in T cells under zinc-deficient conditions.

Methods: Different methods were used in this study. The most important ones comprise the establishment of a T cell line model, which survives short term and long term zinc deficiency, FACS analysis to measure the intracellular zinc content of the T cells, an ELISA assay to determine the amount of produced IL-2 and RT-PCR to analyze gene expression.

Results: In my work, I could show increased expression of the transcription factor *CREMα* (cAMP responsive element modulator α) upon short-term zinc deficiency on RNA levels. CREMα is known to bind to the IL-2-promoter, thereby inhibiting IL-2-production. Increased *CREMα*-expression could moreover be observed after long-term zinc deficiency, being a possible explanation for decreased IL-2-production on RNA and protein levels. Apart from that, differential zinc transporter expression by T cells could be observed under zinc-deficient conditions, going along with reduced intracellular zinc levels.
Methylation analysis of the DNA obtained from long term zinc-deficient T cells and from T cells held under normal conditions revealed that the methylation pattern of the *IL-2* gene shows no significant methylation between zinc-deficient and normal cells, excluding differences in DNA-methylation as a possible explanation of aberrant *IL-2*-transcription.

Conclusion: Summarized, IL-2-production in T cells seems to be influenced by zinc on different levels.

B10
Title: The ZIP/*SLC39A* transporters in β-cell function

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Introduction: Pancreatic β-cells require a constant supply of zinc to maintain normal insulin secretory function. Following co-exocytosis with insulin, zinc is replenished via the Zrt- and Irt-like (ZIP; SLC39A) family of transporters. Since a compromised zinc status is observed in diabetic patients and animal models of diabetes one can hypothesize that altered ZIP expression profiles are associated with disease state. However the ZIP paralogues of particular importance for zinc uptake, and associations with β-cell function remain largely unexplored.

Aim: We aimed to identify ZIP transporters most important for β-cells and to explore their importance in β-cell function.

Methods: We retrieved and statistically analyzed publically available microarray and RNA-seq datasets to perform a systematic review on the expression of β-cell SLC39A paralogues. We complemented results with experimental data on expression profiling of human islets and mouse β-cell derived MIN6 cells. We manipulated the expression of ZIP6, ZIP7 and ZIP9 within MIN6 cells to interrogate their importance in maintaining β-cell phenotype following extracellular zinc stress.

Results: We identified ZIP6, ZIP7, ZIP9, ZIP13 and ZIP14 in human and rodent and ZIP1 in rodent as potentially biologically important for β-cell zinc trafficking. Furthermore, our experimental data suggest a depleted zinc status impairs β-cell viability and transcriptional profile, and ZIP6 knockdown is associated with increased apoptosis and an impaired ability to influx extracellular zinc.

Conclusion: We propose ZIP6 is a key functional orthologue within human and rodent β-cells and highlight this paralogue as an important target for exploring associations between zinc status, β-cell physiology and their decline in Type 2 Diabetes.

B11

Title: Zinc supplementation induces antigen-specific regulatory T cells by upregulation of Forkhead-Box-Protein P3 and Krüppel like factor 10, and downregulation of Interferon regulatory factor 1 expression

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Introduction: Adequate tolerance induction is critical for maintaining immune homeostasis. In this regard, regulatory T cells (Treg) as well as essential trace elements like zinc play a major role. Proper zinc homeostasis is critically important, since its deficiency is associated with severe impairment of the immune response characterized by increased susceptibility to infections, development of allergies, autoimmune diseases, and transplant rejections.

Aim: This study was designed to disclose the influence of zinc supplementation on the T helper cell (Th)1-driven alloreaction in mixed lymphocyte cultures (MLC), the generation of antigen-specific Treg cells and to uncover responsible underlying molecular mechanisms.

Methods: The severity of the allogeneic immunoreaction in MLC was measured by [^{3}H]-thymidine assay and ELISA. Treg cell induction was determined by FACS-analysis. Molecular targets modulated by zinc supplementation was investigated by real-time PCR and Western blot.

Results: Zinc supplementation of antigen-specific T cells in physiological doses (50µM) provoked a significant amelioration of cell proliferation and pro-inflammatory cytokine production after re-activation compared to untreated controls. Zinc administration to MLC resulted in an increased induction and stabilization of antigen-specific Treg cells, which was based on zinc-induced upregulation of Foxp3 and KLF-10 and downregulation of interferon regulatory factor (IRF)-1 mRNA and protein expression.

Conclusion: Taken together, zinc was capable to ameliorate the Th1-driven allogeneic immune reaction by enhancement of antigen-specific induced Treg cells due to modulation of essential molecular targets: Foxp3, KLF-10, and IRF-1. Thus, zinc can be seen as an auspicious tool for inducing tolerance in dysregulated immunoreactions.

B12

Tile: Aluminium Speciation in Serum and Urine after Subcutaneous Immuno Therapy(SCIT) with Aluminium as Adjuvant

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Introduction: Concerns about aluminium exposure with potential adverse health effects are increasing. Several studies did not reveal a specific risk whereas other studies could not exclude such a risk. In SCIT considerable Al-amounts as adjuvant are injected (ca. 1 mg Al per injection, every 4 weeks, for several years) and skin as barrier is circumvented. The question remains whether the Al-deposit slowly dissolves and gets constantly excreted or whether it gets enriched. Since Al analysis, even more speciation, are prone to be affected by contamination, special attention was paid.

Aim: How can Al concentrations reliably be obtained in urine and serum and is an Al-load (which species) reliably monitored after allergen specific subcutaneous immunotherapy?

Methods: Urine and serum were collected from patients after SCIT using Al as adjuvant, and from controls. Total Al was determined with ICP-sf-MS whereas Al-speciation was performed with SEC-ICP-MS. To avoid the likely Al-contamination a couple of pre-cleaning steps and quality control measures were applied.

