EFFECTS OF PHYSICAL TRAINING ON THE MECHANICAL RESISTANCE OF RAT FEMUR PROXIMAL THIRDS

ANDREO FERNANDO AGUIAR, LEANDRO BARILE AGATI, SÉRGIO SWAIN MÜLLER, ODUVALDO CAMARA PEREIRA, MAELI DAL-PAI-SILVA

ABSTRACT

Objective: To analyze the mechanical behavior of rat femur proximal thirds submitted to chronic aerobic and resistance training. Methods: Male *Wistar* rats (80 days of age, weighing 300 to 350 g) were divided into 3 groups ($n=8$ per group): control (CO), aerobic training (AT) and resistance training (RT). At the end of the training, the animals were euthanized and the right femur was collected. Flexion-compression tests were carried out to analyze the mechanical behavior of the femurs. Results: The resistance training promoted a significant reduction in maximum force (F_{max}) of the femur. However, it also promoted a relevant increase (23.7%), though without statistical significance, in maximum force deformation (DF_{max}). The aerobic training did not affect maximum force, however, it caused a considerable reduction in DF_{max} (26.6%), though this was also not statistically significant. Conclusions: The results show that resistance and aerobic training promoted a reduction in the F_{max} and DF_{max}, respectively. The data showed a different response of both physical training models on the mechanical properties of the rat femurs.

Keywords: Exercise. Biomechanical. Femur. Rats.

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INTRODUCTION

The effects of the presence and absence of exogenous load on the mechanical properties of bone tissue have been extensively discussed in literature. According to Bikle et al.,[1] osteogenesis can be stimulated by small deformations in the bone architecture, provoked by mechanical forces applied during normal physical activity, which can act directly on the bone remodeling rate. Several factors associated with physical exercise can affect the bone, metabolic and endocrinal responses, such as training intensity/volume, the number of series and repetitions, the rest period between series of exercises, and the type of muscle contraction. Therefore, different models of exercises can cause different adaptations to the bone tissue and it has been suggested that minimum effective damage to this tissue is necessary for maintenance of the bone.

The mechanical load on the bone is recognized as an important regulator of maturation, maintenance and skeletal strength. Likewise, it is believed that training with weights can contribute toward the increase and preservation of bone mass in young individuals and adults.[2] Experimental and theoretical data suggest that for the load to generate an increase of bone mass, it must have sufficient magnitude to exceed the minimum effective load and be applied in a progressive and intermittent manner.[3]

On the other hand, the absence of load can be a determinant factor for reduction of the bone matrix, whereas one of the molecular mechanisms responsible for this is the induction of IGF-1 resistance. It was demonstrated that the in vivo administration of IGF-I stimulates growth and bone formation during exposure to the load, while no effect was observed in immobilized bones.[4]

Evidence indicates resistance training as a powerful stimulus to promote increase of density and remodeling of the bone tissue.[5] However, contradictory results have been reported by other authors.[6,7] Likewise, this discrepancy has been observed in studies involving aerobic training, in which some report satisfactory results in the structure, recovery and bone strength,[8] while others affirm that more studies are necessary to elucidate the specific effects of this exercise model.[9]

According to Shimano and Volpon.[10] the conventional method of mechanical analysis of the femur presents satisfactory results and well defined methodology, yet the complex format of this region of the femur and its heterogeneous composition cause irregular distribution of forces, which hinders the interpretation of results. In our study, we used the analysis of maximum force (F_{max}) and of maximum force deformation (FD_{max}), by means of flexion-compression tests, as these reflect the mechanical behavior of the region tested.[11] Thus the aim of this study was to investigate the effects of different physical training models on the mechanical resistance of rat femur proximal thirds.

All the authors declare that there is no potential conflict of interest referring to this article.

