Original Article (short paper)

Moderate intensity swimming training on bone mineral density preservation under food restriction in female rats

Taciane Maria Melges Pejon[1], Claudio Alexandre Gobatto[2], Victor Fabrício[3], Wladimir Rafael Beck[1]

[1]Universidade Federal de São Carlos, Departamento de Ciências Fisiológicas, Laboratório de Fisiologia Endócrina e Exercício Físico, São Carlos, SP, Brasil; [2]Universidade Estadual de Campinas, Faculdade de Ciências Aplicadas, Laboratório de Fisiologia Aplicada ao Esporte, Limeira, SP, Brasil; [3]Universidade do Oeste Paulista, Faculdade de Medicina do Jaú, Jaú, SP, Brasil.

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Abstract - Aim: To investigate the effect of moderate-intensity swimming training on bone mineral density under a 20% food restriction (FR) schedule for 12 weeks in female rats. **Methods:** Forty female Wistar rats were distributed into four groups: control (CG), exercised (EG), food restriction (FRG), and food restriction/exercised (FREG). At 95 days, the animals were subjected to aquatic adaptation and then performed the critical load test to individually determine the critical load intensity (CLi, % of body mass). Exercised groups swam 5 days a week, 30 minutes daily with weekly adjustment of the load equivalent to 80% of the CLi. The FR schedule was 20% in relation to CG and started concomitantly with physical training (PT). After 12 weeks, visceral fat weight was recorded and the femur was collected for biophysical and biomechanical analysis. **Results:** FR and exercise training promoted visceral fat reduction ($p<0.01$). FR reduced bone mineral density ($p<0.01$), while exercise training prevented such reduction. On the other hand, FR and exercise training did not promote significant changes in biomechanical parameters of the femur. **Conclusion:** Exercise training at moderate intensity was efficient in preserving bone mineral density despite long term of FR at 20%.

Keywords: aerobic exercise; bone tissue; energy restriction; adipose tissue.

Introduction

A high amount of adipose tissue has been associated with metabolic disorders[1], such as obesity[2,3], diabetes[4,5], and cardiovascular diseases[6]. Food restriction (FR) promotes low energy availability, being an effective model in reducing visceral fat[7]. Despite the benefits, FR has also been associated with bone mineral density (BMD) impairment[8,9] once indirectly induces mechanisms leading to greater osteoclastic action[10,11], due to hormonal changes and mineral and protein distribution[10]. These alterations increase the risk of fractures[8,9] and diseases related to bone health[9] in both genders, but females seem to be more affected, especially those older, characterized by the reduction of steroid hormones that act on bone balance[12]. In this sense, exercise training promotes the reduction of metabolic diseases[11,13] and has been positively correlated to BMD maintenance[14,15].

Regular practice of physical exercise promotes important mechanical impact on bones. Muscle contraction generates tension and bone deformation, leading to metabolic and piezoelectric stimuli responsible for attracting minerals to the bone matrix. For this, sufficient workload application is required[11,14,16-20]. However, physical exercise with high intensity and high impact is usually related to the risk of falling, being contraindicated for populations that already have bone mass impairment[11,21]. Therefore, the exercise prescription for such populations must employ an optimized relationship between intensity and volume, besides preoccupation with the type of exercise.

An alternative to this context may be the application of moderate swimming exercise intensity, which does not involve excessive impact and risk of fall[21,22], but the time of continuous exercise enough for physiological adaptation. Yet, its beneficial role on bone tissue is inconclusive[23], possibly due to the lack of precise exercise prescription regarding intensity and volume. One way to objectively classify the continuous exercise intensity is through the domains of intensity, defined by Faude et al.[24] as low, moderate, and high. The moderate domain is an exercise intensity characterized by the balance between lactate production and removal, keeping stable the lactate blood concentration. So, continuous physical exercise can be maintained for a prolonged period at moderate intensity[24,25]. However, the literature lacks the studies with objective determination of exercise intensity, volume, and individual load adjustment along time on bone tissue. Another relevant concern is regarding the degree of FR. Literature has extensively used severe schedules, such as 30%[8,26] or more, leading to unhealthy consequences.

