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Serum Osteocalcin Concentration in Treadmill-Trained Adult Male Wistar Rats

by
Alicja Nowak[1], Maria Pogrzebna[2], Jan Celichowski[2], Łucja Pilaczyńska-Szcześniak[1]

Mechanical stress is considered to be essential for the regulation of bone mass. The purpose of this study was to determine whether treadmill exercise at moderate intensity induces alterations in blood osteocalcin concentration in rats. Male Wistar rats, aged 5 months, were divided randomly into two groups: trained animals (n = 6) and controls (n = 7). Trained rats were exercised 5 days/week for 4 weeks on a motor-driven treadmill. Each exercise session lasted 60 minutes and the average locomotion speed was 16.2 m/min. After completion of the training period, a blood sample was taken for osteocalcin measurement and the hindlimbs medial gastrocnemius muscles were excised and weighed. Comparative analysis showed significantly lower circulating osteocalcin levels in the exercised rats in comparison to control animals. It is possible that the observed decreased blood osteocalcin concentration is transient in nature. Factors including stress may also influenced the results.
Serum osteocalcin concentration in treadmill-trained rats
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[1] - *Department of Hygiene, University School of Physical Education in Poznań*
[2] - *Department of Neurobiology, University School of Physical Education in Poznań*

Mechanical stress is considered to be essential for the regulation of bone mass. The type of exercise practiced is important in skeletal adaptation. The beneficial effects of weight-bearing exercise on the skeleton have been demonstrated in animals and humans (Brahm et al. 1997, Holy, Zérath 2000, MacDougall et al. 1992, Newhall et al. 1991, Wheeler et al. 1995). On the other hand, the absence or reduction of mechanical stress escalates bone degradation (Fries 1996). Frost (1992) suggested that a minimum effective strain is necessary to stimulate proper bone remodeling. Animal studies have shown that relatively few daily strain cycles are necessary to promote or maintain the balance in bone turnover (Rubin, Lanyon 1983). However, the physical activity threshold for promoting bone formation or maintenance is unknown.

Some reports have suggested that the rat model may mimic the skeletal status of humans (Frost, Jee 1992). Therefore, treadmill exercises have been used to test the effects of physical activity on bone metabolism in rats. In many animal studies, treadmill training at moderate or high speeds produced an increase in bone mineral density (Holy, Zérath 2000, Warner et al. 2006, Wheeler et al. 1995, Yeh et al. 1993), raised the histological bone volume (Holy, Zérath 2000, Sogaard et al. 1994, Yeh et al. 1993) and improved mechanical properties of bone at different skeletal sites (Fujie et al. 2004, Mosekilde et al. 1994, Wheeler et al. 1995). Tissue analysis documented an increase in bone formation levels and a decrease in bone resorption after treadmill exercise (Yeh et al. 1993). Although repetitive loading is believed to be beneficial in stimulating bone modeling and remodeling, the distance covered and speed of locomotion are also important factors in skeletal adaptation. It is known that an excessive loading regime related to very high exercise intensity or long duration may cause an imbalance in the remodeling process and can lead to the accumulation of fatigue microdamage (Verborgt et al. 2000).

As mentioned above, several studies have demonstrated the positive influence of locomotion on bone metabolism. On the other hand, Singh et al. (2002) have suggested that treadmill running used in rat studies is a low impact exercise and may not be an effective training mode to investigate bone hypertrophy. However, physical activity influences the whole body and induces metabolic processes, and running appears to have been effective in investigations of plasticity of motor unit contractile properties in hindlimb skeletal muscles (Pogrzebna, Celichowski 2005, Seburn, Gardiner 1995).

Thus, there are conflicting opinions concerning the response of bone tissue to treadmill training in rats and there are only a few reports describing the effects of this type of exercise on biochemical indices of bone turnover (Goseki et al. 1995, Holy, Zérath 2000, Iwamoto et al. 2004, Li et al. 1991). The purpose of

the present study was to determine whether treadmill exercise at moderate intensity can induce any alterations in the bone formation rate expressed by osteocalcin blood concentration in rats.

Methods

Male Wistar rats, aged 5 months, housed in cages in a 20°C room with a reversed 12 h light/dark cycle (12 h dark period in the day, 12 h light period at night) were divided randomly into two groups: exercised animals (n = 6) and controls (n = 7). All animals were allowed *ad libitum* access to food and water. Trained rats were exercised 5 days/week for 4 weeks on a motor-driven treadmill. Each exercise session lasted 60 minutes and the average locomotion speed was 16.2 m/min (Pogrzebna, Celichowski 2005). Due to the nocturnal activity of rats, they were trained under weak lighting during the daytime. After 4 weeks of training the animals of both groups were anesthetized (sodium pentobarbital 60 mg/kg). A blood sample of approximately 8 ml was taken in 24 h after the last exercise, directly from the heart and the medial gastrocnemius muscle of the hindlimb was excised and weighed. Blood samples were separated by centrifugation at 5000 rpm at 4ºC, then serum was collected and stored at -70ºC. The concentration of osteocalcin in the blood serum was determined by radioimmunological assay using a Rat Osteocalcin IRMA kit (Immunotopics Inc., USA).