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MATERIAL AND METHOD

Animals and experimental groups

The study subjects were 24 male Wistar rats (80 days of age, 300 to 350g), from the multidisciplinary center of biological investigation (CEMIB, UNICAMP, Campinas, São Paulo). The animals were kept in collective polypropylene cages (4 animals/cage) at the Vivarium of Small Mammals of the Morphology Department, in an environment with controlled temperature (22-24 °C) and dark/light luminosity cycle (12/12hrs), in which they received feed and water *ad libitum*. The animals were randomly divided into 3 experimental groups: resistance training/ 8 weeks (RT, $n=8$), aerobic training/ 8 weeks (AT, $n=8$) and Control/ 8 weeks (CO, $n=8$). This experiment was approved by the Committee of Ethics for Experiments with Animals (CEEA) of Instituto de Biociências da UNESP, Botucatu (Protocol N° 017/06-CEEA) and was carried out according to the guidelines proposed by the American College of Sports Medicine on research with experimental animals.

Resistance training

The RT group was submitted to a program of physical resistance training (RT) for eight weeks, following the squat training model proposed by Tamaki et al.[12] (Figure 1) The animals from the control group did not receive any training stimulus during the experimental period. For the training performance, the animals used a leather jacket (1), connected to a 35cm (2) mobile wooden bar on which the weight rings (3) were allocated. The rats wearing the jacket remained seated with their rear legs flexed and resting on the support base (4). Electrical stimulation was performed using an electrical stimulator (5) (Dualpex 961, Quarker), by means of self-adhesive electrodes (ValuTrode, model CFE200 and size 3.2cm) positioned on the rats' tails. The parameters used in the electrical stimulation were: frequency 1Hz, duration 1 millisecond (MS), active cycle 1:2 seconds (s) and current intensity adjusted to allow the performance of movement, ranging from 4 to 15 milliamperes (MA). As a result of the electrical stimulation, the rats performed knee extension repeatedly, lifting the weight fastened to the apparatus. In order to guarantee the same intensity of training from start to finish of the experiment, weekly weight adjustments were performed by means of the maximum repetition test (1MR). The RT protocol consisted of the performance of 4 series of 12 repetitions (3x/week), with overload of 65-75% of 1MR and interval of 40 seconds between series. The training sessions were always held in the same period of the day, between 2 and 4 p.m. The training program started after two weeks of familiarization with the apparatus and with the execution of the exercise. In the first week of this phase, the rats using the jacket were fastened to the apparatus for 20 minutes per day, without the electrical stimulation. The performance of exercises was allowed in the second week, using 2 series of 5 to 10 repetitions with load between 40 and 60% of the body weight for this purpose.

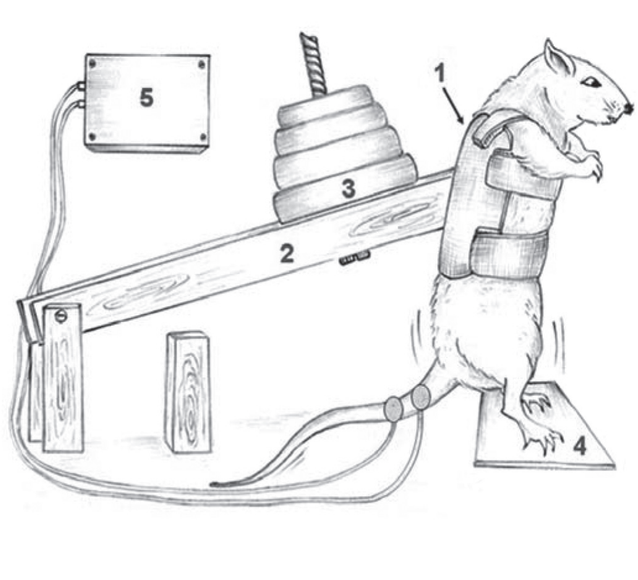

Figure 1 – *Apparatus used for resistance training. (Adapted from Tamaki et al.)[12].*

Aerobic training

The AT group was submitted to an aerobic swimming training program, similar to that proposed by Gobatto et al.[13] The training sessions were held in an aquarium divided up into individualized compartments, containing water at 28-30 ºC with a depth of 35cm. The protocol consisted of a daily session (5 days/ week), for 8 weeks. The volume (time) and intensity (overload) of training were progressive, being equivalent to 10 min, without overload (1st week); 20 min, 1% (2nd week); 25, 30, 35 and 40 min, 3% (from beginning to end of the 3rd week); 45, 50, 55 and 60 min, 5% (from beginning to end of the 4th week) and 60 min, 5% (5th to 8th week), respectively. (Table 1) The training sessions were always held in the same period of the day, between 2 and 4 p.m.

