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ORIGINAL ARTICLE

Comparison of the effects of two resistance training regimens on muscular and bone responses in premenopausal women

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Abstract
Summary A 28-week resistance training with linear periodization was compared with an undulating model in 27 premenopausal women. In both groups, bone mineral density (BMD) was not changed but muscle strength increased, and there were changes in anthropometrical and muscle damage parameters, indicating that in this population, these models are similar concerning these variables.
Introduction This study seeks to compare the effects of resistance training with undulating versus linear periodization on BMD, muscle strength, anthropometrical variables, and muscle damage parameters in premenopausal women.
Methods Twenty-seven females (39.6±0.41 years, mean± standard error), without osteopenia or osteoporosis and without calcium supplementation, were randomly assigned either to a linear periodization group (LPG, n=14) or to an undulating periodization group (UPG, n=13). The subjects were trained three times a week for 28 weeks. Lumbar spine and femoral neck BMDs were measured through dual-energy X-ray absorptiometry. Maximal and submaximal dynamic muscle strengths were measured through the 1-RM and 20-RM tests, respectively. Anthropometrical (body mass, skinfolds, and perimeters) and muscle damage parameters were assessed through serum creatine kinase (CK) and delayed-onset muscle soreness (DOMS).
Results BMD remained unchanged in both groups, despite significant increases in maximal (LPG, 37–73%; UPG, 40–70%) and submaximal (LPG, 82–114%; UPG, 70–102%) muscle strength. The perimeter of the distal thigh was increased (about 1.7 cm) in both groups. CK and DOMS were greater in the first mesocycle than in the subsequent ones. After the 1st training session in each mesocycle, 24 and 48 h CK was increased as compared to pretraining values.
Conclusions The resistance training of 28 weeks increased muscle strength in both training groups with no difference in BMD or in the occurrence of muscle damage.

Keywords Bone densitometry · Creatine kinase · Muscle damage · Osteoporosis · Periodization

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Introduction

It is believed that an appropriate resistance training program is beneficial to women's bone health, even before they reach the menopause and undergo major loss of bone mass [1–3]. A number of resistance training models have assessed the effect on bone mineral density (BMD) [4–7], strength gains [8, 9], and body composition [10, 11]. However, a resistance training model becomes inefficacious if it poses any risk of muscle damage.

A most efficacious model of resistance training (periodization, volume, and intensity) for increasing BMD and thus, preventing osteopenia and osteoporosis without risk of muscle damage, is still unknown. Studies of muscle strength are scarcer among premenopausal women without bone loss and not on calcium supplementation or estrogen replacement therapy [12].

In resistance training, depending on the magnitude of the overload to which the muscle is submitted, there may be muscle damage whose clinical manifestation is soreness. Because such pain usually appears within 24–72 h after the exercise [13, 14], it is known as delayed-onset muscle soreness (DOMS). The risk of damage increases when the training load surpasses the tolerance of the conjunctive and contractile tissue involved in the performed contraction [15, 16]. DOMS is accounted for by tiny lacerations to the muscle tissue, with concomitant release of enzyme creatine kinase (CK) from the muscle into the blood [17, 18]. So, CK serum level is used as an indicator of muscle damage [19, 20].

As there is no consensus on which model of resistance training is more beneficial concerning BMD [7, 12, 21] with minimal risk of muscle damage, models of resistance training to optimize bone health without impairing the musculature have been investigated. Using a 16-week progressive exclusively eccentric resistance training of low or high intensity in 18–28 year-old females, Schroeder et al. [22] found an increase in the maximal force of up to 40% without an increase of BMD, despite a greater increase of the bone mineral content in the spine only in the low-intensity training group (several repetitions). Serum CK was increased in the high-intensity training in the first 4 weeks followed by a decrease in the subsequent weeks, while the increase observed in the low intensity group persisted along the first 8 weeks. The DOMS results were in agreement with the serum CK ones.

It is assumed that resistance training of longer duration including concentric as well as eccentric contractions beginning at a high intensity, such as the one of undulating periodization, promotes a greater increment of BMD and muscle strength than one of linear periodization, perhaps, compromising the DOMS. The purpose of this study was to compare the effects of two 28-week resistance training regimens on BMD, dynamic muscular strength, muscle damage parameters, and anthropometrical parameters of premenopausal Caucasian women.

