

EFFECTS OF HIGH-IMPACT EXERCISE TRAINING ON BONE MECHANICAL PROPERTIES – AN EXPERIMENTAL STUDY IN FEMALE WISTAR RATS



Fernando Fonseca de Almeida e Val¹
Rodrigo Okubo¹
Maurício José Falcai¹
Fábio Senishi Asano¹
Antônio Carlos Shimano¹

1. Medicine School of Ribeirão Preto, University of São Paulo – Ribeirão Preto, SP, Brazil.

Mailing address:

Laboratório de Bioengenharia do Departamento de Biomecânica, Medicina e Reabilitação do Aparelho Locomotor da Faculdade de Medicina de Ribeirão Preto – Universidade de São Paulo.
Av. Bandeirantes, 3.900, Monte Alegre - 14049-900 – Ribeirão Preto, SP, Brasil.
ffaval@gmail.com

ABSTRACT

Introduction and objectives: Physical activity has well-established benefits on bone mechanical properties and is a non-pharmacological treatment strategy against bone weakening processes. Training regimens originate loading stress to bone and promote adaptive responses which enhance bone health. Jump exercises generate high-impact loading on bone structure with positive adaptations. The goal of this study was to investigate the effect of high-impact training regimens with different number of weekly sessions for different time periods in bone mechanical properties of female Wistar rats. **Methods:** fifty-four female Wistar rats, mean age 10 weeks, were randomly allocated in six groups (n=9 each): CGI (four-week sedentary group, control), TGI3 (trained three times per week, four weeks), TGI5 (trained five times per week, four weeks), CGII (eight-week sedentary group, control), TGII3 (trained three times per week, eight weeks) and TGII5 (trained five times per week, eight weeks). The high-impact protocol consisted on the completion of ten 40cm-vertical jumps per session. **Results:** Bone mechanical properties in the groups that underwent higher weekly frequency regimens for longer periods had greater gains in maximum strength and stiffness parameters when compared with the animals that trained less. **Conclusions:** the present results indicate that performance of high-impact training protocol has beneficial effects on bone mechanical properties, even with a low weekly frequency, suggesting hence, that for bone gain, daily work volume is not necessary. However, for greater result, daily exercise does have better outcome.

Keywords: physical activity, mechanical testing, bone tissue.

Received on 3/15/2012, and approved on 11/6/2012.

INTRODUCTION

Throughout life the human musculoskeletal complex (MSC) is subject to multifarious mechanical demands. Physical activities, fractures and immobilization processes are stimuli that cause qualitative and quantitative adaptation responses. Since the MSC is crucial for human movement, protection and anchorage of body structures, Shimano et al., in 2002¹, stated that studies involving this complex are necessary to better understand its complexity. The diversity of processes to which the MSC is submitted varies with physical and mechanical composition of these stimuli. Type of loading to which MSC is submitted (compression, straining, twisting), frequency of application, level and magnitude, exposure period and stimuli imposition/removal velocity, among others, are components of these stimuli. Hence, MSC adaptations are consequence of the kind of mechanical imposition.

These stimuli may come from a multitude of sources and result in different structural adaptations. For instance, those derived from physical activity result in positive dimensional alterations in width, diameter, perimeter, transversal section, bone volume and weight. Concerning bone tissue, Eastell, in 2003², observed that cortical and trabecular regions of bones of trained individuals presented elevated thickness and resistance to external demands. In contrast, absence of stimuli to MSC derived from paralysis, immobilizations and absence of weight bearing, produced negative alterations to bone. These alterations include loss of biomechanical, structural and mineral properties with bone mass loss as consequence^{3,5}.

Natural processes, as aging, or even pathologic processes may cause weakening of bone tissue. Osteoporosis and its consequences, generate loss in MSC integrity. Once these processes have important implications in health, it becomes a public health problem, producing elevated cost to governments. Therefore, efficient treatment strategies are necessary to be developed to counter this fact⁶.