Mechanical trial

The flexion-compression tests were performed in a EMIC®-10000N universal testing machine. (Figure 2) A load cell with maximum capacity of 50kgf was used for obtainment of the

Table 1 – *Aerobic training program.*

Week	Time (min)	Overload (%BW)
1st	10	-
2nd	20	1%
3rd	25 to 40	3%
4th	45 to 60	5%
5th	60	5%
6th	60	5%
7th	60	5%
8th	60	5%

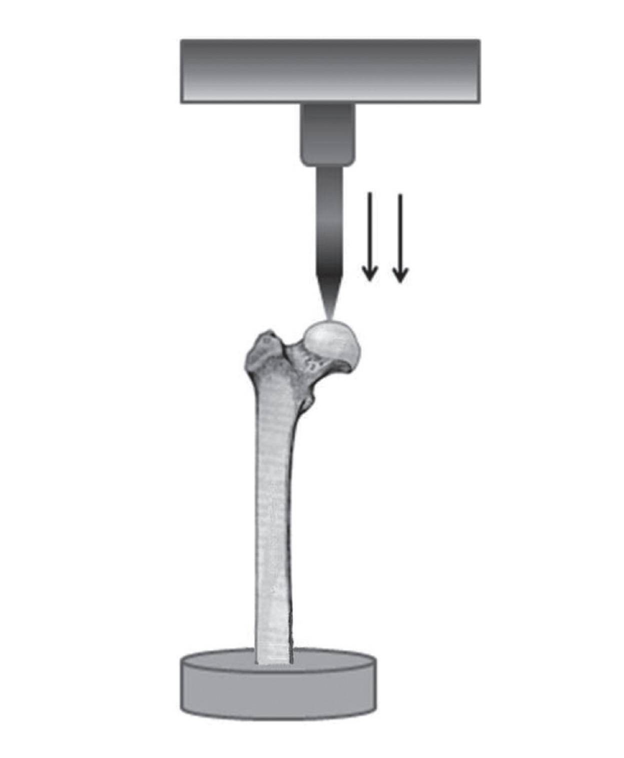

Figure 2 – *Figure representing a femur undergoing the mechanical flexion-compression test.*

forces exerted, and the deformations were captured by the internal displacement sensors of the machine. The acrylic-bone set was fixed in a vice coupled to the base of the universal testing machine. Vertical force was applied with an accessory measuring 2 mm in diameter on the femoral head until fracture occurred. The speed of application of the force was 0.1mm/min. Force x deformation graphs were obtained from the tests, based on which it was possible to obtain stiffness and maximum force as mechanical properties.

STATISTICAL ANALYSIS

The GraphPad InStat® v.3 program was used for the statistical analysis. The statistical procedure was carried out after preliminary variability study, related to the normality and equality of variance among the groups, with statistical power of 80% for the comparisons made. The paired Student's T-test was used to perform the comparison of body weight gain (intra-group relationship) of the experimental groups. Another comparison made was the mechanical analysis of the bone among groups, in this case using the One-way Analysis of Variance (ANOVA), for parametric data, followed by the Tukey's post-hoc test. The value of 5% was used as a significance level in all the analyses.

RESULTS

Body Weight

The values referring to initial body weight (IW) were not statistically different among the groups. (Table 2) All the groups presented significant gain ($p<0.05$) of body weight (BW) from the beginning to the end of the experiment period. The percentage of increase of BW (Δ%) was 26.2%, 30.8% and 33.5% in the CO, AT and RT groups, respectively, yet the values were not statistically different among the groups. (Table 2)

Table 2 – *Initial (IW) and Final (FW) Body Weight, and percentage variation (Δ%) of the body weight of the untrained (CO), aerobic training (AT) and resistance training (RT) groups. Values expressed in mean ± SD.*

Groups	IW (g)	FW (g)	Δ %
CO (n=8)	308.9 ± 27.1	418.6 ± 37.7*	26.2
AT (n=8)	326.0 ± 27.8	471.4 ± 34.9*	30.8
RT (n=8)	302.8 ± 32.3	455.1 ± 61.8*	33.5

* p< 0.05 compared with IW.