So, in order to fine adjust such exercise and FR parameters, looking for better applicability and minor undesirable consequences, this study aimed to apply a model of continuous swimming training at individually determined moderate intensity to investigate its effects on the bone tissue of animals subjected to 20% of FR. We hypothesize that a less severe FR regimen than usual would be enough to impact body mass, visceral fat, and BMD, and moderate-intensity exercise training will prevent such BMD reduction.

Methods

Animals

This study used 40 female Wistar rats *(Rattus norvegicus albinus)* obtained from the central bioterium of the Federal University of São Carlos. The animals were housed in the Neuroendocrinology Laboratory bioterium following cycles of 10 hours light and 14 hours dark, at 22±2ºC, and receiving commercial food for rodents and water "ad libitum" until the beginning of the experiment (45 days old). The experimental procedures were conducted after approval of the institutional Ethics Committee on the Use of Animals to research under protocol no.: 1556060417.

Experimental design

The animals arrived at the bioterium with 45 days old to familiarize with the environment and were randomly distributed into four groups: control group (CG: n=10), food restriction group (FRG: n=10), exercised group (EG: n=9), and food restriction/exercised training group (FREG: n=11), containing a maximum of 5 animals per cage. The estrous cycle was not observed once we performed only chronic analysis for quite stable parameters. At 95 days old, EG, and FREG initiated an aquatic environment adaptation, and subsequently at 108 days old, were subjected to the critical load test (CLT). Immediately after that, the animals FRG and FREG started the 20% food restriction schedule for the amount ingested by the control group. The CLT was reapplied in the fifth and ninth week for exercise intensity adjustment.

Food intake measurement and FR schedule

Twenty-four-h food intake was measured to determine 20% restriction of food for FRG and FREG in relation to the control group. Data were relativized by body mass, being weekly adjusted along with the experiment. The food was systematically available from 6 p.m. every day.

Aquatic adaptation

The EG and FREG were submitted to the aquatic environment adaptation using a protocol adapted from previous studies[27],[28]. The aquatic adaptation lasted for 6 days, with progressive time (5 to 20 minutes), depth water (10 to 80 cm), and overload exposure (0 to 3% of body mass). The procedures were performed in an individual opaque cylindrical tank with an 80 cm column of water, 30 cm of diameter, and temperature maintained at 31±1°C, following the guidelines of the American Physiological Society[29].

Critical load determination and swimming training protocol

To determine the critical load intensity (CLi) for exercise prescription, the animals were individually subjected to the CLT. The animals performed 4 efforts to exhaustion between two and ten minutes, with the criterion for exhaustion being determined by Beck & Gobatto[30]. The time and load data were subjected to linear regression based on the third model proposed by Gobatto et al.[27], in which the linear and angular coefficients corresponded to the anaerobic swimming capacity and intensity corresponding to the critical load, respectively. We opted to accept only the coefficient of linear determination (R^2) higher than 0.95. The CLT was applied 3 times to adjust the CLi, in the 1st, 5th, and 9th week.

The animals swam with 80% of the CLi, 5 days a week, 30 minutes daily for 12 weeks, being training load adjusted weekly.

Obtaining and storage of biological material

At the end of 12 weeks, the animals were euthanized by decapitation, according to the American Veterinary Medical Association[31]. Immediately to euthanasia, the right femur of each animal was collected, which was kept in saline solution 0.9% and stored at -20ºC for subsequent biophysical and biomechanical analyses. The retroperitoneal, mesenteric, and perigonadal (visceral) white adipose tissues were extracted to record the total mass (g).