For statistical comparison of somatic parameters and osteocalcin concentrations between groups of animals, the Mann-Whitney U test was used. $P < 0.05$ was considered statistically significant.

Results

The body mass and the mass of the medial gastrocnemius muscles for the exercised and control rats are presented in Table 1. There were no significant differences in these parameters between the two groups of animals.

Osteocalcin concentrations in the blood serum are presented in Figure 1. Comparative analysis showed significantly lower circulating osteocalcin levels in the exercised rats compared to control animals ($P = 0.035$).

Table 1

Mean values (± SD) of total body and medial gastrocnemius muscle mass for the trained and control rats

	Trained group (n = 6) $\bar{x}$ ± SD	Control group (n = 7) $\bar{x}$ ± SD	P
Total body mass (g)	480.0 ± 59.58	530.0 ± 59.67	0.1797
Medial gastrocnemius muscle mass (mg)	1207.8 ± 225.93	1125.0 ± 28.28	0.4286

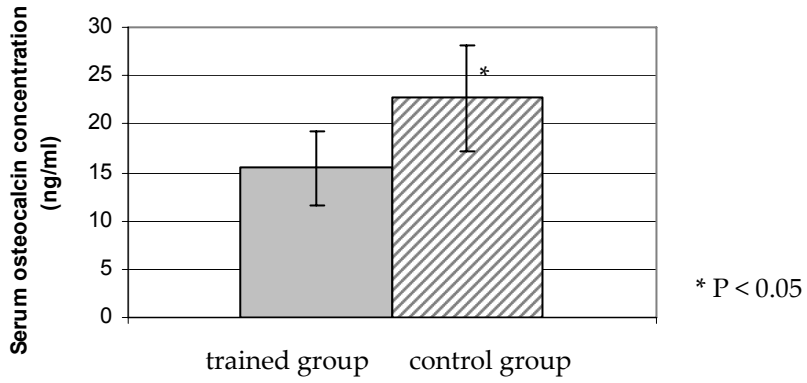

Fig. 1

Mean values (± SD) of serum osteocalcin concentration in the trained and control rats. The comparative analysis showed a significant difference in circulating osteocalcin levels between two groups of animals.

Discussion

Bone is a dynamic tissue that is continuously undergoing both processes formation and resorption throughout life. Physical activity is one of the factors influencing this bone remodeling. The positive effects of exercise are to increase bone formation and reduce bone resorption. In the present study we found that, unexpectedly, the level of osteocalcin, a bone formation marker, decreased in trained animals in comparison to control subjects.

Osteocalcin is a noncollagenous matrix protein of bone, comprising 1-2% of total bone proteins, produced mainly by osteoblasts and odontoblasts (Eriksen

et al. 1995). It inhibits precipitation of hydroxyapatite and may also be involved in the regulation of bone resorption because it chemotactically attracts osteoclast precursors. Osteocalcin is released into the circulation following *de novo* synthesis of bone. Therefore, it reflects the activity of the mineralization phase of the newly formed bone matrix. As this marker was apparently decreased in treadmill-trained rats, it may be concluded that the applied pattern of systematic exercise slowed the bone mineralization process.

Exercise studies using human and animal models have yielded inconclusive or conflicting results regarding the influence of exercise on osteocalcin production. In studies on human osteoblast cultures, Kaspar et al. (2000) showed that cyclic strain applied over two days at physiological doses (30 min. per day, frequency 1 Hz, strain magnitude 1000 με strain) led to an increase in proliferation and early osteoblast activities related to matrix production, which was reflected by elevated levels of collagen type I carboxyterminal propeptide. However, the activity of alkaline phosphatase and concentration of osteocalcin were decreased. These authors concluded that reinforcement of the proliferation phase leads to the increased matrix production that is necessary to functionally adapt bone to mechanical stress, but matrix mineralization is reduced by mechanical loading and does not recommence until the strain magnitude is decreased. On the other hand, in a study using young rats (4-week-old), Holy and Zérath (2000) observed accelerated bone formation, expressed as an increase in bone mass and raised activity of alkaline phosphatase – a marker of matrix maturation – without simultaneous changes in the level of osteocalcin, after a period of systematic voluntary spontaneous running on a rotating wheel (speed range 19.6 to 40.8 m/min., running time 3.5 to 5.5 h/d., 7 days/week, 4 weeks). Iwamoto et al. (2004) demonstrated that treadmill exercise (25 m/min., 1 h/d., 5 days/week, 11 weeks) stimulated an increase in circulating osteocalcin concentration and suppressed bone resorption, resulting in increased bone mass and longitudinal bone growth in young (6-week-old) Wistar rats.

