BIOMECHANICAL ADAPTATIONS OF MICE CORTICAL BONE SUBMITTED TO THREE DIFFERENT EXERCISE MODALITIES

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ABSTRACT

Objective: To compare the adaptive effects of three non-weight bearing exercise on bone mechanical properties. Methods: 24 male Balb/c mice (22-25g), were randomly divided into four groups (n=6): sedentary group (S); swimming group (N) which performed sessions five times per week for 60 min progressively; resistance group (R), which performed climbing exercise with progressive load, three times per week; and combined group (C), which performed the same protocols aforementioned being three times a week according to N protocol and two times a week the R protocol during eight weeks. Biomechanical tests, load until failure and stiffness evaluation of shinbone was performed after animals have been sacrificed. Results: Stiffness values were statistically higher only in the isolated modalities groups (N and R, 41.68 ± 10.43 and 41.21 ± 11.38 N/mm, respectively) compared with the S group (28.48 ± 7.34 N/mm). However, taking into consideration the final body mass, relative values, there was no difference in the biomechanical tests among the groups. Conclusion: Data from the present investigation demonstrated a favorable influence of muscle contraction in lower impact isolated exercise modalities on absolute stiffness values, i.e.groups N and R, whereas the combined group (C) did not present any statistical significant difference compared to sedentary group. ***Level of Evidence II, Prospective Comparative Study.***

Keywords: Exercise. Biomechanics. Tibia. Mice.

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INTRODUCTION

The influence of physical activity on the dynamics of bone tissue has caused a growing interest of the scientific community, particularly in the treatment and prevention of the risk of fractures resulting from osteoporosis.[1] It has been found that the bone structure adapts to the type of applied mechanical loading[2] and that the exercise would act as an agent for mechanical loading in the bone tissue.[3,4] Experimental models of high-impact activities, such as jumping, demonstrated benefits to bone mechanical properties, especially for bone mass gain.[5,6] However, low impact exercise modalities showed that the mechanisms of bone adaptation could differ from those by high impact modalities.[7,9]

The mechanical testing of bone tissue allowed us to show more accurately the potential of reduced or no impact activities on the maintenance and improvement of the properties of bone tissue in response to these atividades.[10,11] In addition, the bone tissue of male mice subjected to swimming training showed good sensitivity to stimuli triggered by the exercise.[12] Although the mechanism is not fully elucidated, it has been speculated that the osteogenic response to low impact exercise was influenced by mechanical interaction between bone and muscle contraction signaling a cascade of events in the bone metabolism, including the elevation of intracellular calcium levels, growth factors and increased bone matrix production.[13]

The programs of physical exercise which include aerobic and resistance exercise modalities are recommended by the American College of Sports Medicine (ACSM) as strategies for prevention and treatment to various populations.[14] Experimental models of swimming and resistance exercise have led to observe distinct adaptations to each model training on the mechanical properties of the bone tissue.[15] However, there is no

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evidence of the effect of combined modality on these properties. Combined exercise is defined as the inclusion of aerobic and resistance modalities performed sequentially (competitor's mode) or alternated sessions (combined).[16,17] Many athletes and rehabilitation programs use this exercise strategy expecting to maximize physiological adaptations offered by each specific modality.[17] However, the superiority or efficacy of the combined exercise on physiological markers still remains unclear.[18,19]

Moreover, from the neuromuscular and metabolic point of view, the combined exercise template performed in sequence (competitor's mode) showed distinct cell signaling pathways or even antagonistic when compared to isolated modalities,[20,21] demonstrating a phenomenon as previously described by Hickson[22] as "interference" between them. Thus, it was shown that performing both types separated by a resting period of at least 6-8 hours allows the recovery of glycogen stores and reduces residual fatigue.[23] Thus, a better understanding of these exercise modalities on bone tissue will favor the adequate prescription of exercise for many people, especially those at higher risk of fractures. Therefore, we have proposed to investigate and compare the effects of adaptive swimming and resistance exercise modalities, and their combined effect performed on alternate days (resting period of 24 hours) on the biomechanical properties of bone tissue of healthy mice.

MATERIALS AND METHODS

Animals

Twenty four Balb/c male mice, initially weighing 25g (± 3g) provided by the Central Animal Facility of the USP campus at Ribeirão Preto were used in this study. All procedures were approved by the Comitê de Ética em Experimentação Animal (Ethics Committee on Animal Experiments) of Faculdade de Medicina de Ribeirão Preto, Cetea / FMRP (Protocol N° 13/2011). The animals were randomized into four groups ($n = 6$), namely sedentary group (S), the swimming exercise group (N), the resistance exercise group (R) and the combined exercise group (C). All animals were housed in special mice cages, day and night cycles of 12 hours and a controlled average temperature of 22° C.