Bone tissue analysis

The bones were kept in distilled water and remained 24 hours in a desiccator for the air removal from inside the trabecular bone. Subsequently, it was measured wet and immersed weight for biophysical parameters determination. Subsequently, bones were submitted to a three-point bending test performed at Instron 4444, which records the data in the Instron Series IX software referring to the applied force and deformation of the bone. The femur remained bisupported in the metaphyseal regions, being load applied at the center of each bone in the anteroposterior direction. Force was applied perpendicularly to the longitudinal axis at a speed of 0.5 cm/min until bone rupture. A graph was obtained corresponding to bone deformation to determine the maximum load, displacement at maximum load, tenacity and stiffness values. Subsequently, samples were exposed to 100ºC for 24 hours to dehydration and dry weight identification. To obtain the ashes weight, bones were incinerated at 800ºC for a period of 24 hours in a muffle[32,33]. The mineral density was obtained by calculating the equation:

ashes weight (g/cm³) / volume ($BMD = AW / VOL$),

While the volume was calculated according to:

Wet weight - immersed weight (cm³) / water density ($VOL = WW – IW / WD$).

The calculations were based on the Archimedes principle[34].

Statistical analysis

The results were presented as mean±standard deviation. The data were submitted to Shapiro-Wilk's normality test, allowing parametric statistics usage. The data from critical load intensity were subjected to two-way factorial analysis of variance for the main effects of exercise training (three levels: first, second and third assessments) and food restriction (FR: two levels: EG and FREG). The data from bone tissue were subjected to two-way factorial analysis of variance for the main effects of PT (two levels: CG and FRG vs EG and FREG) and FR (two levels: CG and EG vs FRG and FREG). The data from body mass were subjected to a three-way factorial analysis of variance for the main effects of exercised training (two levels: CG and FRG vs EG and FREG), FR (two levels: CG and EG vs FRG and FREG) and, time (before vs after interventions). When appropriated was used the post hoc of Newman-Keuls. The effect sizes (ES) were calculated according to Cohen[35]. A significance level of 5% was established for all analyses and used the Statsoft Statistica software.

Results

Critical load intensity

The FR did not promote difference in critical load intensity ($F=1.59$; $p=0.21$), but there was PT ($F=5.87$; $p<0.01$) in the course of the three tests (Table 1).

Body mass and visceral fat

Figure 1 presents the body mass measured before and after 12 weeks of FR and PT. At the end of 12 weeks of the experiment, FR significantly reduced body mass ($F=126.0$; $p<0.01$), with no PT significant effect ($F=1.5$; $p=0.22$).

Figure 2 shows the visceral fat extracted at the end of 12 weeks of the experiment. FR and PT promoted reduction of absolut ($F=220.3$; $p<0.01$ and $F=28.0$; $p<0.01$, respectively) and relative visceral fat ($F=152.5$; $p<0.01$ and $F=25.7$; $p<0.01$), respectively).

Bone parameters

Figure 3 shows the results obtained from femur bone mineral density. The animals submitted to FR suffered a reduction in BMD ($F=12.84$; $p<0.01$), with no effect for PT ($F=0.11$; $p=0.74$).

Table 2 represents the biomechanical outcomes corresponding to femur. FR did not promote significant alteration for maximum load, displacement at maximum load, stiffness and tenacity ($F=1.18$; $p=0.28$; $F=0.53$; $p=0.47$; $F=1.54$; $p=0.22$ and $F=0.23$; $p=0.63$, respectively). PT did not promote significant alteration for maximum load ($F=0.84$; $p=0.36$), displacement at maximum load ($F=0.20$; $p=0.65$), stiffness ($F=0.49$; $p=0.48$) and tenacity ($F=0.06$; $p=0.79$).

Table 1 - Three critical load tests (CLT) applied to determine critical load intensity (%body mass) in exercised (EG) and food restriction/exercised (FREG) female rats.