Physical activity has beneficial effects on bone. Maintenance of bone mineral density (BMD), or even its increase, are observed effects due to training regimens. Bone overload derived from physical activity in training regimens becomes and attempt to stop degenerative processes of bone tissue⁷.

In experimental models, performance of physical activity demonstrated increase in bone health during training periods. Although maintenance of results occurred, it happened only for a limited time after cessation of training stimuli⁸.

Swimming (minimum impact generation) and running (medium impact generation) have beneficial effects on biomechanical, mineral and structural properties of bones. Animals submitted to exercise protocols with greater weight sustaining, such as running, evidence greater BMD in specific carrying sites when compared to those submitted to a lesser weight sustaining protocol⁹. Greater benefits are seen in bone tissue submitted to high-impact exercise modalities. It is believed that due to higher mechanical stress, with elevated tissue deformation and dynamism and also having short-duration and being a high-intensity stimulus, high-impact modalities deserve special attention¹⁰. This modality presents two high generating tension phases, an initial acceleration component

triggered by vigorous muscle concentric contraction, and a final deceleration phase, where impact absorption takes place. Both generate elevated energy loads to bone tissue¹¹.

However, it's not well established in the literature, which would be a minimum high-impact training regimen for significant bone gain. Therefore, the main objective of this study was to investigate the effect of different high-impact training regimens on bone mechanical properties and verify which regimen would be the most efficient on promoting positive osteogenic effects.

METHODS

Animals

Fifty-four female Wistar rats (*Rattus norvegicus albinus*), mean age 10 weeks were kept in collective cages at controlled temperature (25±2°C) and received water and food ad libitum. All animals were donated by the Central Animal Facility of the Ribeirão Preto Campus - University of São Paulo (USP-RP). They were allocated in the animal facility of the Bioengineering Laboratory – Medicine School of Ribeirão Preto (FMRP/USP-RP). This project was approved by the Animal Experimentation Ethics Committee (CETEA/FMRP/USP-RP).

Experimental groups

All animals were randomly allocated in six experimental groups, divided as follows: control groups (CGI and CGII), animals that trained for four weeks (TGI3 and TGI5) and animals that trained for eight weeks (TGI3 and TGI5). Total training period, four or eight weeks, characterized the training duration. The control groups remained sedentary for a period of four weeks (CGI) or eight weeks (CGII); the exercise groups that trained during four weeks were divided either in groups that trained three (TGI3) or five times per week (TGI5); animals that trained during eight weeks were divided similarly, in groups with three (TGI3) or five weekly training sessions (TGI5). All groups were composed by nine animals (n=9), with a total of 54 animals in the experiment.

High-impact training technique

The high-impact training technique consisted of each animal successfully jumping from the bottom of a 40cm deep wooden box to its top edge. The front limbs were used to grip to the top of the box after the jump. In order to initiate each jump, the animals were electrically stimulated. After having completed the each jump, the animals were manually positioned at the bottom of the box for the next jump.

Experimental protocol

The training protocol consisted in the completion of ten jumps per session. The weekly training frequency characterized the training volume of each group. Three seconds was the time between

jumps, and a period of no longer than one minute was necessary for the completion of one session. The electrical stimulus was only necessary for the first training sessions. After this short period, the animals no longer needed the electrical stimulus to execute the jump. At training protocol completion, each animal was euthanized with an excessive chloral hydrate dose applied to the ventral region. Left femurs were dissected, identified and stored in saline. Dissection only began after ten minutes post excessive chloral hydrate application and confirmation of animal death.

Sample preparation and mechanical assays

In order to analyze mechanical properties of femoral neck, we performed flex-compression assays through axial compression of the femoral head of left femurs. Femur distal extremity was fixed in a methyl-methacrylate sphere to better accommodate the body-test in the assay machine. A plumbline was used to assure verticality. The body-test set was then attached to a bench vise on the base of the assay machine. Flex-compression assays were performed in a universal assay machine (EMIC®-DL 10000) at the Bioengineering Laboratory of the Medicine School of Ribeirão Preto. Capacity of the load cell was 50kgf, application velocity of 10mm/min with a pre-load of 5N and 30 seconds of accommodation time. A load/deformity chart was plotted. Maximal strength and relative stiffness were calculated through TESC software, version 1.4.