Results: Three consecutive approaches were performed. The first sample set (n=41) showed no contamination in urine but also no differentiation between cases and controls. Contrary, serum samples showed significant difference (p=0.007) and strong correlation between number of injections (*NI*) and total Al or Al-citrate. However, even controls showed exceptional high Al values, suggesting contamination in serum. Thus the second approach used HNO_3-washed monovettes (*HwM*) instead of the typically used gel-monovettes (*GM*) for blood sampling. Again, serum samples showed significant difference (p=0.019) between groups, but now no correlation between *NI* and any species was observed. Al concentrations of controls were within the concentration range of non-exposed persons, i.e. no contamination was suspected. The 3rd approach aimed to clarify whether the type of monovettes caused the difference in Al-concentrations in previous two approaches. Samples were drawn with *HwM* and *GM* in parallel. Serum samples from *GM* showed an averaged contamination of 37 µg/L, which is about 8 fold of concentration from controls. Comparing only results from non-contaminated sampling, in 3rd attempt, too, Al-concentrations in serum differed significantly between controls and SCIT patients (p=0.017). A correlation between *NI* and total Al may be suggested but has to be confirmed due to the limited sample size in 3rd approach. An evaluation of normalized Al-species pattern (as percentage of total amount) from samples taken with *GM* vs. *HwM* revealed no difference in distribution. This indicates that the surplus Al amount from contamination was not attached to one specific ligand. Therefore speciation results from the 1st approach had to be ignored.

Conclusion: Al-contamination remains a critical issue -mainly during sampling -even when paying special attention. After SCIT Al values are significantly elevated in serum. Whether the indicated correlation between Al and injection frequency actually exists has to be further examined.

B13

Title: About the impact of Arsenolipids on tight junctions of brain barriers

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Introduction: In the last years a group of fat-soluble arsenicals also known as arsenolipids (AsL) has gained much attention due to their significant cytotoxicity in various cell lines such as human neurons (LUHMES) [1], liver (HepG2) and bladder (UROtsa) cells [2]. Recently, it was shown that arsenic-containing hydrocarbons (AsHC), a subgroup of AsL, are not only able to cross *in vitro* barrier models (Caco-2) [3] but also can accumulate in the brain area of the fruit fly [4].

Aim: We investigated the impact of AsL on protective *in vitro* brain barrier models, namely the blood-brain (BBB) and blood-liquor barrier (BLB). We studied the cytotoxicity of various AsL and their metabolites, the transport of selected AsL across these barriers as well as the effects on the barrier integrity including tight junctions.

Methods: For the *in vitro* brain barrier models, porcine brain capillary endothelial cells (PBCECs; model for the BBB) and porcine choroidal plexus epithelial cells (PCPECs; model for the BLB) were isolated from freshly slaughtered pigs. To examine the cytotoxic potential of the test compounds (AsHC 332, 360, 444; arsenic-containing fatty acid 388, 362) and of their metabolites (thio/oxo-dimethylpropionic acid, dimethylarsinic acid), WST-8 reduction and neutral red uptake were chosen. For barrier studies, the transendothelial electrical resistance (marker for the barrier integrity) as well as the capacitance (marker for the cell surface area) were continuously measured by impedance spectroscopy *via* CellZscope® for 72 hours. In the *in vitro* BBB, the transport of AsHC 332 and AsHC 360 was assessed after 6 hours *via* microwave-assisted acid digestion followed by ICP-QQQ-MS analysis. Furthermore, an immunostaining of various tight junction proteins was performed to gain information on morphological changes. In all experiments, arsenite (iAs^{III}) served as the (toxic) reference.

Results: The *in vitro* BBB is multiple times more sensitive towards the applied test substances than the *in vitro* BLB. The potency of AsHCs is up to 5-fold higher than that of iAs^{III} in PBCECs. Incubation of AsHCs for 72 hours caused the disruption of the *in vitro* brain barriers at (subcytotoxic) concentrations where iAs^{III} showed no effect. Immunostaining in PBCECs revealed strong morphological alterations, while in PCPECs no visible changes were noticed. At concentrations that did not affect the barrier integrity, in case of the AsHCs a slight transfer of arsenite across the *in vitro* BBB after 6 hours could be observed in the same range as for iAs^{III}.

Conclusion: The selected AsHCs were more cytotoxic than the reference iAs^{III} and caused severe disruption of the barriers. Thereby they possibly enhance the transfer of accompanying toxic substances from the food into the brain so further experiments are required to elucidate whether AsHCs are metabolized *in vivo*.

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B14

Title: Structural and functional features of the C-terminal cytoplasmic domain of ZnT8

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Introduction: One of the four human vesicular zinc transporters, ZnT8, supplies the millimolar zinc concentrations of insulin granules in pancreatic β-cells, affecting insulin processing, crystallisation, and secretion. ZnT8 has a transmembrane and a C-terminal cytoplasmic domain; the latter purportedly mediates protein-protein interactions, senses cytosolic zinc, and/or channels zinc to the transport site in the transmembrane domain. A common mutation (W325R) in the C-terminal domain (aa267-369) increases the risk to type-2 diabetes and affects autoantibody specificity in type-1 diabetes.

Aim: To understand how a mutation in the cytoplasmic C-terminal domain of ZnT8 affects zinc transport and alters diabetes susceptibility.

Methods: We recombinantly expressed the two ZnT8 C-terminal domains in bacteria and analysed them using solution techniques such as circular dichroism, microscale thermophoresis, fluorescence and ICP-MS.