Methods

Subjects

The women were recruited through invitations by the author and gynecologists who knew about the research, as well as through posters placed around Erechim, the city where the study took place (Erechim, Rio Grande do Sul, Brazil). The volunteers were informed about the procedures of the study and signed a written free consent form approved by the Ethical Research Committee of the Federal University of Rio Grande do Sul. Thirty females were randomly assigned to one of two training groups: linear periodization (LPG, $n=15$) and undulating periodization (UPG, $n=15$). All women were Caucasian, between 35 and 44 years old, and premenopausal. None of them was a smoker, a regular drinker (two to three doses of alcohol per day), or was on hormonal replacement therapy. All of them were out of the range for osteopenia or osteoporosis, i.e., Z-score≤ -2.0, according to the standards of the International Society for Clinical Densitometry [23]. Also, they had not participated in any systematic physical training in the previous 6 months, and they did not have any physical condition that would contraindicate the training. Table 1 shows the physical characteristics of each group who were similar in age, height, and body mass.

Screening

About 10 days before the training, the volunteers were screened through the PAR-Q-YOU questionnaire. They were also questioned about the regularity of their menses as well as 3 months after the training was begun so as to ensure that none of the participants was entering the menopause.

The anthropometrical assessment, which was repeated after the training, consisted in measuring body mass (scale ARJA, precision 0.1 kg), three (triceps, supra-iliac, and abdomen) skinfolds (adipometer SANNY, precision 0.5 mm), and waist, abdomen, hip, and proximal, mesofemoral, and distal thigh circumferences (metric ribbon SANNY, precision 1 mm).

A cardiologist performed a resting electrocardiogram and consented for the practice of resistance training. A nutritionist carried out a food intake survey of 3 days prior and after the training period. The participants were individually instructed to continue their usual diet during the study. We calculated the mean 3-day intake of carbohydrates, proteins, lipids, calcium, phosphorus and magnesium, as well as the energy consumption in kilocalories (kcal), using the software NutWin ("NutWin 1.5, Editora Metha Ltda).

Bone densitometry

Bone densitometries were measured within 10 days before and after the training. The DXA technique (LUNAR, model DPX-alpha) was used to evaluate BMD of lumbar spine L1–L4 (DMO_{L1L4}) and right femoral neck (DMO_{neck}).

Maximal and submaximal strength tests

To evaluate the maximal dynamic muscular strength, women were submitted to the 1-repetition maximum (1-RM) test for each exercise of the training. The same exercises were used to test the 20-RM. The concentric and eccentric phase of each trial was controlled (metronome SEIKO, resolution 1 Hz) to last 2 s. The recovery time between each trial (three at most) was 4–5 min, controlled by digital chronometer (TECHNOS,

Table 1 Pretraining anthropometrical characteristics and age by group

Variables	LPG(n=14) Mean±SE (minimum–maximum)	UPG(n=13)	p
Age, years	39.5±0.60 (36–44)	39.7±0.59 (35–43)	0.8212
Height, cm	158.8±1.64 (149–167)	162±1.29 (152–171)	0.1306
BM, kg	58.9±1.69 (45.6–69.7)	58.2±1.41 (50.4–65.1)	0.6950
BMI, kg m^{-2}	23.3±0.49 (19.2–25)	22.1±0.22 (19.6–24.8)	0.0289

SE standard error, *LPG* linear periodization group, *UPG* undulating periodization group, *BM* body mass, *BMI* body mass index

model YP2151), as recommended [24, 25]. If the three trials were insufficient to estimate the load, the test was repeated 48 h later. Three sessions of familiarization with the 1-RM and 20-RM tests were performed in all weight lifting equipments (GERVASPORT, resolution of 1 kg), as inexperience can compromise the test quality [26]. To estimate the starting loads in the 1-RM tests, we used the percentages of body mass, as recommended [27]. After the first trial, the load was revised according to the indexes proposed by Lombardi [28].

Muscle damage parameters

The serum CK concentration was measured at the first training session of each mesocycle, i.e., on the day the load was increased. Blood collections were performed before (pre) and after 24 (post 24 h) and 48 (post 48 h) h of the training session. Five millimeters of blood were drawn from the antecubital vein and centrifuged (CELM, model LS3plus) for the CK analysis (BIOSYSTEM commercial Kit) in serum (ROCHE, model COBAS MIRA PLUS; ROCHE standard and calibrator). The BIOSYSTEM Kit uses a measure system in units per liter and reference values of 26–155 U L^{-1} for women.