Statistical analysis

Data were analyzed with SIGMASTAT software version 3.5 and presented as means ± SD. Data from mechanical assays were considered normal and one-way ANOVA was used, followed by a post-hoc analysis (Tukey multiple comparison test). Student's T-test was applied for intergroup comparisons. Statistical significance was considered if p<0.05.

RESULTS

The four-week training regimen caused adaptations to femur maximal strength parameter in both groups (table 1). Regarding this parameter, and taking into account the training volume (weekly training frequency) there was statistical significance between the eight-week training groups CGI, TGI3 and TGI5 (p<0,05) (figure 1A). Statistical significance was also found between the control groups CGI and CGI (p=0,008), groups that trained with a three-weekly sessions frequency for four and eight weeks (TGI3 and TGI3 respectively, p=0,003) and groups that underwent training with a five-weekly session frequency for four and eight weeks (TGI5 and TGI5 respectively, p<0,001) (figure 1B).

Adaptations due to different training regimens were also found for the stiffness parameter (table1). There was no statistical difference between groups that trained for four weeks; though comparison between trained and sedentary groups that trained for eight weeks showed significant bone stiffness adaptations (p<0,05) (figure 1C).

Table 1. Values (MD ± SD) of the mechanical properties of maximal strength and relative stiffness of the femur bones of the experimental groups.

	CGI (n = 9)	TGI3 (n = 9)	TGI5 (n = 9)	CGII (n = 9)	TGI3 (n = 9)	TGI5 (n = 9)
Maximal strength (N)	98,07 ± 8,9	96,94 ± 11,09	98,62 ± 12,98	110,81 ± 6,12	111,26 ± 9,84	127,60 ± 8,42
Relative stiffness (N/mm)	140,75 ± 51,74	158,94 ± 86,11	150,50 ± 65,46	139,57 ± 30,95	239,36 ± 48,77	332,12 ± 61,77

Values in means ± SD. CGI, control/1 month; CGI control/2 months; TGI3, 3x/week/1 month; TGI5, 5x/week/1 month; TGI3, 3x/week/2 months; TGI5, 5x/week/ 2 months.

There was also statistical significance between groups with different training duration, but with equal number of weekly sessions ($p < 0,05$) (figure 1D).

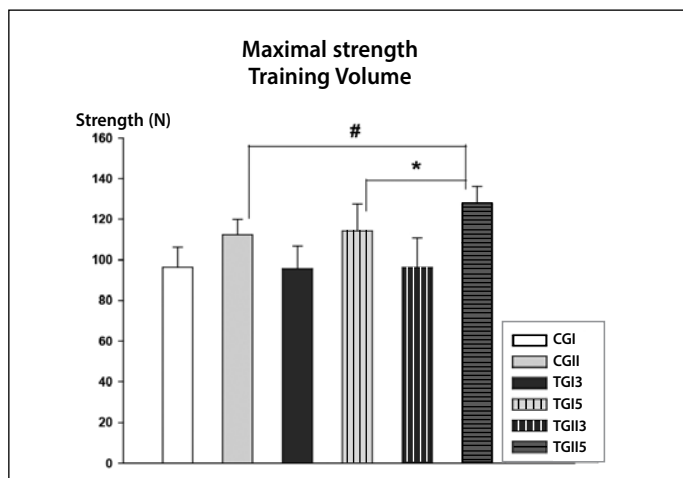


Figure 1A. Measurements of the mechanical assays performed. Training volume and maximal strength. ANOVA revealed time effect performance on maximal strength parameter (# for $p = 0.002$ and * for $p = 0.003$) without training. CGI, control/1 month; CGII control/2 months; TGI3, 3x/week/1 month; TGI5, 5x/week/1 month; TGI3, 3x/week/2 months; TGI5, 5x/week/2 months.