EXERCISE PROTOCOLS

Swimming Protocol

The animals of group N performed swimming exercise held in reservoirs adapted to its practice with water temperature kept at 32 (± 1^{o} C). The swimming training consisted of a weekly increase of 20 min until 60 min was achieved in the third week and maintained throughout the protocol, five days per week for eight weeks without the inclusion of additional charge, as adapted from Venditti and Di Meo.[24] Swimming was selected due to its natural environment for rodents and because it generate less interference due to impact with the ground preventing interference with the interpretation of the results.[25]

Protocol of Resistance Exercise

The exercise equipment consisted of a resistance climbing apparatus (80^{o} slope, 1 inch space between each step, and 0.5m height) in which the animals were adapted to climb. The vertical dimension of the ladder allowed the animals to perform 8-12 dynamic movements, preserving the original model for rats.[26] The parameters of the protocol used corresponded to six to eight climbing (repetitions), with a two minute interval between each repetition, three intercalated sessions per week during eight week (24 sessions). In the first week, the animals were trained to the climbing equipment without any additional weight. In the second week of the protocol, tests were started to determine the initial training load, similar to a load test in humans. To this end, an additional weight equivalent to 50% of the body weight was attached to the proximal portion of tail by a latex strap (Fulgor®) and in the following repetitions, loads equivalent to 75, 90 and 100% of body weight were used. After this intensity protocol, an extra load of 3g was added until failure to perform exercise. Failure was considered as the inability of the animal to reach the top of the device. In order to determine the maximum load considering the initial body weight, the load of the next session corresponded to 50% of the maximum load of the last session followed by 90%, 100% and 100% + 3.0 g increases up to failure or to achieve maximum of eight repetitions.[27]

Protocol of combined exercise

The group undergoing the combined protocol performed intercalated sessions on alternate days in the week, three times the group N protocol and twice a week the group R protocol for eight weeks. At the end, the animals in group C underwent 24 sessions of protocol N intercalated with 16 sessions of protocol R. The animals did not train on weekends.

Mechanical assays

Mechanical assays were performed in a universal testing machine (Emic®), model DL 10000, Laboratory of Bioengineering FMRP/ USP. The speed of load application was 1mm/min with preload of 1N, load cell of 500N, settling time of 30 seconds and distance between the points of 10mm. (Figure 1)

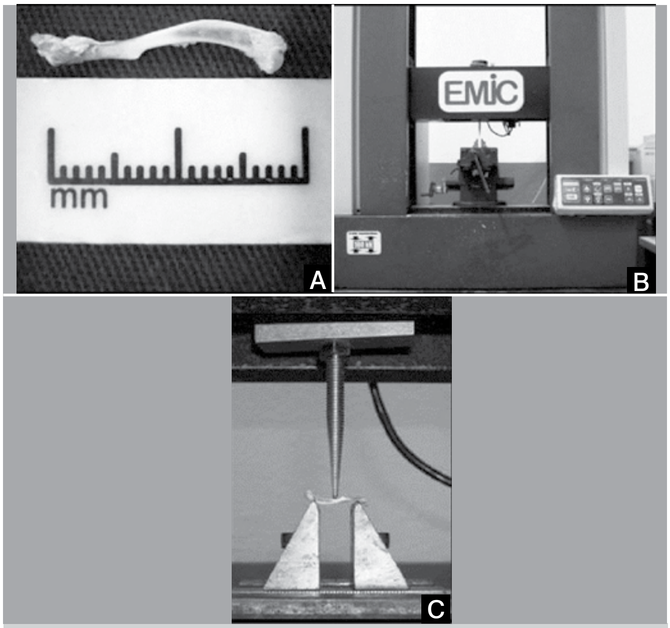

Figure 1. Mechanical assay of the right tibia of the experimental groups. 1A) bone length in mm; 1B) mechanical assay machine; 1C) device prepared for three-point mechanical assay.

STATISTICAL ANALYSIS

The values obtained were tested for normality according to the Kolmogorov-Smirnov test to determine if their distribution was parametric or nonparametric. In case of a parametric distribution, the data were submitted to the One-Way ANOVA test, followed by the Tukey's post-test. In case of a non-parametric distribution, the One-way ANOVA test was followed by Dunn's post-test. For each analysis, we used the GraphPad Prism V 5.1 Software. Results were expressed as mean ± standard deviation. In all analysis, we used a significance level of 5% (p ≤0 .05).