	EG	FREG
1º CLT	7.54±1.01	7.51±1.25
2º CLT	7.69±0.59	7.75±0.88
3º CLT	7.11±1.21	5.97±1.29*

Values expressed as mean and standard deviation. *p<0.05 in relation to all other groups and, for comparisons with significant difference, lower ES in relation to EG (3º CLT) = 0.91.

Table 2 - Biomechanical outcomes of the femur in control (CG), exercised (EG), food restriction (FRG), and food restriction/exercised (FREG) from female rats.

	CG	EG	FRG	FREG
Maximum load (N)	170.75±13.93	168.90±11.61	177.99±12.85	171.52±12.48
DML (mm)	0.76±0.20	0.73±0.12	0.79±0.10	0.78±0.17
Stiffness (N/mm)	500.18±66.07	486.11±44.19	519.02±34.02	509.27±47.58
Tenacity (J)	0.12±0.02	0.12±0.03	0.11±0.02	0.12±.0.03

Values expressed as mean and standard deviation. DML: displacement at maximum load; N: newton; mm: millimeter; J: joules.

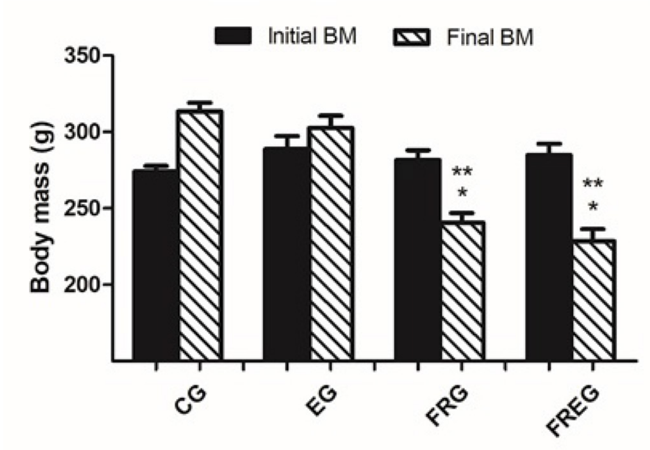

Figure 1 - Data from initial body mass showed no difference between groups (p>0.05) in control (CG), exercised (EG), food restriction (FRG), and food restriction/exercised (FREG) from female rats. Comparing data from final body mass, *p<0.05 in relation to CG and **p<0.05 in relation to EG, showing the lower ES of 3.19. When analyzed initial versus final for the same group were found significant differences for all (p<0.05). BM: body mass; g: grams; mg: miligram.

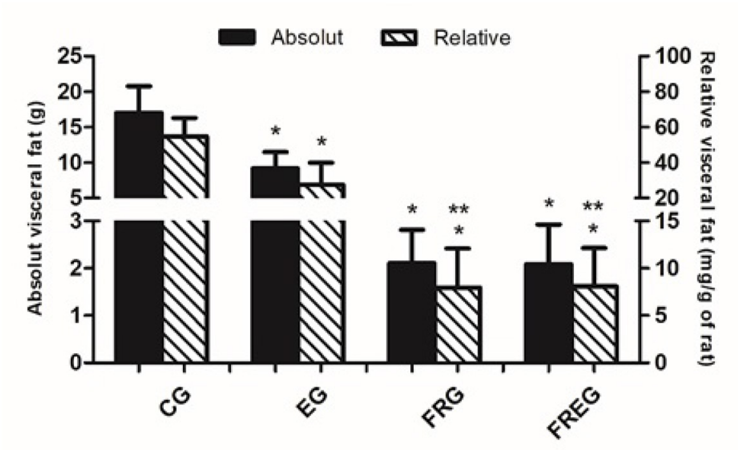

Figure 2 - Data from relative and absolut visceral fat in control (CG), exercised (EG), food restriction (FRG), and food restriction/exercised (FREG) from female rats. Values expressed as mean and standard deviation. *p<0.05 in relation to CG and **p<0.05 in relation to EG. g: grams; mg: milligrams. All comparison among groups at the same moment. The lower ES for the absolut visceral fat (EG in relation to CG) = 2.61. Lower ES for the relative visceral fat (FREG in relation to EG) =