Results: The ZnT8 C-terminal domains fold independently of the transmembrane domain. Remarkably, the W325 variant (diabetes protective) is less thermostable than the R325 variant (diabetes risk). Determinations of monomer/dimer equilibria demonstrate that the W325 monomers associate with higher affinity. Both variants bind zinc with a stoichiometry that differs from those of the bacterial proteins for which 3D structures are available.

Conclusion: The relatively small but reproducible differences between the variants begin to provide a molecular basis for the different diabetes risk associated with the two ZnT8 variants.

B15

Title: Zinc availability to fish: salt and speciation matters

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Introduction: In Atlantic salmon, bio-availability of Zn can be influenced by the source of dietary Zn and variation in the gut ionic concentration arising after shifting from freshwater to seawater. Although not convincingly demonstrated, amino acid chelated organic Zn forms have been suggested to have a higher bioavailability than their inorganic forms to fish (Antony Jesu Prabhu et al., 2016). However, the physiological understanding on the influence of gut ionic concentration and Zn forms on the bio-availability of dietary Zn in fish is very limited. The complexity of the environment and multiple dietary interactions have been the limiting factors with in vivo experiments. Recently, an intestinal epithelial cell line named RTgutGC, was used to characterize the intestinal absorption of nutritive and non-nutritive metals in fish (Minghetti et al., 2017). This study thereby intended the application of such models to address and understand practical limitations faced in fish nutrition.

Aim: Examine the uptake of Zn as affected by gut ionic concentration found in freshwater (FW) and seawater (SW) acclimatized salmonids and the effect of zinc-methionine (Zn-Met) chelation using RTgutGC cell line *in vitro*.

Methods: RTgutGC cells were aseptically cultured in a complete Leibovitz' L-15 medium added with 5% fetal bovine serum (L15/FBS) at 19 °C in normal atmosphere as previously described by Kawano et al., (2011). The cells were seeded to 24 well plates with commercial L15/FBS at a density of about 0.5 x 105 cells/ml and incubated at 19 °C for 48h to form a monolayer. Three exposure media were designed based on the ionic concentration of L-15 medium, namely (i) L15/ex, adapted from (Schirmer et al., 1997), (ii) FW and (iii) SW both conceptually designed from (Bucking and Wood, 2007, 2006) and Dabrowski et al. (1986) to closely represent the luminal gut saline of the freshwater (FW) and seawater (SW) acclimatized salmonids, respectively. The viability of the cells to different media composition and graded level of Zn concentrations were examined through alamar blue assay. Amino acids namely L-methionine (Sigma), D-methionine (Sigma), and DL-methionine (Alfa Aesar) were used as chelating ligands; 2-Aminobicyclo [2.2.1] heptane-2-carboxylic acid (BCH, Sigma) was used as the amino acid transport inhibitor. The cells were exposed to different media compositions with different ligands and ^{65}Zn (as ZnCl2, PerkinElmer, USA) at specified concentrations and ^{65}Zn influx was measured in a gamma counter (Wallac, 1282 Compugamma).

Results: Influx of ^{65}Zn in SW treated cells were lower compared to those treated with FW media. The uptake kinetics revealed that both V_{max} and K_m were reduced in SW media treated cells compared to cells treated with FW. Presence of methionine (L-Met and DL-Met) significantly increased the V_{max} of uptake under both FW and SW media conditions, magnitude being higher in FW; K_m was not significantly affected. Cells exposed to methionine along with BCH showed a significant reduction in ^{65}Zn uptake when compared to control cells without BCH exposure, the effect was more pronounced in SW media composition than in FW.

Conclusion: Luminal ionic concentration of gut and Zn-met speciation affects Zn uptake in RTgutGC, an in-vitro fish intestinal epithelial cell model which could have practical implications for Zn nutrition in Atlantic salmon.

B16

Title: Ferric nitrilotriacetete augments 7,12-dimethyl benz(a)anthracene-initiated and benzoyl peroxide-promoted skin carcinogenesis

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Introduction: Ferric nitrilotriacetete (Fe-NTA) is renal carcinogen and little is known about its effects on skin cancer.

Aim: We report that Fe-NTA augments benzoyl peroxide (BPO)-mediated tumor promotion in 7,12-dimethyl benz(a)anthracene (DMBA)-initiated mouse skin.

Methods: Swiss mice were topically applied with Fe-NTA and tumors were initiated by topical application of DMBA. Then animals were given weekly application of BPO for 46 weeks. Appearance of papilloma and number of tumors were recorded.

Results: In comparison to other groups, Fe-NTA caused an increased incidence of tumors and carcinoma at various time intervals. In Fe-NTA group, tumors appeared earlier, also number of tumors and carcinoma were higher. Further, BPO-mediated induction in ODC activity and [3H]thymidine uptake were higher in Fe-NTA treated group.

Conclusion: we propose that Fe-NTA increases tumor promotion and progression potential of BPO and oxidative stress generated by Fe-NTA is responsible for BPO-mediated cutaneous tumorigenesis.

B17

Title: Trace element species and amyotrophic lateral sclerosis with disease associated genetic mutations

Authors: Nikolay Solovyev[a,b,*], Jessica Mandrioli[c], Marco Vinceti[d], Carlotta Malagoli[d], Marianna Lucio[b], Bernhard Michalke[b]

Affiliation: [a] St. Petersburg State University, Institute of Chemistry, St. Petersburg, Russia; [b] Helmholtz Zentrum München – German Research Center for Environmental Health GmbH, Research Unit Analytical BioGeoChemistry, Neuherberg, Germany; [c] Department of Neurosciences, St. Agostino-Estense Hospital, Azienda Ospedaliero Universitaria di Modena, Modena, Italy; [d] CREAGEN Research Center for Environmental, Genetic and Nutritional Epidemiology, Department of Biomedical, Metabolic and Neurosciences, University of Modena and Reggio Emilia, Modena, Italy; * n.solovyev@spbu.ru

Introduction: Amyotrophic lateral sclerosis (ALS) is a motor neuron disease with mostly unknown etiology. Certain genetic mutations are associated with the disease; however, the role of environmental factors, such as exposure to metals and organic pollutants is also widely discussed in the literature. ALS, as other neurodegenerative disorders, is related to the brain oxidative stress, so the disturbance of redox homeostasis may be anticipated for such elements as selenium (Se), copper (Cu), iron (Fe), and manganese (Mn).