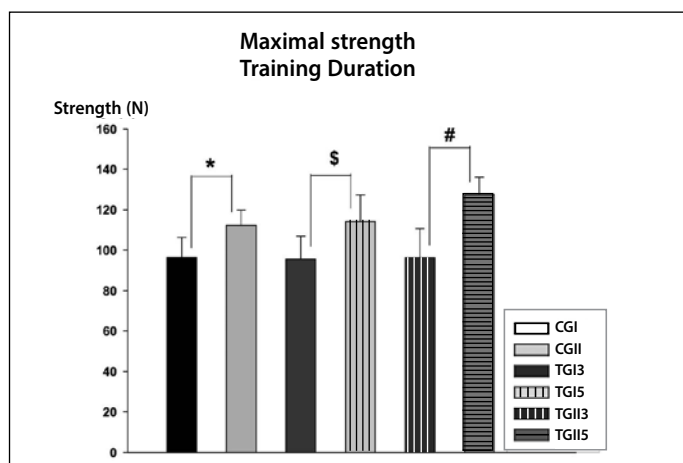


Figure 1B. Measurements of the mechanical assays performed. Training duration and maximal strength. ANOVA revealed the effect of different training protocols on the maximal strength parameter (* for $p = 0.008$, \$ for $p = 0.003$ and # for $p < 0.001$). CGI, control/1 month; CGII control/2 months; TGI3, 3x/week/1 month; TGI5, 5x/week/1 month; TGI3, 3x/week/2 months; TGI5, 5x/week/2 months.

DISCUSSION

The data evidence that the high-impact protocol described in this study positively affected the bone tissue, even for shorter training periods. However, the groups that underwent longer and more frequent training protocols presented more expressive gains in the analyzed biomechanical parameters.

The training volume (three or five weekly sessions) did not significantly affect the group that underwent a four-week training regimen. Nevertheless, an eight-week training regimen presented higher effectiveness in promoting osteogenic alterations. This was evidenced by comparisons between groups that trained with three weekly sessions, five weekly sessions and also sedentary/control groups.

Regarding the duration of the training protocol (four or eight weeks), and taking into account the strength parameter, we observed

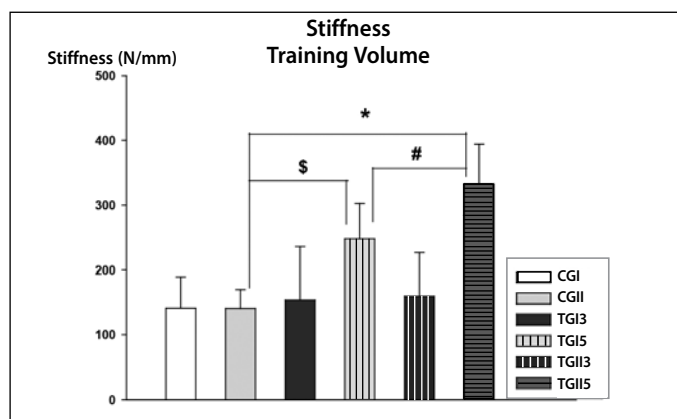


Figure 1C. Measurements of the mechanical assays performed. Training volume and relative stiffness. ANOVA revealed the effect of different training frequencies on stiffness (* for $p < 0.001$ and # for $p = 0.005$ and \$ for $p = 0.003$). CGI, control/1 month; CGII control/2 months; TGI3, 3x/week/1 month; TGI5, 5x/week/1 month; TGI3, 3x/week/2 months; TGI5, 5x/week/2 months.