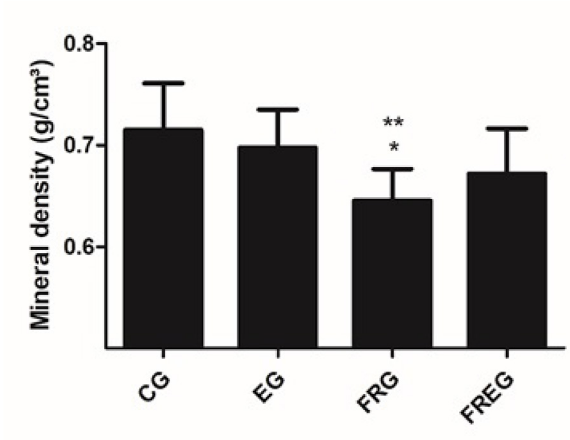

Figure 3 - Data from femoral mineral density in control (CG), exercised (EG), food restriction (FRG), and food restriction/exercised (FREG) from female rats. Values expressed as mean and standard deviation. *p<0.01 in relation to CG (ES = 1.80) and **p=0,02 in relation to EG (ES = 1.51)

Discussion

The main findings of this study were the reduction of BMD in female animals subjected to 20% FR and its prevention when combined with a 12-week PT. Such an issue is particularly important to be studied in the female. Despite the development of bone diseases in males, the female is more affected by them[12].

The reduced supply of food involves a lower intake of proteins, fats, carbohydrates, and minerals that, according to duration and intensity, promotes substrates redirection among tissues, being one factor to reduce the bone mass[36]. In addition, the lower energetic flux is noticeable to the sympathetic nervous system and may have as consequence a reduction of insulin and thyroid hormones secretion. These hormonal changes decrease the cellular metabolic rate to save energy, inducing a reduction in spontaneous physical activity[37]. It may also reflect in a greater expression of PPARy in mesenchymal stem cells and receptor activator of nuclear kappa-B ligand (RANKL), stimulating bone resorption by osteoclasts[10,11]. These are possible explanations of the 9.8% reduction in BMD when comparing FRG with CG, as well as Talbott et al.[10] reported a reduction of 32-35% applying energetic restriction more intense of 40% in female rats after 9 weeks. This demonstrates the consequences of bone tissue may be proportional to the intensity of FR.

Although the present study found a difference in BMD in the animals submitted to restriction, the 20% protocol did not cause significant alterations in the biomechanical bone parameters like the maximum load, displacement at maximum load, stiffness, and tenacity. Despite the reduction of BMD, the bone maintained the structural capacity to withstand load and deformation with conditions similar to the healthy control group, corroborating with the study conducted by Hattori et al.[38], that applied in male rats 30% of FR and voluntary running training which was measured from the daily rotations for 13 weeks. These results showed that the mechanical force of the bone is not easily altered despite other changes.

Over 12 weeks, it was possible to demonstrate a reduction of body mass in animals submitted to FR of 20%, as expected, corroborating with other studies such as Aikawa et al.[8], Beck et al.[37] and Yanaka et al.[26] who applied a more intense protocol than our, using 30%, 50%, and 30%, respectively. It is known the relationship between body mass reduction and BMD reduction[10,14], possibly because it exerted less impact with the consequent decrease of the piezoelectric effect[19], which could explain FR result regarding BMD. One of the contributing factors for body mass reduction was the absolute visceral fat reduction of FRG in relation to the CG ($p<0.01$) and EG ($p<0.01$), showing the efficiency of the protocol of 20% FR for such aim. The animals submitted to swimming exercise training did not present significant alterations in body mass, but significantly showed a reduction of absolute ($p<0.01$) and relative visceral fat ($p<0.01$), as well as FR reduced it ($p<0.01$). The positive influence of both effects, exercise training and FR, is of massive importance once visceral fat is associated with increased risk of diseases such as atherosclerosis, diabetes, hypertension, among others that have a growing global index[6].