The interpretation of *in vivo* results is difficult because many factors are involved in the adaptation of bone to exercise, including age and experimental conditions such as activity duration, intensity and type of training. Moreover, bone adaptation to mechanical stimuli is site specific and the site of bone response may reflect the local strain environment that varies according to the type and intensity of the exercise (Bourrin et al. 1994). Therefore, several factors should be taken into account in the analysis of the results of the present study:

1/ Physical activity influences bone by inducing mechanical loads (bone deformation) which can occur as a result of muscle contraction or gravitational force. The body weight, which influences bone tissue directly, by the magnitude

of the applied mechanical load, is an important factor influencing the skeletal system (May et al. 1994). Frost (1997) has suggested that the greatest loads on bones are the result of muscle activity. Muscle stretching results in strain on bone tissue and generates streaming potentials which stimulate bone formation. Studies on humans and animals have revealed the relationship between strength and muscle mass as well as bone mass (Frost, Schönau 2000, Ferretti et al. 2001, Schönau et al. 1996). In the present study no significant differences in body or medial gastrocnemius muscle mass were found between systematically trained and control animals. The observed differences in serum osteocalcin concentration in these two groups of rats were not therefore due to changes in these somatic factors.

2/ The effect of exercise may depend on the stage of growth and development when training is undertaken. In mammals, bone modeling is less active in adults than in younger individuals (Jee et al. 1991). The rats used in our experiment were young adults (5-month-old) so their bones may be less responsive to mechanical loading than those of younger animals. Moreover, in adults, the pattern of bone mineralization following exercise is also related to age. McDonald et al. (1986) showed that older rats (19-month-old) underwent total skeletal mineralization in response to treadmill exercise compared to the more local adaptation seen in younger, 7-month-old animals.

3/ It is unlikely that the decreased circulating concentration of a bone formation marker found in the present study was an adverse effect of the training regime. Li et al. (1991) reported that exercise at an intensity exceeding 80% of aerobic capacity had a negative effect on bone remodeling and increased bone resorption. However, in the present study the training intensity was rather low because the locomotion speed did not exceed 16.2 m/min, whereas the maximum locomotion speed for rats can exceed 75 m/min. (Cohen, Gans 1975). The average distance covered by the rats in our study was 5 km/wk and corresponds to a low range of performance for these animals. Several investigators have shown that rats submitted to daily spontaneous running activity using wheel devices can cover 5-15 km per day (Holy, Zérath 2000, Tokuyama et al. 1982). Therefore, we conclude that the duration and intensity of exercise used in our experiment were low and could not have induced adverse effects on bone formation.

4/ Bone turnover markers are sensitive to mechanical as well metabolic factors. Physical training in rats by treadmill running usually follows precisely standardized protocols of duration and intensity. Holy and Zérath (2000) concluded that rat biorhythms may be disrupted by this kind of exercise, which

could induce adverse, stress-related effects. Therefore in the present study, the decreased osteocalcin level in active animals may be an effect of stress.

5/ It is possible that the decreased levels of circulating osteocalcin seen in trained rats in the present study is an effect of skeletal adaptation and a transient response to the physical exercise. Studies conducted on horses have shown that bone turnover may vary during the course of training. Untrained Quarter Horses placed in race-training showed a transient decrease, followed by an increase, in serum osteocalcin levels (Nielsen et al. 1998). The same bone turnover reaction was found in young Thoroughbred horses after 4 weeks of race-training (Carstanjen et al. 2005).

6/ The level of physical activity before the start of training may be an important factor influencing bone adaptation during exercise. Pedersen et al. (1995) demonstrated that blood osteocalcin levels were significantly increased following five days bed rest in humans. They concluded that this elevation in osteocalcin may be caused by an increase in osteoblast activity or the release of bone-incorporated osteocalcin from resorbing sites. In the present study, the physical activity of untrained caged rats probably has a low impact on the skeleton and is unlikely to be sufficient to maintain a balanced bone metabolism. Therefore, the high osteocalcin concentrations found in control animals may be a result of increased bone degradation compared to the exercised rats. However, in the present study we did not measure bone resorption.

In conclusion, we found decreased serum osteocalcin concentrations in rats after 4 weeks of treadmill exercise in comparison to control animals. This result might be an effect of the low sensitivity of the animal's skeleton to moderate intensity physical effort of a few weeks duration. Other factors such as stress have probably influenced the results. It is also possible that the observed osteocalcin decrease is transient in nature.

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Corresponding author:

dr hab. Alicja Nowak
Chair of Physiology, Biochemistry and Hygiene, Department of Hygiene
University School of Physical Education
Królowej Jadwigi str. 27/39, 61-871 Poznań
E-mail: anowak@awf.poznan.pl
Phone: +4861 8355170,
Fax: +4861 8330087

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