DOI : 10.35124/bca.2019.19.2.3549
*Biochem. Cell. Arch.* Vol. 19, No. 2, pp. 3549-3552, 2019 | www.connectjournals.com/bca | *ISSN 0972-5075*

# EFFECT OF TRAINING ON FEMUR MINERAL DENSITY OF RATS

**Nikolay G. Belyaev[1], Igor V. Rzhepakovsky[1], Lyudmila D. Timchenko[1], David A. Areshidze[2], Alexander N. Simonov[3], Andrey A. Nagdalian[1], Igor A. Rodin[4], Matvey I. Rodin[4], Sergey N. Povetkin[1] and Marina E. Kopchekchi[5]**

[1]Institute of Life Systems, North Caucasus Federal University, Stavropol, Russia.
[2]Researching Laboratory of Experimental Biology and Biotechnology, Moscow State Regional University, Moscow, Russia.
[3]Department of Epizootology and Microbiology, Stavropol State Agrarian University, Stavropol, Russia.
[4]Department of Anatomy, Veterinary Obstetrics and Surgery, Kuban State Agrarian University, Krasnodar, Russia.
[5]Department of Morphology, Animal Pathology and Biology, Saratov State Agrarian University, Saratov, Russia.
e-mail: geniando@yandex.ru

**(Received 12 May 2019, Revised 11 July 2019, Accepted 30 July 2019)**

**ABSTRACT : Reducing the mineral density of bone tissue of athletes in carrying out training loads, bordering on the functional capabilities of the body at the first stages, is asymptomatic and difficult to diagnose. A comprehensive study of the possible mechanisms underlying the fall in bone mineral density in athletes is of great practical importance. Therefore, the study was conducted using rats with the 12 weeks of training. The training process was composed of five cycles: 1-4 weeks – preparatory cycle; 5-6 – transitional cycle; 7-8 – main cycle; 9 – active rest cycle, 10-12 – heavy loads cycle. On the 8th and 12th weeks of training the mineral density of this bone part was lower by 22% (P<0.001) and 26.6% (P<0.001), respectively as compared to control group. It is concluded that the decline in bone mineral density is the main reason for its mechanical strength reduction leading to injuries or stress fracture during the stages of prolonged intensive muscle loads.**

***Key words :*** Muscular load, parathyroid hormone, calcitonin, bone mineral density.

## INTRODUCTION

The desire to achieve high results often urges an athlete to execute a training load by utilizing maximum functional capacity. A rapid increase in muscle exercise is not an uncommon thing during the initial stage in amateur sports. Such training conditions, especially in combination with other stress factors, might lead to the adaptive mechanism failure. The damage often affects the cardiovascular system, the blood system and the musculoskeletal apparatus, in particular, the bone tissue *i.e.*, the organs and the organ systems that suffer from the biggest load while adapting to the specific sport activity.

The most common form of the bone tissue disorder among the athletes is the mineral density reduction, which in its turn leads to the mechanical strength failure, making them more vulnerable to injuries. The athlete's mineral bone density decrease in the early stages, usually being asymptomatic, hence difficult to diagnose. Therefore, a comprehensive study on the possible reduction of the bone tissue mineral density is of great practical importance.

Given that the main mineral component that provides the bone mechanical strength are the calcium salts, a comprehensive metabolism study of this very inorganic component in the body might prove informative.

## MATERIALS AND METHODS

The experimental animals in our study were four month old "Wistar" line rats. All the animals were handled in accordance with the EU Directive 86/609/EEC and the Russian law regulating experiments on animals. The research was conducted during 12 weeks of training. The training process was comosed of five cycles: 1-4 weeks – preparatory cycle; 5-6 – transitional cycle; 7-8 – main cycle; 9 – active rest cycle, 10-12 – heavy loads cycle. The experiment involved 120 animals, 10 rats in the experimental and control groups for each training cycle.

To model the muscle exercises, a treadmill was used. The intensity of the performed exercises in the experiment was regulated by varying speed and angle of the treadmill. In the initial training stages, the belt speed was set to 10 m/min with further speed and angle increase in the transition cycle the animals performed running at the speed of 20 m/min, and in the heavy load cycle of 25 m/min, in the active rest cycle the belt speed was decreased to 20 m/min, and the load duration to 6 min. In the intense load cycle, the initial running speed was set to 25 m/min with 15 min duration. The belt speed and the running were consequently increased on a daily basis – by 1 m/min and then by 5 min correspondingly. After 10 days of experiment the animals were able to perform running at a speed of 35 m/min for 65 minutes. This load was maintained in the further stages of the training.