Aim: The aim of the study was to evaluate a possible alteration of trace element (Se, Cu, Mn, and Fe) homeostasis in the ALS patients with disease associated gene mutations.

Methods: We analyzed cerebrospinal fluid (CSF) samples from 9 patients with ALS-associated mutations (C9ORF72, SOD1, FUS, TARDBP, ATXN2, and TUBA4A) and 42 age- and gender-matched controls. Advanced speciation techniques were used to quantify redox forms of Cu (I/II), Mn (II/III), and Fe (II/III) and Se species (selenoprotein P, glutathione peroxidase, thioredoxin reductase, selenite, selenate, and human serum bound-Se). For the separation of Se species strong anion exchange chromatography (SAX) was used, whereas Cu, Mn, and Fe redox forms were separated by strong cation exchange (SCX). For the species detection, inductively coupled plasma sector field mass spectrometry (ICP-sf-MS), operated at high resolution for Se or medium resolution for Cu, Fe, and Mn was employed. Standard compounds and spikings were used for peak assignment. External calibration vs. matching to the total content of the elements, measured by inductively coupled plasma dynamic reaction cell mass spectrometry, was used for species quantification.

Results: The analytical schemes of species quantification, using SAX-ICP-sf-MS [1] and SCX-ICP-sf-MS [2], have been optimized. The difference in Cu(II) and some Se species were found to be altered in the CSF of the ALS patients with disease-associated mutations. Also, since multi-element speciation had been performed for the same set of CSF samples, some inter-element correlations were observed (between Fe and Se species, Mn and Fe, Mn and Cu).

Conclusion: Despite the limited sample size, we could presume a distortion in trace element metabolism, reflected the altered speciation of Cu and Se in the CSF. However, more insight is required to understand if these findings are an innocent bystander to the pathological changes in the ALS brain or has its own relevant role in the etiopathogenesis of the disease.

References: [1] Mandrioli et al., Neurodegener. Dis., 17 (2017) 171-180; doi: 10.1159/000460253.
[2] Solovyev et al., Anal. Chim. Acta, 973 (2017) 25-33; doi: 10.1016/j.aca.2017.03.040.

B18

Title: Analysis of the cellular mechanism of toxicity of zinc oxide nanoparticles aiming at their application in tumor therapy

Authors: Nadine Wiesmann*[1], Martin Klünker[2], Muhammad Nawaz Tahir[2], Wolfgang Tremel[2], Jürgen Brieger[1]

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Introduction: Zinc oxide nanoparticles (ZnO-NP) were shown to exert selective cytotoxicity against tumor cells. Up to now the cellular mechanism of toxicity of ZnO-NP is not fully elucidated and ongoing subject to discussions. Most likely the generation of reactive oxygen species (ROS) and subsequent oxidative stress are involved but also cytoskeletal damage has been discussed.

Aim: Our aim was to elucidate the mechanism of toxicity of ZnO-NP and evaluate their applicability as radiosensitizer to improve the irradiation response of tumor cells.

Methods: We assessed the viability of A549 cells after treatment via an AlamarBlue assay, the genotoxicity of ZnO-NP was analyzed by γH2AX foci analysis and the performance of ZnO-NP as radiosensitizer was assessed by a colony formation assay.

Results: We could demonstrate that ZnO-NP exert cytotoxicity to human tumor cells, which was conveyed by dissolved Zn^{2+} ions as well as by the particles themselves, which are probably taken up into the cells. Treatment with ZnO-NP resulted DNA damage in the form of double-strand breaks. The colony formation assay showed that treatment with ZnO-NP in combination with irradiation with 2 or 4 Gray, according to typical, clinically applied irradiation dosages, resulted in reduction of clonogenic survival and enhancement of tumor cell death.

Conclusion: We were able to show that ZnO-NP exert a cytotoxic and genotoxic effect on human tumor cells and combined treatment of tumor cells with ZnO-NP and irradiation reduced tumor cell survival. All in all, the study shows that ZnO-NPs could probably be a promising anticancer agent, to improve the irradiation response of tumor cells.

B19

Title: Metals and metabolites in cerebrospinal fluid of Parkinson's disease patients

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Introduction: Parkinson´s disease is a severe, progressive neurodegenerative disease. Many risk factors are suggested to be involved in disease onset and progression, e.g. exposure to metals, increased oxidative stress and neuroinflammation. Despite much research effort, underlying mechanisms are not completely uncovered.

Aim: We want to get a comprehensive view to metal-homeostasis, metabolites and corresponding pathways which are affected in Parkinson´s disease, to find possible starting points for proper medication.

Methods: We used inductively coupled plasma-sector field-mass spectrometry (ICP-sf-MS) to investigate the total metal-content and size exclusion chromatography-inductively coupled plasma-dynamic reaction cell-mass spectrometry (SEC-ICP-DRC-MS) for the determination of metal species fractions in CSF. The amino acids were separated by anion exchange chromatography (AEX) and quantified using integrated pulsed amperometry (IPAD), fractions of each amino acid were collected for further metal-determination by ICP-sf-MS. Metabolite determination was done by electrospray ionization-Fourier transform-ion cyclotron resonance-mass spectrometry (ESI-FT-ICR-MS).