RESULTS

Body mass

The normality test of the values obtained in the experiment showed a parametric distribution of data. Although there was no significant difference in the average body mass of the groups at the start of the experiment, there were significant differences in the final weight of the groups. (Figure 2) If compared to group S, the final body weights of the groups submitted to protocols N and R were higher (10.19%) and (14, 96%), respectively. However, only the R group showed a significant difference compared to the S group, p <0.05, while group C decreased by 3.05% the body weight in relation to group S.

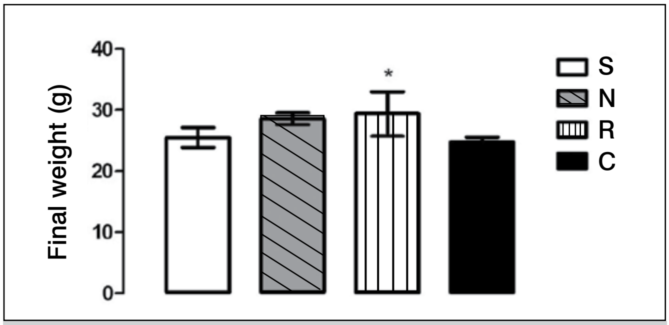

Figure 2. Final body mass of the groups, mean values ± standard deviation, n = 6. (S) Sedentary group; (N) Swimming group; (R) Resistance exercise group; (C) combined exercise group.

Maximum Force

After eight weeks of training protocol specific to each group, the mean of the absolute values of maximum force were (10.40 ± 2.37) N to S group (12.14 ± 1.87) N for group N, (13.72 ± 3.2) N for the R group and (10.63 ± 1.67) N for group C. Despite the increase (15.72%) and (30.79%) in the protocols swimming and resistance exercise isolate, respectively, it has not been observed any statistical differences between groups, p> 0.05. (Figure 3) Such difference remained unchanged for the standardization of the final body mass, maximal strength, p> 0.05. (Figure 3)

Stiffness

The mean absolute stiffness values found were: (26.49 ± 6.13) N / mm for the S group; (41.68 ± 10.43) N/mm for group N; (41.21 ± 11:38) N/mm for the R group; and (35.34 ± 2.97) N/ mm for C group. Statistical analysis showed that there was a significant increase in groups N and R compared to the S group, p <0.05. However, the values of relative stiffness revealed no statistical difference between the groups, p> 0:05. (Figure 4)

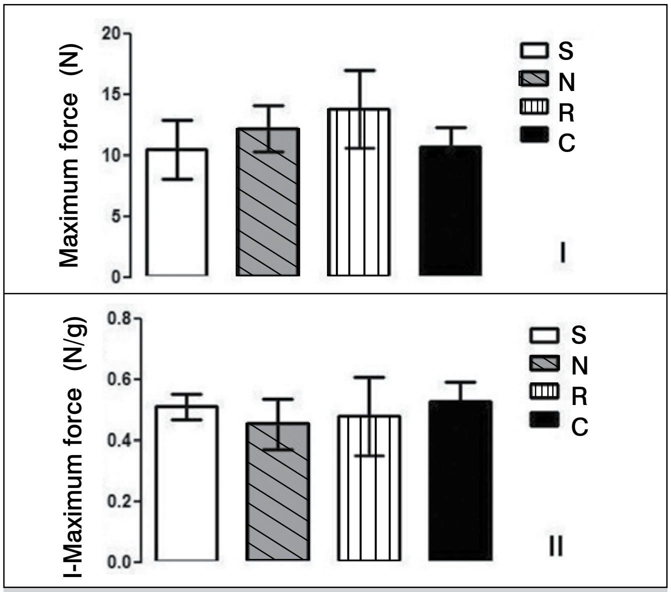

Figure 3. Maximum force (I) and of maximum force index corrected by body weight (II). Values expressed as mean ± standard deviation, n = 6. (S) Sedentary group; (N) Swimming group; (R) Resistance exercise group; and (C) combined exercise group.