To acquire the information about adaptive changes in the animals' body during the experiment, the value of maximum efficiency was determined – running up to the obvious fatigue signs onset (running to failure). Indicator of failure was determined as the inability of animals to run against the moving belt, despite the electrical stimulation. As a measure of training level, we've also used the data for detection of changes in the blood system, data on the heart mass registration, testosterone concentration and 11-oxycorticosteroids (11-OCS) and testosterone.

The concentration of total calcium (CA), calcitonin (CT) and parathyroid hormone (PTH) in blood at different stages of the training cycle were estimated. The peripheral blood state was assessed using an automatic hematological analyzer "Medonic-M20C" (Boule Medical AB, Sweden). The percentage of CA was determined by titration, the testosterone level (11-OCS) CT and PTH by ELISA using enzyme immunoassay analyzer "Uniplan AIFR-01" (Russia) and with corresponding kits for laboratory rats diagnosis "Rat Elisa Assay Kit". The bone mineral density assessment was performed using x-ray microtomograpgh SkyScan 1176 (Bruker-microCT, Belgium) in accordance with the official manufacturer recommendations. Polymer disks (0.25 g/cm$^3$ and 0.75 g/cm$^3$ hydroxyapatite density) were used as reference standards to determine the relative densities and were scanned with the same parameters as the rats' femurs (Areshidze *et al*, 2017a).

The femoral bones of white rats have been scanned *ex vivo* and remodeled with a computer program Nrecon 1.7.4.2, Bruker-microCT, Belgium. Data analysis, visualization, structure determination, mineral density of cortical and trabecular areas of the femurs were conducted using the software CTAn 1.18.14.0, Bruker-microCT, Belgium (Areshidze *et al*, 2017a; Bouxsein *et al*, 2010). The use of x-ray provided the internal microstructure of three-dimensional visualization of the investigated object. Student's t-test was used to analyze the data.

## RESULTS AND DISCUSSION

During the systematic execution of the running loads, the efficiency of animals increased significantly. Rats were able to perform running on a treadmill for a 3.1±0.20 hours at the end of the training cycle, the maximum running time increased by 71.3 per cent and amounted to 5.27±0.42 hours.

The ability of rats for the prolonged intensive muscular exercises provided by several morphological and functional changes in oxygen-transport systems. It occurs due to increase in the number of erythrocytes and hemoglobin level. A slight increase in the level of hemoglobin and number of erythrocytes was registered after 4 weeks but statistically significant increase was registered after 9 weeks of training. By the end of the experiment, the concentration of red blood cells increased up to 6.81±0.22×10$^{12}$/l from 5.23±0,32×10$^{12}$/l. The hemoglobin level increased from 158,4±2.1 g/l to 169,3±2.7 g/l. Increasing the number of erythrocytes and hemoglobin under the influence of 12 weeks of training was highly significant ($P<0.001$). In the process of systematic running exercises, a pronounced heart's hypertrophy was noticed. Thus, at beginning of the experiment the mass of the heart was 240±2.1 mg/100 g, and it 359±3.0 mg/100 g by the end of the experiment. Rats in the control group have shown no significant changes in the concentration of erythrocytes, hemoglobin level and weight over the same time period. Thus, the process of the body's adaptation to muscular loads is accompanied by a number of deep morphological and functional changes in oxygen-transport systems.

Significant changes were observed in the hormonal status of the experimental group animals. Thus increasing the concentration of 11-OCS in the process of training cycles showed the growth of functional capabilities of the entire hypothalamic-pituitary-cortical system. The testosterone level dynamics was cyclic: showing decrease during training loads intensification and reliable decrease during active rest cycle. As of today there is no reliable data, that can confirm that training leads to the testosterone and its free fraction level increase in blood. At the same time, we register a high concentration of testosterone 28,7±0.85 nmol/l in the period recreation and 12, 4±0, 73 nmol/l at the beginning of the experiment, which indicated increasing secretory capabilities of the gonads and adrenal glands. A range of adaptive changes in the hormonal status and vegetative systems in the animals' body enabled high efficiency.

