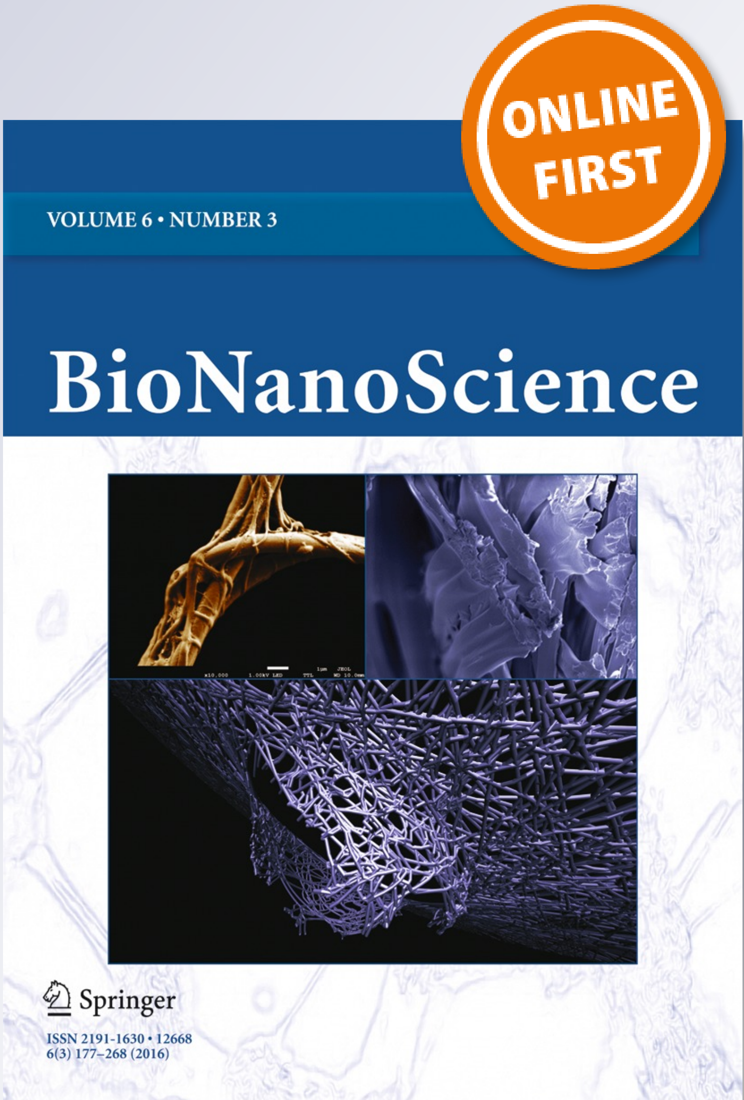
The Influence of Hindlimb Unloading on Bone and Muscle Tissues in Rat Model

Tatyana Baltina, Nafis Ahmetov, Oskar Sachenkov, Artur Fedyanin & Igor Lavrov

BioNanoScience

ISSN 2191-1630

BioNanoSci.
DOI 10.1007/s12668-016-0288-8



Springer

BioNanoSci.
DOI 10.1007/s12668-016-0288-8

The Influence of Hindlimb Unloading on Bone and Muscle Tissues in Rat Model

Tatyana Baltina [1] · Nafis Ahmetov [1] · Oskar Sachenkov [1] · Artur Fedyanin [1] · Igor Lavrov [1,2]

Abstract Numerous results indicate on interactions between muscles and bones. Thus, mechanical, genetic, endocrine, and age-related factors influence both tissues at the same time. Nevertheless, the details of physiological mechanisms of interaction between muscles and bones are still unclear. The purpose of this study was to evaluate the changes in low extremity muscles and bones during gravitational hindlimb unloading in rats. After hindlimb unloading during 7, 14, 21, and 30 days, muscles from the low extremities were collected and measured to estimate the muscle weight and perform cross-sectional analysis. Femoral bones were collected in order to evaluate weight, density, and geometrical parameters of the bone. Additionally, a test with a three-point bending was carried out to evaluate biomechanical bone properties. Results show that loss of muscle weight can be observed already at 1 week of hindlimb unloading with the maximum changes at 14 days. Changes in bone tissue showed the maximum loss of bone weight at 21 day. By 30 days of unloading, the density and rigidity of a bone were decreased; however, the most profound changes were observed in reduction of bone durability. These data support a hypothesis that the atrophy of skeletal muscles may promote the subsequent bone deterioration.