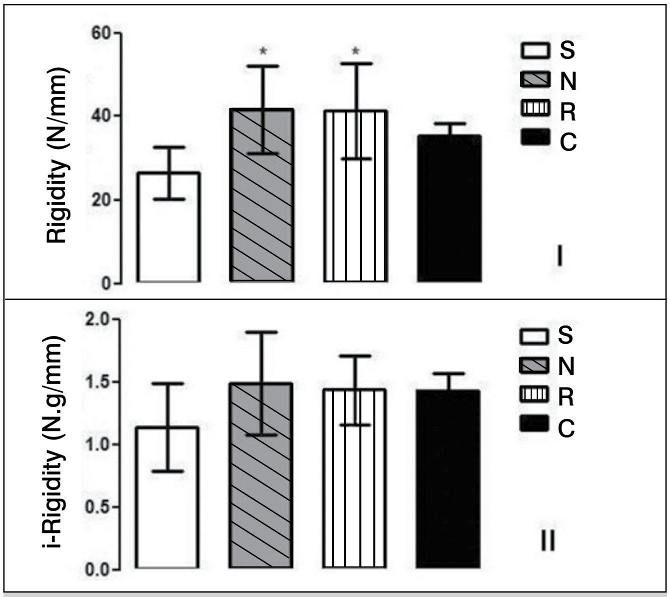

Figure 4. Stiffness values (I) and stiffness index corrected by body weight (II). Values expressed as mean ± standard deviation, n = 6. (S) Sedentary group; (N) Swimming group; (R) Resistance exercise group; and (C) combined exercise group.

DISCUSSION

It is recognized that the mechanical properties of bones can be accurately measured by mechanical testing with load applications to compression, bending, torsion and flexion-compression. In the present study, bone quality was analyzed using absolute and relative maximum strength and stiffness of the tibia, obtained by biomechanical testing of three-point bending. Our data showed that the groups N and R showed an increase in absolute stiffness values. However, group C showed no significant effects on such measured properties when compared to the S group.

The prescription of aerobic and resistance exercise is recommended by ACSM and AHA.[14] However, there are few conclusions regarding the mechanisms of interaction and cell signaling to the combined exercise modality, especially in bone tissue. The "competitor" effect of this modality on the development of muscle hypertrophy was been previously investigated.[28,29] Phosphorylation of the enzyme adenosine monophosphate-activated protein kinase (AMPK) which acts as a regulator of energy metabolism in skeletal muscle is appointed as an agent able to block the cascade of protein synthesis mediated by the pathway Akt/mTOR in competitor's training.[30] In this study we showed that the intervention by combined training were not different from group S regarding the biomechanical properties of bone tissue, supporting the theory of a possible interference of signaling pathways in this modality, also reflecting the mechanical adaptations of bone tissue. These evidences suggest a low adaptive effect of combined training on bone metabolism (e.g. relationship between the activity of osteoblasts and osteoclasts). However, we suggest that further studies can investigate these possible ways of interactions in the combined modality and its effect on bone metabolism.

We observed that only the R group showed a higher final body mass compared to the control group. Thus, when we consider this variable in the interpretation of results (relative values), the values of maximum force and stiffness between the groups showed no significant difference. Previous studies with Wistar rats considered that body mass was a statistically relevant variable[31] and heavier animals of the same species had higher values for maximum strength, stiffness and geometry of the transversal section.[32] However, three-dimensional analysis of these heavier animals' bones revealed that gains in mechanical strength and stiffness occurred in response to the mechanic -static effect which favors a greater cross-sectional area, whereas the mineral quality of the cortex was inversely impaired. Our model study with mice suggests a direct relationship between body mass and final values of absolute stiffness, (Figure 3) although we cannot say that the mineral quality of bone groups subjected to isolated protocols have followed the same relationship. Bone biomechanical testing on small rodents, particularly mice, have fewer evidence compared to rats, possibly due to the intrinsic methodological limitation to the method of mechanical testing of mice bone.[33] However, these animals show a natural advantage of conducting three or four points fracture test of more evenly throughout the bone tissue, accelerated senescence process and greater homogeneity in the species which contributes to the reduced number of specimen.[34,35] Animal models subjected to no impact activities have shown that mechanical stimuli generated by muscle contraction is able to improve mineral bone quality.[11,36] The result would be an adaptation in its trabecular structure favoring resistance to tension loads.[37] Our study presents some methodological limitations such as the lack of imaging technique such as Micro-CT and histomorphometry to confirm the changes to the trabecular structure. Furthermore, the N, R, and C groups had a different total volume of exercises at the end of the study. Such limitations require caution in the interpretation of this study. We propose, however, that the mechanical adaptations promoted by different modalities of exercise or by weight gain are significant, but structurally distinct.

CONCLUSION

Summarizing, the biomechanical analysis of this study shows that mechanical adaptations to the modalities swimming and resistance exercise isolated were not observed in the combined exercise, and that the final body mass exerted a prominent factor in this interpretation. In this sense, our data indicate for a reduction of the adaptive effect in the modality that combines low-impact exercise on bone resistance to fractures. Additionally, we point to future research on the importance of body weight in the interpretation of biomechanical adaptations of bone tissue in animal models and clinical studies.

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