Calcium was considered as a versatile agent for physiological processes (Mironova *et al*, 1982). There was no reliable Ca concentration changes at the initial stages (Fig. 2). At the same time, during the training load intensification (6-7-8 weeks), we have found the

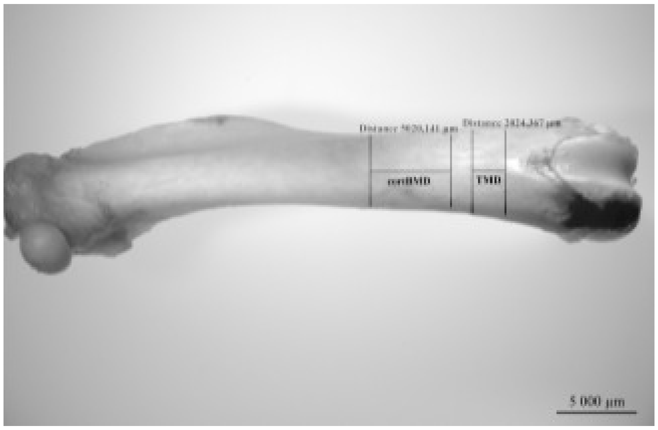


**Fig. 1 :** Areas of the femur that were used to analyze mineral density (A – cortical part; B – trabecular part).

hypercalcinemia interchanges with normalization together with increase after the 12 weeks of the training. The mechanisms for such dynamics of the studied inorganic blood components are not clear. On the one hand, during the intensive exercise cycle, the concentration of PTH was low. While hypercalcemia has not stimulated any thyroid gland C-cells secretory activity. At this stage of training there was a significant decrease in calcitonin concentration (Fig. 2). Accordingly, it is possible to assume that during adaptation to intensive muscular load, some mechanisms for the long-hypercalcemia without increased secretory activity of the parathyroid glands operate.

The decisive factor, ensuring the long-lasting hypercalcemia apparently is the change in acid-base balance. Acidosis is a side effect in terms of submaximal muscle load and high power. The increased acidity boosts the dissolution of the bone matrix and, as a result, increasing the level of CA in liquid media. We cannot exclude the one way effect of reducing testosterone and CT on the Ca metabolism in the body. With its powerful anabolic effect, testosterone is involved in the formation of the inorganic component of the bone tissue. The evidence for this is the male sex hormone gipocalziemicescoe effect, manifested and mainly mediated through increase of the thyroid C-cells secretory activity (Belyaev, 2012; Belyaev and Bolotova, 2012). Reducing the testosterone and the CT concentration, the increased acidity of the body fluids in total contributed to hypercalcemia during the training loads intensification.

The question arises, which parts of the bone provide a rapid increase in total calcium concentration in liquid medium of an organism. In this research, we carried out the evaluation of mineral density of the diaphysis and epiphysis. The diaphysis is the cortical part of the bone, thick and compact the "old bone". While the epiphysis is the bigger part of the trabeculae or cancellous part of the