Results: Neither the determination of total metal amount nor the species-fractionation yielded significant differences. But ratios between metals and species fractions showed highly distinct changes and gave a first hint for further experiments in the direction of amino acids. We found two amino acids to be different in cases and controls. Additional metabolomics investigations showed significant differences in Parkinson´s disease for markers of oxidative stress and neuroinflammation.

Conclusion: The combination of metallomics and metabolomics approaches is a powerful combination to get a deeper insight into ongoing disease mechanisms.

B20

Title: Effects of sodium fluoride in immune responses of macrophages in hypoxic and normoxic conditions

Authors: Nuray Yazihan, Durul Seyma Sen, Gokberk Metin, Mehmet Ozkok, Ilker Dincer,Yigit Dilaver

Affiliation: Ankara University, Faculty of Medicine, Pathophysiology Department Ankara, Turkey, Email: nurayyazihan@yahoo.com

Introduction: Fluoride is one of the basic elements that has important roles in bone metabolism. It is found in a variety of foods, industrial products, oral care products and basically drinking water. Both deficiency and overdose of fluorine can lead to disorders in bone metabolism. In the human body, monocytic cells differentiate to macrophages through appropriate stimulation to the tissues and contribute to the immune response along with tissue macrophages. While blood cells function in the circulation at high O2saturation, they need to maintain their function in low O2 saturation when they pass into tissues. Sodium floride (NaF) is a limited and contradictory study of the effects of inflammatory cells and cells in the immune system. The effects of NaF on macrophages in hypoxic conditions are not yet known.

Aim: The aim of our study was to investigate the effect of NaF administration at different doses on proinflammatory and antiinflammatory cytokines in macrophages.

Methods: THP-1 cells, which are human monocytic leukemic cell line is administered with sodium fluoride (2.5-10-25-50-75 µg / ml) in cell culture medium after differentiation of macrophages by PMA (phorbol myristicacid) application. The changes in the secretions of proinflammatory (TNF-α, IL-2, IL-4 and IL-6) anti-inflammatory (IL-10) cytokine levels were assessed by ELISA. The experiment was repeated in normoxic, hypoxic (1% O2) conditions w/o lipopolysaccharide (E. coli, 0111, 1 µg / ml, LPS).

Results: Our study showed that NaF administration has different effects as dose-dependent manner. At the doses of 50-75 µg / ml NaF decreased the number of PMA-stimulated human monocytic cells and th effect was more prominent in hypoxic conditions. NaF administration (50-75 µg / ml dosage and 24 h) decreased IL-10 secretion significantly ($p < 0.000$). Overall, low doses of NaF administration reduce inflammatory cytokines while high doses induce.

Conclusions: The results of our study have shown that NaF administration significantly changes macrophage functions, the effects of NaF on gene and protein expression in macrophage cells at different doses should be studied more extensively, and that dose-dependent NaF administration suppresses IL-10 secretion significantly. This may lead to the augmentation of the pathological processes, especially in patients with diseases in which autoimmunization plays a role in the pathogenesis, and to the reduction of immunosuppressant treatment responses.

Acknowledgement: This study is supported by TUBITAK 2209-A programme (Project number:1919B011603617).

B21

Title: Genetic determination of the content zinc and other essential elements in the hair of children living in industrial region in Russia

Authors: Elena V. Zhukovskaya[1], Galina N. Kireva[2], Tatyana S. Lisitsa[1,3], Tatyana V. Nasedkina[1,3]

Affiliation: [1] National Scientific Center of Pediatric Hematology, Oncology and Immunology, Moscow, Russia, [2] Regional children hospital, Chelyabinsk, Russia, [3] Engelhardt Institute of Molecular Biology of Russian Academy of Sciences, Moscow, Russia, Corresponding author: elena_zhukovskay@mail.ru

Introduction: Modern biomonitoring content of trace elements in biological samples need to go the identification of options for genetic determination of metabolism, but there are many unknown aspects in it.

Aim: To study the dependence of the content of zinc and other essential trace elements in the hair from genetically determined variants of metabolism in children living in industrial regions of Russia.

Methods: The ratio of the elemental composition of hair was carried out by the method of ISP-MS in 258 children from Karabash and control areas from Chelyabinsk region. DNA was isolated in 86 children (51 girls, 35 boys) from 9 till 18 years (M 14.423 ± 3.21) from leukocytes of peripheral blood by means QIamp DNA Mini kit (Qiagen, USA) according to manufacture protocol. The genotyping was performed using PCR and allele-specific hybridization on biochip, which allowed to identify 17 allelic variants in the genes CYP1A1, CYP2D6, CYP2C9, CYP2C19, ABCB1 (MDR1), NAT2, MTHFR, TPMT.

Results: The most pronounced individual differences in the levels of heavy metals and trace elements in the study group were revealed in analyzing of the microelement composition of the hair. The frequencies of alleles and genotypes in groups of children with decreased and increased levels of trace elements in the hair were determined compared with normal level. It was found that the A allele of rs3892097 (1934G>A, c.506-1G>A) in CYP2D6 gene was more frequent in children with increased level of As compared with normal control (25% vs 13.54%, p=0.075) and in children with decreased level of Ca compared with normal group (29.55% vs. 14.84%, p=0.042). The T allele of rs1799853 (c.430C>T, p.Arg144Cys) in CYP2C9 gene was more frequent in children with increased level of Pb compared with normal control (16.07% vs. 6.03%, p=0.0482). Also the A allele of rs1799930 (c.590G>A, p.Arg197Gln) in NAT2 gene was more frequent in children with increased level of Pb compared with children having normal level (44.54% vs. 23.28%, p=0.007) and in children with decreased level of Zn compared with normal level group (50% vs. 27%, p=0.031).