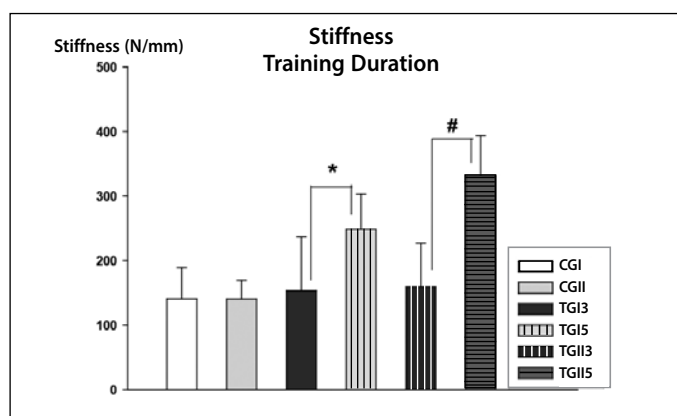


Figure 1D. Measurements of the mechanical assays performed. Training duration of and relative stiffness. ANOVA revealed the effect of different training protocols (* for $p < 0.007$ and # for $p < 0.001$). CGI, control/1 month; CGII control/2 months; TGI3, 3x/week/1 month; TGI5, 5x/week/1 month; TGI3, 3x/week/2 months; TGI5, 5x/week/2 months.

statistical difference between groups that underwent different weekly sessions. Nevertheless, statistical difference was also observed for this parameter between the control groups CGI and CGII. This may be explained by a possible interference of the age factor itself, although it was not possible to quantify its influence on osteogenic process. Duration of training regimen similarly affected the bone stiffness parameter. No statistical differences were found between control groups, suggesting no interference of age in this manner. Consequently, we suggest that alterations in this parameter are originated from high-impact exercise mechanical stress.

In healthy conditions, bone tissue is able to adapt to external stimuli. Pressure modifications and loads from external demands activate bone remodeling through cellular pathways¹³. A study conducted by Umemura et al¹⁴, showed that longer intervals between training sessions, or even between each jump, led to greater bone mass in hind limbs of experimental groups. The authors suggest that longer intervals may change the dynamic of extracellular fluids and hence, bone anabolic response to mechanical loading. Therefore, it was suggested that the already increased bone sensitivity due to jumps with 30-second intervals apart, could cause the bone tissue to perceive greater load magnitudes when compared to a protocol with jumps separated by shorter intervals (three seconds).

Robling et al¹⁵, showed that an eight-hour interval was sufficient to restore tissue cell mechanical sensitivity when compared to shorter periods. This demonstrates the importance of recovery periods to maximize osteogenic effects of mechanical overloads, such as those created by exercise.

The present study was conducted with similar interval between jumps for all experimental groups. Interval differences between groups consisted of those originated from the number of weekly sessions. For those groups that underwent three weekly sessions, the intervals consisted of 48 hours; for those that underwent five weekly sessions, interval consisted of 24 hours. All training sessions were undertaken at the same time of day. Training periods of four and eight weeks, and also time between sessions might have diminished the influence of these factors on bone tissue sensitivity. Other factor that affects bone remodeling is the type mechanical load that is given to the tissue. Due to the adopted training protocol, only concentric muscle contraction was the source of mechanical stress in the hind limbs. No eccentric muscle contraction stimuli were given to the hind limbs of the animals. This is mainly due to the fact that the animals used their front limbs to pull themselves to the top of the box after completion of the jump, and also because they were gently repositioned back to the bottom of the box for the next jump.

Studies that evaluated correlations between load magnitude and bone growth, as well as muscle influence over bone tissue, showed that eccentric muscle activity is more effective in promoting BMD gains than concentric muscle contractions. This means that exercise which concentrate more muscle activity in the eccentric phase of the movement are more osteogenically effective than those concentrating muscle activity in concentric phases. Moreover, they also demonstrate that the load magnitude is the main mechanism by which muscle activity influences bone mass adaptations^{16,17}.

In the present study, the adopted protocol almost exclusively obligated the animals to use concentric muscle contractions. This type of muscle contraction demonstrated to be effective in enhancing both maximum strength and relative stiffness parameters of the analyzed femoral necks. Despite being scientifically proved that eccentric muscle contraction generates higher tension, - and therefore, present higher osteogenic gains - the adopted protocol on this study showed that its possible to obtain significant bone gains exclusively through the application of only one type of muscle contraction.