The individualized moderate-intensity exercise training proposed was effective in preserving the BMD caused by FR, showing that such exercise protocol possibly acted by suppressing the responsible pathways to activate the osteoclasts, displaying a more potent effect despite the energy deficit[11]. As already mentioned, though most of the literature brings opposite results regarding this modality[17,39,23], we found that our swimming protocol with moderate intensity was efficient, probably due to the activation of the bone mechanotransduction process. During the muscle contraction, mechanical signals are emitted through cytoskeleton proteins and integrins up to the cellular level, this intracellular signal can stimulate the genetic machinery within the nuclei. In this context, the propagation of signals through the WNT-β-catenin pathway increases the osteogenic effect. Besides, physical exercise can inhibit the transcription factor FOXO (responsible for suppressing the WNT pathway) by activating PI3K – AKT – mTOR and increasing the flow of fluids into and out of the matrix[11]. Based on our bone tissue results, seems that our training protocol was enough for such cellular activation.

Not all physical activities or exercise training models indeed conducted to positive results on BMD. Aikawa et al.[8], Yanaka et al.[26], and Yanaka et al.[9] submitted the female rats to food restriction of 30% and voluntary running on the wheel in individual cages for 7, 18, and 18 weeks, respectively, and found no protective effect of running exercise on bone tissue. Possibly such physical activity features were not enough to promote benefits, then female bones were still significantly affected by FR. Hattori et al.[38] did not report a statistical difference between groups regarding BMD after 13 weeks of 30% FR and voluntary running in male rats, reinforcing the idea that when bone tissue is the target, regardless of sex, the physical training must be accordingly prescribed instead just elicit physical activities exposition. A previous study mentions that swimming does not allow enough stimuli to be beneficial to the bone[40].

Likely, the results found by some experiments occurred due to the lack of individualization and load adjustment throughout the training, besides the absence in establishing a daily training duration, as found in our exercise protocol. The 80% CLi is considered moderate-intensity once is slightly under maximal lactate steady state[23,41]. Certainly, such intensity can be indicated for most any population, even those with bone impairment, such as osteoarthritis[21] or osteoporosis, which could have aggravated damage if subjected to exercises with high impact, high overload, and which offers the risk of fall[22]. Not only by the individualized intensity we used but also by the daily training duration, periodic overload adjustments, and the recovery of one session to another were advantageous factors for such results and that should be considered according to Burr et al.[42], besides a 12-week training period.

Despite exercise training benefits on bone and visceral fat outcomes, we did not find improvements in aerobic capacity in our study. One possible explanation is that animals were kept in standard cages that do not resemble the natural habit, inducing animals to reduce their physical activities and consequently compromising the improvement of aerobic capacity[43]. These pieces of evidence correlate with the studies conducted by Scariot et al.[43] and De Araujo et al.[44], that reports on not having found improvement in the aerobic performance of their animals even after carefully and individualized exercise training prescription. The physical training proposed by De Araujo et al.[44] involved four intensities, being 3 aerobic (60%, 100% and 120% of the minimum lactate) and 1 anaerobic (260% of the minimum lactate) divided into 3 mesocycles for 12 weeks/ 6 days of training per week. While in the study of Scariot et al.[43] the animals swam 40 minutes/5 days/12 weeks with 80% of the anaerobic threshold. Despite not improving the aerobic capacity, our exercise training was enough to maintain such parameter even under circumstances that commonly induce regression[43,44]. Despite FR did not lead to a significant effect on CLi, FREG was lower than all other groups, showing the potential effect of low energy availability on aerobic capacity[45].