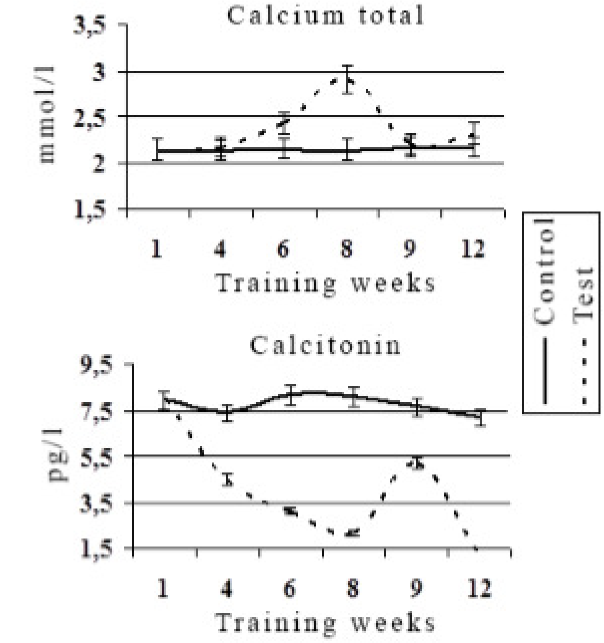


**Fig. 2 :** Total calcium concentrations dynamics (mmol/l) and calcitonin (pg/ml).

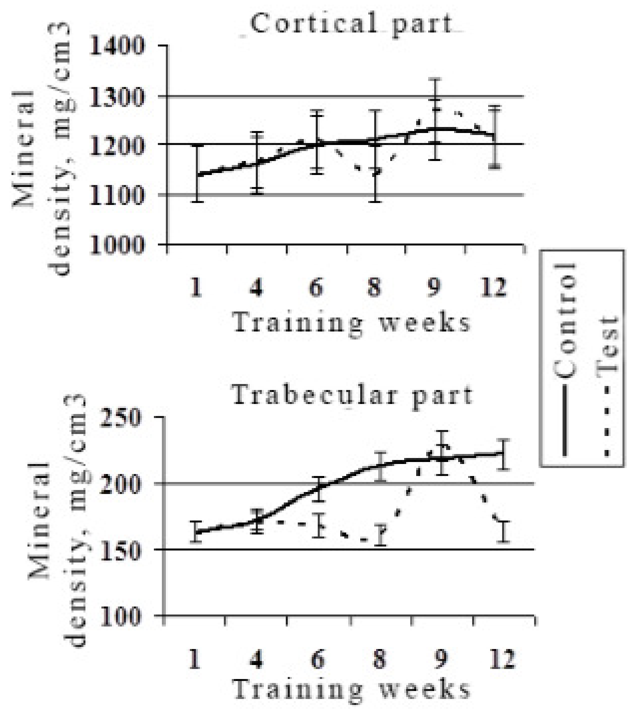


**Fig. 3 :** Bone's cortical and trabecular parts mineral density dynamics (mg/cm$^3$).

bone. The main inorganic component of bone are the hydroxyapatites, which are formed by calcium and phosphates. In the bones, along with calcium and phosphorus, there are various of amounts sodium, magnesium, potassium, chlorine, fluorine, carbonate, and citrate. In total, the trabecular part of the inorganic matrix ammounts to 60-65% of bone mass, and the cortical about 70%. Salt of Ca phosphate in the bone tissue are presented in the 2 different forms: the easily exchanged pool and the part of the salts in the "old bones" in the form of hydroxyapatite crystals, which is relatively stable and is difficult to extract from the bone tissue. At the same time to dissolve the bone matrix of the cortical part and to release Ca, PTH is necessary. As on the 6th, 7th, 8th and 12th weeks of training a parathyroid glands' low secretory activity was registered. The computer microtomography revealed the animals' femur mineral density. In the control group throughout the experiment a gradual increase in mineral density was registered, which displayed the age-related changes in the inorganic component of bone composition. The rats' bone mineral density increased during the first year of life and decreased further as the animal ages.

Adequate physical activity is a determinant of the bone mechanical strength. But positive adaptive changes were recorded at 6-8 weeks of training. In the control group, the bone mineral density increased at the 9 weeks of the experiment and it was equal to 7% ($P<0.05$) for the cortical portion of the bone and 33.7% ($P<0.001$) for the trabecular bone in the experimental group. However, over the same time period, the bone density increased by 11.4% ($P<0.01$) and 39.8% ($P<0.001$), respectively. Intergroup differences were not significant. They were characterized by a tendency to a greater bone mineral density increases, but affected by muscular activity. In periods of increasing volume and intensity of running workloads (8th, 12th weeks of the experiment), a decrease in mineral density in the cortical and in trabecular bones parts was showed, with the most statistically significant reduction in trabecular part (Fig. 3). On the 8th and 12th weeks of training, the mineral density of this bone part was lower by 22% ($P<0.001$) and 26.6% ($P<0.001$), respectively, compared with the data determined in the control group.

## CONCLUSION

In the context of adaptation to intensive muscular loads, calcium concentration fluctuations in the blood, especially hypercalcemia was associated with easily exchanged pool, located in the trabecular part of the bone. This assumption was based in the fact that the trabecular part of the bone was metabolically more active and the body's mineral substance needs were fulfilled by this part's inorganic components. The next important thing in the metabolism of Ca was the possibility to extract it from trabeculae without the PTH. This process was activated when the blood reaction moved to the acid side. At the same time, the bone mineral density decrease was the main reason for its mechanical strength reduction which showed a high susceptibility to injuries – stress fracture during the stages of prolonged intensive muscle loads. This type of injury frequency varied from 7% to 15% of all types of sports injuries.

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