Keywords Hindlimb unloading · Muscle atrophy · Bone density · Rats

✉ Tatyana Baltina
tvbaltina@gmail.com

[1] Kazan Federal University, 18 Kremlevskaya Str., Kazan 420008, Russia

[2] Mayo Clinic, 200 First St. SW, Rochester, MN 55905, USA

1 Introduction

Studies performed in conditions of a microgravity or on models designed to minimize influence of gravity support the hypothesis that influence of gravitational load on a various parts of the musculoskeletal system can be a consequence of evolution of biomechanical structures [1]. It was reported that in conditions of microgravity, postural muscles have the greatest loss in weight [2–5]. It was also demonstrated that changes in muscular force can be observed without increase in muscle mass. Similar results were reported for bone tissue; such increase in bone weight does not necessarily lead to improvement in mechanical properties [6]. These results support the importance of future evaluation of the functional changes in muscles and bones in order to estimate the influence of the gravitational load. Numerous data indicate on the interactions between muscles and bones in normal condition and in pathological states; nevertheless, the physiological and pathological mechanisms of this interaction remain unclear and require future investigations [7, 8]. The goal of this study was to estimate the atrophic changes in low extremity muscles and changes in bone properties in the conditions of hindlimb unloading in rats.

2 Material and Methods

All tests were conducted on nonlinear laboratory rats (180–200 g). As a model of gravitational unloading, we used antiortostatic support model [9]. All experiments were performed according to bioethical standards and were approved by local ethical committee of the Kazan Federal University. Chloral hydrate was used for anesthesia (5 mg/kg, intraperetoneal, Sigma-Aldrich).

Published online: 03 October 2016

After influence of experimental conditions, low extremity muscles were dissected and evaluated. First weight of each muscles was determined, and then, the muscular samples were fixed with a fabric Tissue-Tek® glue (OCT™ Compound 4583) on a cardboard and immediately frozen with a liquid nitrogen. Serial cryostatic cuts (10 μm) were made from each muscular sample. The area of cross-section was measured for at least 100 fibers with an image analysis system QUANTIMET-500 (Leica, Germany) with a colored digital video camera of JVC TK-1280E. The femoral bones were dissected from all the tested rats with following weight measurement, density evaluation, and measurement of geometrical parameters. At the end, the stress tests with a three-point bending were performed. The three-point bending technique was described in details elsewhere [10]. During this test, the assessment of rigidity and durability of a diaphysis of a femur bone was performed. To estimate the Young's modulus, the differentiation of a linear site of each bone was performed. This differentiation was consisted in consecutive reconstruction of the linear regression based on a method of the smallest squares during changes at the right border of a linear site, up to reliable approximation of 0.99.

In this case, the equation for calculation of Young's modulus from the equation of the elastic line can be presented as:

$$E = \frac{kL^3}{192J}.$$

The critical stress and proportional limit were calculated based on equations:

$$[\sigma] = \frac{F_{\max}Ld}{16J},$$

$$\sigma_{lin} = \frac{F_{\text{int}}Ld}{16J},$$

where L—length of the sample, J—the moment of inertion of a cross-section of the sample, d—diameter of the sample, k—coefficient of linear regression, $F_{\max}$—force in the point B on the chart, and F_{int}—a point of intersection between the calculated linear regression and an experimental loading curve.

Two groups were investigated: control and "hypogravitational." The confidence intervals were calculated for each parameter with confidential probability of 95 %.

3 Results and Discussion

After 7 days of antiortostatic support, the mass of the soleus muscle (SOL) was decreased and was 67 ± 8 % ($p < 0.05$) of the control animals. After antiortostatic support for 14 days, the mass of SOL was 57 ± 5 % ($p < 0.05$) in comparison to the intact animals. The mass of the gastrocnemius muscle (GM) at 7 days after antiortostatic support was only slightly decreased compare to the control rats, 96 ± 3 % ($p < 0.05$). With increasing the time of positioning to 14 days, the weight to GM decreased to 68 ± 8 % ($p < 0.05$). The mass of the tibialis anterior muscle (TA) after 7 days of positioning was only slightly different from the control rats and was 101 ± 6 %. However, after 14 days of gravitational unloading, the mass of TA muscle decreased to 61 ± 5 % ($p < 0.05$) from the control. The area of cross-section of SOL after 7 days of unloading decreased to 48 ± 2 % ($p < 0.05$). The area of cross-section of GM and TA after 7 days of unloading changed less, 19 ± 5 % and 2 ± 3 % for GM and TA accordingly. However, after 14 days, it was consistent with 31 ± 6 % ($p < 0.05$) for SOL, 73 ± 5 % for GM, and 81 ± 6 % for TA ($p < 0.05$).

The average density of a femoral bone at 30 days of positioning in the control group was 1.43 ± 0.06 g/cm^3, and in experimental group—1.38 ± 0.10 g/cm^3. Based on the average values of the Jung's module, the reduction in bone rigidity at 35 % was observed after 30 days of antiortostatic support. The bone strength in the control group was 55.4 ± 12.22 MPas, and in experimental group—18.12 ± 2.64 MPas, that indicates the decrease in bone durability by 64 % ($p < 0.05$), which can increase the risk of low-energy fractures.