CONCLUSIONS

Through analysis of both maximum strength and relative stiffness parameters, obtained through mechanical assays of femoral necks derived from animals that underwent high-impact training protocols, we showed that daily training frequencies for longer periods are beneficial to bone health. We also show that lesser weekly sessions associated to a shorter period is capable of inducing alterations in these proprieties. This demonstrates that frequent daily exercises are not necessary to evoke alterations in bone tissue and bone health, although higher frequencies and longer periods have additional benefits.

ACKNOWLEDGEMENTS

The authors would like to thank the Bioengineering Laboratory of the Medicine School of Ribeirão Preto, University of São Paulo (FMRP/USP-RP) for the infrastructure and the agencies FAPESP and CNPq, for granting financial aid and scholarships.

All authors have declared there is not any potential conflict of interests concerning this article.

REFERENCES

1. Shimano MM, Shimano AC, Volpon JB. Histerese de fêmures de ratas submetidos a ensaios de flexão em diferentes faixas etárias. *Rev Bras Eng Biomed* 2002;18:89-97.
2. Eastell R. Management of osteoporosis due to ovarian failure. *Med Pediatr Oncol* 2003;41:222-7.
3. Bain SD, Rubin CT. Metabolic modulation of disuse osteopenia: endocrine-dependent site specificity on bone remodeling. *J Bone Miner Res* 1990;5:1069-75.
4. Smith EL, Gilligan C. Mechanical forces and bone. *Bone Miner Res* 1989;6:139-73.
5. Wronski TJ, Lowry PL, Walsh CC, Ignaszewskin JA. Skeletal alterations in ovariectomized rat. *Calcif Tissue Int* 1985;37:32-8.
6. Fuchs RK, Snow CM. Gains in hip bone mass from high-impact training are maintained: A randomized controlled trial in children. *J Pediatr* 2002;141:357-62.
7. Ocarino NM, Serakides R. Effect of the physical activity on normal bone and on the osteoporosis prevention and treatment. *Rev Bras Med Esporte* 2006;12:149-52.
8. Honda A, Sogo N, Nagasawa S, Kato T, Umemura Y. Bone benefits gained by jump-training are preserved after detraining in Young and adult rats. *J Appl Physiol* 2008;105:849-53.
9. Huang TH, Lin SC, Chang FL, Hsieh SS, Liu SH, Yang RS. Effects of different exercise modes on mineralization, structure, and biomechanical properties of growing bone. *J Appl Physiol* 2003;95:300-7.
10. Turner CH. Three rules for bone adaptation to mechanical stimuli. *Bone* 1998;23:399-407.
11. Nagasawa S, Umemura Y. Bone hypertrophy in rats: effects of jump number and height. *Adv Exerc Sports Physiol* 2002;8:87-92.
12. Umemura Y, Nagasawa S, Honda A, Singh R. High-impact exercise frequency per week or day for osteogenic response in rats. *J Bone Miner Metab* 2008;26:456-60.
13. Burger EH, Klein-Nulend J. Responses of bone to biomechanical forces in vitro. *Adv Dent Res* 1999;13:93-8.
14. Umemura Y, Sogo N, Honda A. Effects of intervals between jumps or bouts on osteogenic response to loading. *J Appl Physiol* 2002;93:1345-8.
15. Robling AG, Burr DB, Turner CH. Recovery periods restore mechanosensitivity to dynamically loaded bone. *J Exp Biol* 2001;204:3389-99.
16. Hawkins SA, Schroeder ET, Wiswell RA, Jaque SV, Marcell TJ, Costa, K. Eccentric muscle action increases site-specific osteogenic response. *Med Sci Sports Exerc* 1999;31:1287-92.
17. Heinonen A, Oja P, Kannus P, Sievanen H, Haapasalo H, Manttari A. Bone mineral density in females athletes representing sports with different loading characteristics of the skeleton. *Bone* 1995;17:197-203.