Conclusion: The A allele of CYP2D6 gene (rs3892097) and the T allele of CYP2C9 gene (rs1799853) code the cytochrome variants with reduced enzymatic activity. The A allele of NAT2 gene codes the enzyme variant with slow acetylating rate. In our study, the presence of allelic variants with reduced enzymatic activities was associated with accumulation of heavy metals (As and Pb) and loss of essential microelements (Ca and Zn) in the hair of the children subjected to heavy metals exposure in industrial zones of Chelyabinsk region.

B22

Title: Toxicity of arsenolipids in human neurons: RONS and genotoxicity

Authors: Vanessa Ziemann[1], Franziska Ebert[1], Sandra M. Müller[1], Lena Woelk[1], Linda Wietholz[1], Barbara Witt[1], Kevin A. Francesconi[2], Tanja Schwerdtle[1*]

Affiliation: [1] Universität Potsdam, Institut für Ernährungswissenschaft, Abteilung Lebensmittelchemie; [2] Karl-Franzens-Universität Graz, Institut für Chemie; *tanja.schwerdtle@uni-potsdam.de

Introduction: Arsenic occurs in inorganic and organic forms. Inorganic arsenic is especially present in water, soil as well as in terrestrial food and has been classified as a human carcinogen (IARC, 2012). In marine organisms, organic arsenicals predominate, which can be grouped in water- and lipid-soluble arsenicals. Arsenic-containing hydrocarbons (AsHCs) are a subclass of arsenolipids and have been recently shown to exert substantial cellular toxicity in postmitotic, fully differentiated human neurons (LUHMES, *Lund human mesencephalic cells*). Even though exerting cellular toxicity in a similar concentration range like arsenite, the underlying toxic mechanisms seem to differ between AsHCs and arsenite (iAs^{III}).

Aim: Here we aim to further characterize cellular toxicity of arsenolipids in human neurons with a special focus on reactive oxygen and nitrogen species (RONS) and genotoxicity.

Methods: As marker for cytotoxicity we assessed cell number (*via* Hoechst staining) and cell viability (*via* Resazurin reduction assay). Cellular RONS levels were measured applying two fluorescence-based methods, the dihydroethidium and the 5-(and-6)-carboxy-2',7'-dichlorfluorescein diacetate assay (Carboxy-DCFH-DA). As an indicator for DNA double strand breaks (DSB) we quantified phosphorylation at serine 139 histone variant H2AX, *via* γH2AX foci and γH2AX slot blot analysis.

Results: Both iAs^{III} and a selected arsenolipid (AsHC 332) did not increase the cellular amount of RONS. Very interestingly, AsHC 332 strongly increased the number of γH2AX foci after 48 h incubation, while arsenite exerted only moderate effects. Nevertheless, arsenite induced much more single stand breaks as compared to AsHC 332.

Conclusion: These data provide first evidence for *in vitro* genotoxicity of arsenolipids in human neurons. Further studies have to (1) clarify whether arsenolipids are indeed genotoxic in human neurons and (2) identify the underling mechanism of the potential genotoxicity of arsenolipids.

References: IARC. Arsenic, metals, fibres and dusts: A review of human carcinogens. *IARC Monogr.* 100CA, 41–93 (2012).

HEINZ-ZUMKLEY-PRIZE AWARDEES

Curriculum Vitae Nikolay Solovyev

Nikolay Solovyev

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Research interests

Primary	Speciation analysis, Atomic Spectrometry, Mass Spectrometry, Hyphenated Techniques, Metallomics and Metabolomics, Trace Elements, Selenium, Transition metals, Role of Trace Elements in Brain and Brain Disorders,
Secondary	Metabolism, Brain Signaling, Redox balance, Validation and Quality Control

Professional Experience

2016 – present	St. Petersburg State University, Institute of Chemistry, Department of Analytical Chemistry; Associate Professor
2014 – 2016	St. Petersburg State University, Institute of Chemistry, Department of Analytical Chemistry; Assistant Professor
2008 – 2014	Institute of Toxicology of Federal Medico-Biological Agency; Research engineer

Education

Doctorate	Candidate of Science (PhD) in Analytical Chemistry from St. Petersburg State University

Professional membership	Member of Royal Society of Chemistry (since 2014) Member of German Society for Minerals and Trace Elements (GMS) (since 2016)

Main research collaborations

Helmholtz Zentrum Muenchen – Deutsches Forschungszentrum fuer Gesundheit und Umwelt, Neuherberg, Germany

Universita degli Studi di Modena e Reggio Emilia, Modena, Italy

RSCH Trace Element Laboratory, Royal Surrey County Hospital, Guildford, Surrey, UK

Lappeenrannan Teknillinen Yliopisto, Lappeenranta, Finland N.N. Petrov Research Institute of Oncology, St. Petersburg, Russian Federation

Curriculum Vitae Martina Maywald

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Date of Birth	18th May 1988, Kassel, Germany

Work Experience

06/2017 - today	Institute of Immunology, RWTH Aachen, Post-Doctoral Fellow
03/2013 - 06/2016	Institute of Immunology, RWTH Aachen, Ph.D. Student
03/2013 - 06/2015	Institute of Immunology, RWTH Aachen, Scientific Employee
10/2012 - 03/2013	Work and travel Australia
09/2011 - 09/2012	Institute of Immunology, RWTH Aachen, Scientific Research Assistant
12/2008 - 08/2010	South Westphalia University of Applied Sciences, Scientific Employee
09/2008 - 09/2010	South Westphalia University of Applied Sciences, Treasurer of the Student Council