These data show that the loss of muscle mass can be observed already at 1 week after unloading with the maximum changes at 14 days. Changes in muscles proceed to the changes in bones where the maximum changes were observed at 21 day. These results may indicate that the muscular atrophy can promote the subsequent change in bone properties during unloading. These results support a dominant role of muscle over a bone in a homeostatic control over these two types of tissue [11] and the fact that the size of muscles can correlate with bone size and durability [12]. Other results also suggest that "myokines" can influence activity of bone cells [13], for example, suppressing apoptosis of osteocytes [14].

4 Conclusions

These results emphasize that the muscle atrophy precedes the change in a bone tissue after unloading and support our hypothesis that the muscle atrophy can promote the subsequent deterioration of a bone structure. This further emphasizes that rehabilitation strategies directed on preservation of muscle loss also help to support the bone structure during gravitational unloading.

Acknowledgments This work was supported by grant №16-04-00772 from the Russian Foundation for Basic Research.

Compliance with Ethical Standards All experiments were performed according to bioethical standards and were approved by local ethical committee of the Kazan Federal University.

References

1. Richardson, C. A. (2002). The health of the human skeletal system for weight bearing against gravity: the role of deloading the musculo-skeletal system in the development of musculoskeletal injury. *Journal of Gravitational Physiology, 9*(1), 7–10.
2. Edgerton, V. R., & Roy, R. R. (1994). Neuromuscular adaptation to actual and simulated weightlessness. *Advances in Space Biology and Medicine, 4*, 33–67.
3. Fitts, R. H., Riley, D. R., Widrick, J. J. (2001). Functional and structural adaptations of skeletal muscle to microgravity. *Journal of Experimental Biology, 204*(Pt 18), 3201–3208.
4. Kozlovskaya I., Dmitrieva I., Grigorieva L., Kirenskaya A., Kreidich Yu.V. Gravitational mechanisms in the motor system. Studies in real and simulated weightlessness // In: Stance and Motion. Facts and Concepts / Eds. Gurfinkel V.S., Ioffe M.Ye., Massion J. Springer Science Business Media New York 1988. P. 37-48.
5. Kuznetsov, M. V. Effect of vibrostimulation of foot and supporting afferentation on functional state of shin muscles in rats during hindlimb unloading/M. V. Kuznetsov, M. E. Baltin, A. O. Fedyanin, A. A. Eremeyev, and T. V. Baltina// Biophysics, 2014. V. 59, № 5. P. 806-809.
6. Shirazi-Fard, Y., Kupke, J. S., Bloomfield, S. A., Hogan, H. A. (2013). Discordant recovery of bone mass and mechanical properties during prolonged recovery from disuse. *Bone, 52*(1), 433–443.
7. Bonewald, L. F., Kiel, D. P., Clemens, T. L., Esser, K., Orwoll, E. S., O'Keefe, R. J., et al. (2013). Forum on bone and skeletal muscle interactions: summary of the proceedings of an ASBMR workshop. *Journal of Bone and Mineral Research, 28*(9), 1857–1865.
8. Lloyd, S. A., Lang, C. H., Zhang, Y., Paul, E. M., Laufenberg, L. J., Lewis, G. S., et al. (2014). Interdependence of muscle atrophy and bone loss induced by mechanical unloading. *Journal of Bone and Mineral Research, 29*(5), 1118–1130. doi:10.1002/jbmr.2113.
9. Morey-Holton, E.R. Hindlimb unloading rodent model: technical aspects [Text]/ E.R. Morey-Holton, R.K. Globus// J. Appl. Physiol. – 2002. - V. 92, №4. - P. 1367-1377.
10. Sachenkov O., Kharislamova L., Shamsutdinova N., Kirillova E. and Konoplev Yu. Evaluation of the bone tissue mechanical parameters after induced alimentary Cu-deficiency followed by supplementary injection of Cu nanoparticles in rats // IOP Conference Series: Materials Science and Engineering 98 (2015) 012015 doi:10.1088/1757-899X/98/1/012015
11. DiGirolamo, D. J., Kiel, D. P., Esser, K. A. (2013). Bone and skeletal muscle: neighbors with close ties. *J Bone Miner Res, 28*(7), 1509–1518.
12. Edwards, M., Gregson, C., Patel, H., Jameson, K., Harvey, N., Sayer, A. A., et al. (2013). Muscle size, strength and physical performance and their associations with bone structure in the Hertfordshire Cohort Study. *Journal of Bone and Mineral Research, 28*(11), 2295–2304.
13. Pedersen, B. K., & Febbraio, M. A. (2012). Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nature Reviews Endrocrinology, 8*(8), 457–465.
14. Jahn, K., Lara-Castillo, N., Brotto, L., Mo, C. L., Johnson, M. L., Brotto, M., et al. (2012). Skeletal muscle secreted factors prevent glucocorticoid-induced osteocyte apoptosis through activation of beta-catenin. *European Cells & Materials, 24*, 197–209.