Maximum Force (F_{max}) and Maximum Force Deformation (FD_{max})

The apparatus used for analysis of F_{max} and FD_{max} is shown in Figure 2, and the corresponding data are presented in Figures 3 and 4, respectively. After eight weeks of resistance training, the RT group presented a significant reduction ($p<0.05$) of F_{max} compared with the control group (CO: 191.6±29.7 N and RT: 143.1±33.9 N, dif.%= -25.3%). However, the AT group did not present significant difference ($p>0.05$) in the values of F_{max} in relation to the CO group (CO: 191.6±29.7 N and AT: 180.3±13.0 N). (Figure 3)

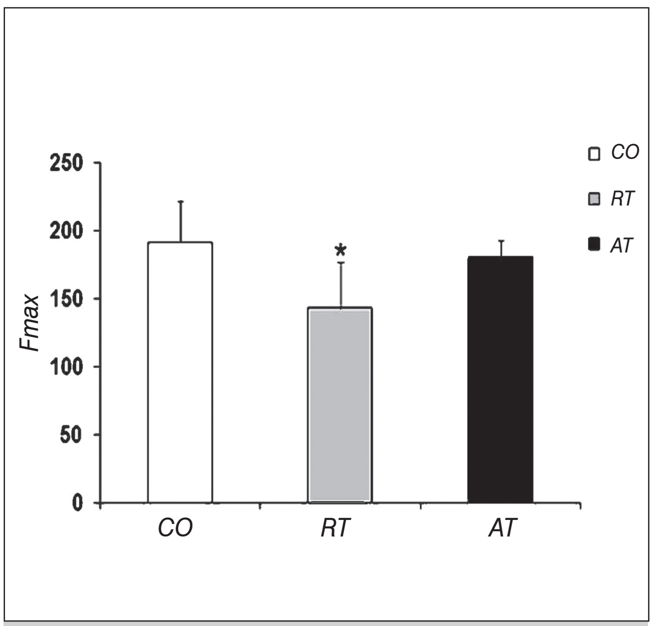

Figure 3 – *Maximum force (F_{max}) of the femur of the control (CO, n=8), resistance training (RT, n=8) and aerobic training (AT n=8) groups. Values expressed in mean±SD. * p<0.05 compared with the CO group.*

According to the FD_{max} data, there was an increase of 23.7% and adecrease of 26.6% in groups RT and AT, respectively. These values were not statistically different ($P>0.05$) in relation to the CO group. (Figure 3) When compared with each other, the RT and AT groups exhibited a significant difference ($p<0.05$) in the FD_{max} (RT: 1.65±0.37 mm and AT: 0.98±0.14 mm, dif.%= 40.7%). (Figure 4)

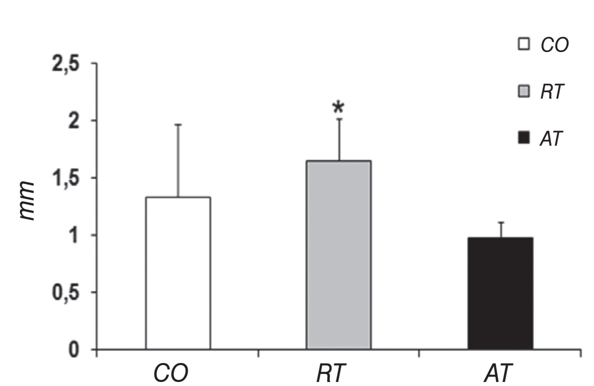

Figure 4 *– Maximum force deformation (FD_{max}) of the femur of the control (CO, n=8), resistance training (RT, n=8) and aerobic training (AT n=8) groups. Values expressed in mean±SD. * p<0.05 in relation to AT group.*

DISCUSSION

The use of noninvasive techniques (for example: bone densitometry, dual-energy x-ray absorptiometry, DXA) facilitates the investigation of bone mineral density (BMD) in different species. On the other hand, the study of the mechanical behavior of this tissue becomes impracticable in humans, as it involves mechanical tests conducted directly on the bone structure. Due to the precision of the data and easy application in experimental animals, the mechanical test has been used as a valuable tool to understand the mechanical behavior of bone tissue in situations of physical exercise and pathological conditions, like osteopenia. In this study, the use of an animal model allowed the direct analysis of force and deformation of the femur, by means of a mechanical load applied directly on this tissue. Human studies can be influenced by motivation, movement technique and food consumption during the training period. Our animal model provides a training method independent on the motivation of the subjects and guarantees total control over food consumption and movement technique during the training.