Education

03/2013 - today	Institute of Immunology, RWTH Aachen Dr. rer. nat. Immunology (Ph.D. Immunology) (summa cum laude; with highest distinction) Ph.D. thesis: Induction of immunological tolerance by zinc administration
10/2010 - 09/2012	RWTH Aachen University, M.Sc. in Molecular and Applied Biotechnology (awarded 10.09.2012) (Grade: 1.4)
09/2007 - 09/2010	University of Applied Sciences South Westphalia, Campus Iserlohn, B.Sc. in Bio- and Nanotechnology (awarded 09.09.2010) (Grade: 1.7)

Qualifications and Achievements

- 2014 poster presentation: Zinc-Net Meeting; The COST action for zinc biology 1st scientific conference in London, Great Britain
- 2016 oral presentation: Zinc-Net/Zinc-UK Meeting in Belfast, Ireland
- 2017 poster presentation: ISZB Meeting UCLan Facility, Pyla, Cyprus, 1st poster price awarded

Scholarships

11/2015 - 01/2017	Mentoring program TANDEMdok RWTH Aachen
02/2016 - 02/2017	Online-scholarship e-fellows.net

DIRECTIONS

Aachen

By Train from Düsseldorf Airport (Düsseldorf Flughafen):

Take Regional Express direction Aachen Hbf (ride time approx. 1 ½ hours). This is a direct connection. Other connections require changing.

By Train from Cologne Airport (Köln/Bonn Flughafen):

Take Regional Express direction Minden (Westf) or Mönchengladbach respectively towards Cologne main station (Köln Hbf). S-Bahn S13 (direction Sindorf) is also possible.
At Cologne main station, take Regional Express direction Aachen Hbf (ride time approx. 1 hour).

For further information and train connections see: www.bahn.com, DB Navigator App.

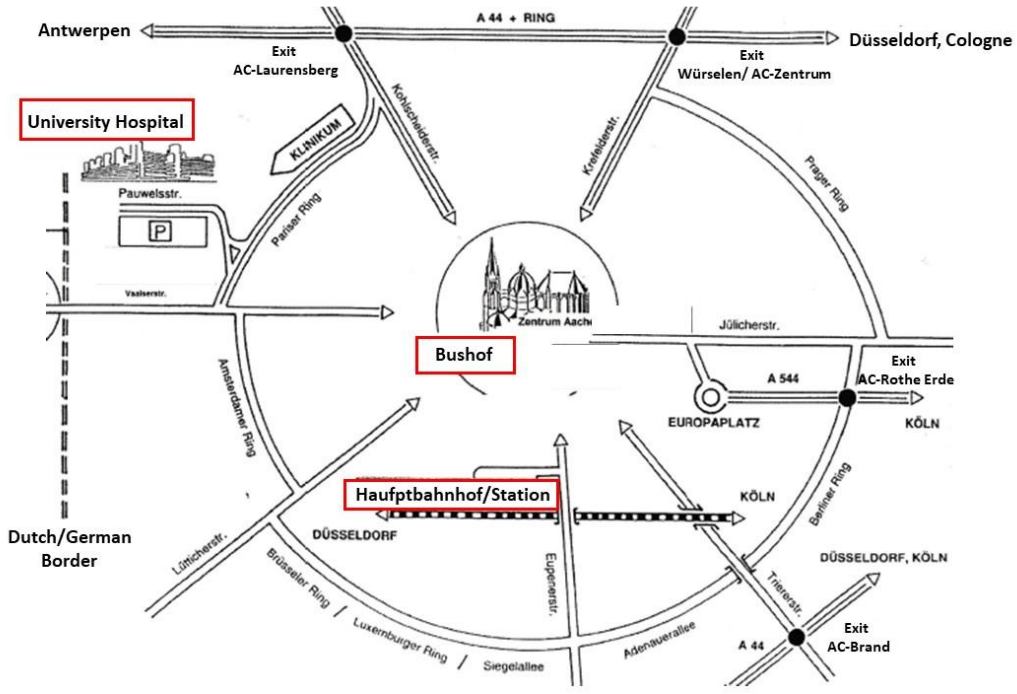
Address University Hospital RWTH Aachen

Universitätsklinikum Aachen, AöR
Pauwelsstraße 30
D-52074 Aachen, Germany

Directions

By car:

At Aachener Kreuz take the Holland-Linie (A4) towards Antwerpen/ Heerlen. You leave the motorway at the exit No. 2 Aachen-Laurensberg, turn right, then continue towards Maastricht and follow on a 4-lane feeder road the signs "Uniklinik". Parking (pay) is in front of the building.



By bus from Aachen main station:

On the opposite side of the street to the main station, you take at the bus stop H2 line **3B**. This will take you directly to the portal of RWTH Aachen University Hospital. Other connections require changing. Ask the driver for alternative connections.