Despite methodological care, our study is not out of criticism. The estrous cycle of the animals was not verified due to the objectified analyses to be chronic and on stable parameters, therefore, the acute influence of the cycle does not interfere in the final result. Regardless we used animal model, it is known about physiological similarities when compared to the human, making promising the clinical advances concerning bone diseases[46].

Conclusions

20% FR positively modulates the adipose tissue, however, conducted to impairments on bone tissue of the female rats. When we reconciled with the proposed swimming training of individualized intensity and periodically adjusted overload, we found fat loss and avoided the loss of BMD in a highly safe exercise protocol.

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Corresponding author

Wladimir Rafael Beck
Federal University of São Carlos, Department of Physiological.Washington Luis - Km 25. 13565-905. São Carlos/SP. Telephone: +55 16 3351-8964.
Email: beckwr@ufscar.br

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Erratum

In the article "***Moderate intensity swimming training on bone mineral density preservation under food restriction in female rats***", published in volume 26, number 4, 2020: DOI: http://dx.doi.org/10.1590/S1980-6574202000040062 and identification e10200062.

In the ***Affiliations***:

Where it reads: [3]Universidade do Oeste Paulista, Faculdade de Medicina do Jaú, Jau, SP, Brasil.
Should be: [3]Universidade do Oeste Paulista, Faculdade de Medicina do Jaú, Jaú, SP, Brasil.

In the ***Abstract***:

Where it reads: At 95 days.
Should be: At 95 days,

In the ***Experimental design***:

Where it reads: At 95 days old, EG, and FREG
Should be: At 95 days old, EG and FREG

Where it reads: in the fifth and ninth week
Should be: in the 5th and 9th week

In the ***Critical load determination and swimming training protocol***:

Where it reads: in the 1st, 5^{th}, and 9th week
Should be: in the 1st, 5th, and 9th week

In the ***Bone tissue analysis***:

Where it reads: measured wet and immersed weight for biophysical
Should be: measured wet (WW) and immersed weight (IW) for biophysical

Where it reads: To obtain the ashes weight,
Should be: To obtain the ashes weight (AW),

Where it reads: ashes weight (g/cm³) / volume (BMD = AW / VOL), While the volume was calculated according to: Wet weight - immersed weight (cm³) / water density (VOL = WW – IW / WD).
Should be: ashes weight / volume; g/cm³ (BMD = AW / volume; g/cm³). While the volume was calculated according to: wet weight - immersed weight / water density; cm³ (Volume = WW – IW / water density; cm³).

In the ***Bone parameters***:

Where it reads: stiffness and tenacity
Should be: stiffness, and tenacity

Where it reads: stiffness (F=0.49; p=0.48) and tenacity
Should be: stiffness (F=0.49; p=0.48), and tenacity

In the ***Table 1***:

Where it reads: Three critical load tests (CLT) applied
Should be: Three critical load tests applied

Where it reads: lower ES in relation to EG (3º CLT) = 0.91.
Should be: lower ES in relation to EG (3º CLT) = 0.91. CLT: critical load test.In the ***Figure 1***:

Where it reads: body mass; g: grams; mg: miligram.

Should be: body mass; g: grams.

In the ***Figure 2*****:**

Where it reads: (FREG in relation to EG) =
Should be: (FREG in relation to EG) = 2.333.

In the ***References*****:**

Where it reads: 19. Menkes A, Mazel S, Redmond RA, Koffler K, Libanati CR, Gundberg CM, et al. Strength training increases regional bone mineral density and bone remodeling in middle-aged and older men. Journal of Applied Physiology. 1993;74(5):2478-2484, 1993.
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Where it reads: 28. De Lima AA, Gobatto CA, Messias LHD, Scariot PPM, Forte LDM, Santin JO, et al. Two water environment adaptation models enhance motor behavior and improve the success of the lactate minimum test in swimming rats. Motriz: Revista de Educação Física. 2017; 23.
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