With the external variables controlled, significant body mass gain was observed from the beginning to end of the experimental period. (Table 2) However, the percentage of body weight (BW) increase was not statistically different among the groups (Table 2). The absence of variation in BW gain indicates that both training models did not subject the animals to the overtraining state and did not interfere in their somatic growth either. In addition, the results of this study showed that the F_{max} was reduced only in the group that was submitted to resistance training, suggesting that this training model has reduced bone resistance in relation to the control group . (Figure 3) The aerobic training promoted significant decrease of bone flexibility (FD_{max}) in the AT group, compared with the control group. (Figure 4)

Maeda et al.[14] observed significant loss of spongy bone in rats submitted to 6 weeks of immobilization. Likewise, Zerwekh et al.[15] demonstrated an increase of resorption together with a decrease of bone formation in humans submitted to 12 weeks of bed rest. The results of these studies demonstrated that immobilization can directly affect the mechanical and biological components of the bone tissue, suggesting that the presence of mechanical load is a determinant factor for the maintenance of the functional integrity of this tissue. Although the evidence suggests that the conditions of absence of mechanical load exert a profound impact on the mechanical properties of bone tissue, the effects of different physical exercise models on these variables remain unclear. Up to now, most studies have been performed on human subjects submitted to physical exercise for subsequent analysis of bone density. In this investigation, the use of an animal model presents the advantage of conducting the direct analysis of the mechanical behavior of bone, by means of flexion-compression testing. According to the results, resistance training (RT) for eight weeks brought about a substantial increase (+23.7%) of the FD_{max} of the femur. (Figure 4) As observed previously in our laboratory, Pereira et al.[16] also described that the FD_{max} is higher in rats submitted to the resistance exercise of high intensity. In humans, several studies report an increase in bone mineral density (BMD) of young individuals that practice high impact activities.[17,18] Although the BMD analysis was not conducted in this study, the increase of FD_{max} observed in the RT group demonstrates that the application of force training can be an adequate strategy to promote increase of femoral bone flexibility. In addition to the increase of BMD after chronic resistance training,[19] the increase of elasticity up to the break point may be related to the increase of collagen content. Interestingly, the increase of FD_{max} was associated with a significant reduction ($p<0.05$) of F_{max}, in relation to the CO group. (Figure 3) The results show, for the first time, that the increase of bone flexibility may be directly related to the reduction of the F_{max} of this tissue. While the molecular events that support our findings remain unknown, these observations give rise to questions about the functional adaptations of bone tissue in responses to training with weights. Unlike what was observed in the RT group, aerobic training (AT) for eight weeks promoted a considerable reduction (-26.6%) of the FD_{max}, without any change in F_{max}. (Figures 3 and 4) Although some questions persist in relation to the mechanisms involved in the decrase of FD_{max} during aerobic training, the reduction of bone flexibility may be associated with the decrease of the organic matrix, comprised mainly of type I collagen. Based on the knowledge that the mechanical load is the primary factor to promote increase of BMD,[17] our results suggest that this may be the determinant factor for the maintenance of collagen synthesis of bone tissue. Consistent with this idea, McCulloch et al.[20] suggest that the increase of bone mass is more evident in activities on land with impact and body support, than in water activities. Thus the gravity decrease resulting from aerobic training in the form of swimming could be a stimulant factor for decrease of bone flexibility, due to the decrease of type I collagen synthesis.

CONCLUSION

The results demonstrate a differential beneficial action of both physical training models on the mechanical properties of young adult rats. The data suggest a specific application of exercise/physical training according to the physical activity or type of sport, since the chronic adaptations of the bone tissue present peculiar characteristics in response to the stimulus applied during the activity.

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