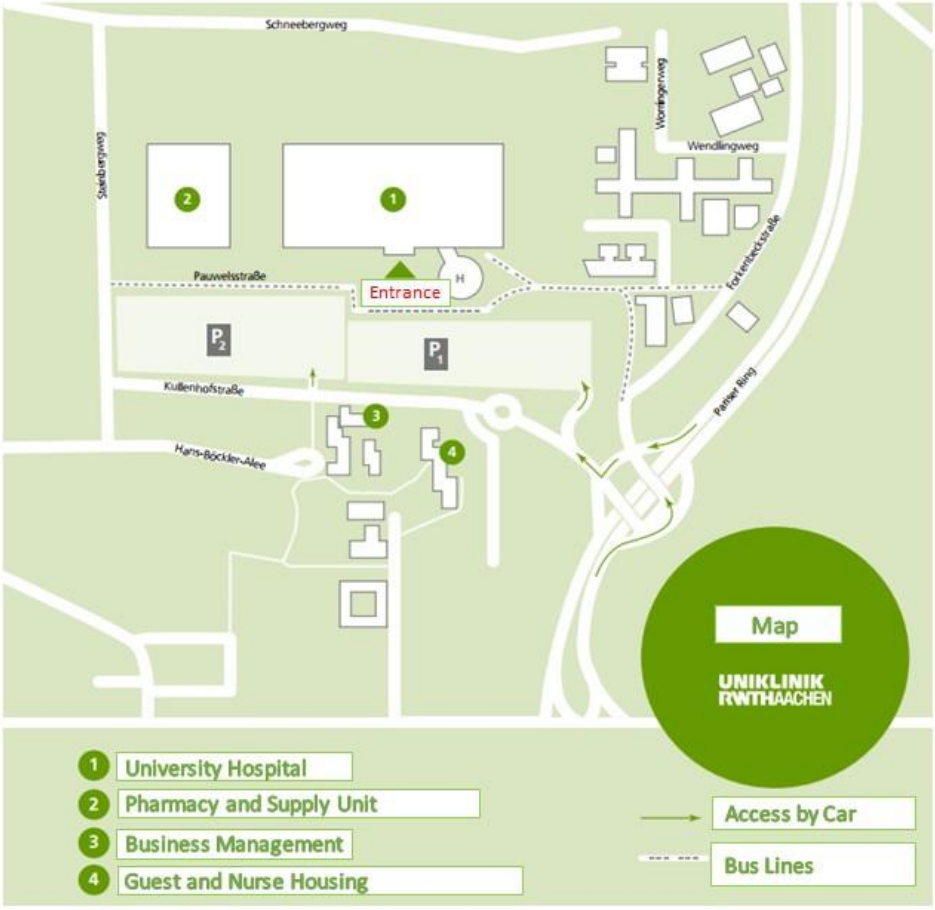
By bus from Aachen Bushof or Westbahnhof:

Either take bus connection line 33 (direction Vaals) or 73 (direction Uniklinik) at bus stop H14, which is located at Kurhausstrasse or bus connection line 5 (direction Uniklinik) at bus stop H11 (located at Peterstraße). Those will take you directly to the portal of RWTH Aachen University Hospital. Other connections require changing.

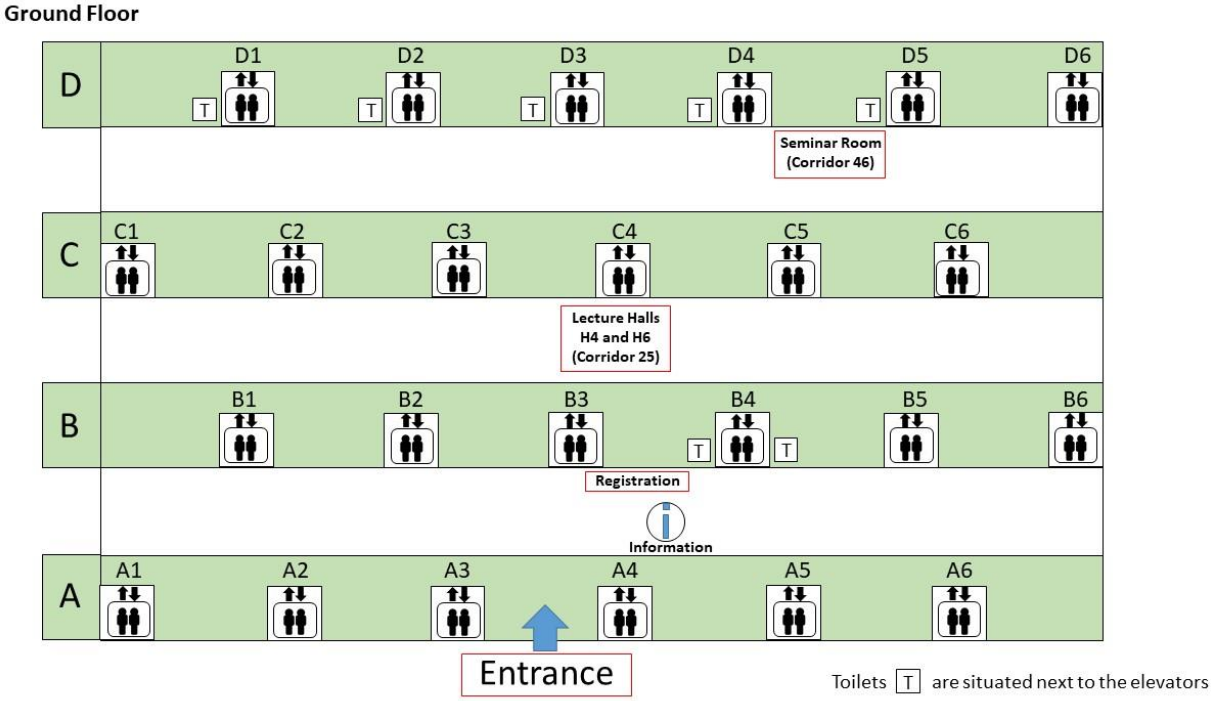
By bus from Hansemannplatz:

Either take the bus connection line **3A** (direction Ponttor/Uniklinik) at bus stop H3 or bus connection line **3B** (direction Kaiserplatz/Uniklinik) at bus stop H2. Both connection lines are circle-lines and will eventually reach university hospital. **3A** takes 12 min, **3B** 20 min. Alternatively, take bus connection line 5 (direction Uniklinik). All bus stops are located at Heinrichsallee.

For further information and bus connections: www.aseag.de, ASEAG mobile App.



Floor Plan University Hospital Aachen



Registration: Foyer (between elevator B3 and B4)

Workshops: Lecture Halls H4 and H6

Oral Presentations: Lecture Hall H4

Poster Presentations, Industrial Exhibition, Coffee Break, Lunch, Dinner: Seminar Room

NOTES