To evaluate the DOMS, we used Borg's CR10 scale suggested by Borg and Kaijser [29]. The volunteer reported the degree of perceived muscle soreness at rest during an isometric contraction and by palpation, 48 h after the first training session of each mesocycle, according to Kauranen et al. [18].

Strength training

In both groups, the training occurred three times a week on alternate days for 28 weeks. Each training session lasted from 70 to 90 min, depending on the mesocycle. Before the strength exercises, the women warmed up (10 min walk), stretched (5 min), and performed a set of 15 repetitions with 50% of the load of each exercise, as recommended [3]. The 10-minute stretching was repeated at the end of each session. Each woman was always supervised by the researcher or a trained student to ensure the quality and safety of the training.

In the first half of the training period (14 weeks), the exercises for both groups followed one and the same order: leg press 45°, abdominal chair, hack 45°, waist (cross over), hip abduction, bench press, hip adduction, and rowing. Then, for the sake of motivation, the order of the exercises was changed.

The volunteer proceeded to the next exercise only after completing all sets of the previous exercise. The multiple sets system was followed using the exhaustion set technique, and the time of recovery between the sets of the same exercise was 2 min, except for the arm exercises, which was of 1 min. In the first mesocycle and in the adaptation phase, there was a rest of 1 min between the sets of each exercise; but, there was no rest between the exercises [3, 11]. The speed of each repetition, controlled by metronome, was performed with for 1 s per concentric phase and 2 s per eccentric phase.

At the beginning of each microcycle, a session of tests of 1-RMs for each exercise was performed based on the previous weight for prediction of the next one [30]. To avoid any effect on the pre-CK of the initial session of each mesocycle, the volunteers did not train for about 90 h.

Each model of periodization was based on Baker et al. [31] and characterized as follows: (1) in LPG, the intensity was progressively increased and the volume was reduced over the training and (2) in the UPG, short periods of high volume were alternated with short periods of high intensity over the training. Table 2 shows the volume and intensity of the training in both groups in each training microcycle.

The total training load was measured by summing the weights, sets, repetitions, and sessions of all the volunteers in tons and was similar between groups. For LPG, the mean (±standard error) of the total load was 1,713±93, and for

Table 2 Periodization of 28 weeks of training for LPG and UPG

Mesocycles	Weeks	LPG	UPG
1	1–4	3×20–18-RM	3×20–18-RM
2	5–8	3×18–16-RM	3×12–10-RM
	9–12	3×16–14-RM	3×8–6-RM
3	13–16	3×14–12-RM	4×12–10-RM
	17–20	3×12–10-RM	4×8–6-RM
4	21–24	3×10–8-RM	4×12–10-RM
	25–28	3×8–6-RM	4×8–6-RM

LPG linear periodization group, *UPG* undulating periodization group, *3×* three series, *4×* four series, *RM* repetition maximum

UPG, it was 1,690±81 t, remaining similar at the end of 28 weeks of training.

Statistical analysis

For quantitative variables with repeated measures, we applied the theory of mixed models (MIXED) of statistical software SAS [32], considering the effects of group, time, and group–time interaction. The same was used for serum CK but considering the model with the effects of group, mesocycle, collection, and all interactions between these factors. The detailed analysis for the effects of mesocycle and collection was done through Bonferroni's test.

Since more than 70% of the DOMS responses were negative, we characterized the responses as negative or positive, analyzing them through logistic regression (LOGISTIC procedure of SAS [32]) and considering the effects of group and mesocycle.

The LPG and UPG groups were compared in the increase of loads in percent in the 1-RM and 20-RM tests and the total training load through the Student's t test. The level of significance used for all tests was $p<0.05$.

Results

The study began with 15 women in each group, but two were lost in the UPG and one in the LPG for the following reasons: (1) gestation during the 2nd mesocycle; (2) emergency surgery (unrelated to the training) during the 2nd mesocycle; and (3) personal reasons at the beginning of the 4th mesocycle. Thus, 27 women completed the 28 weeks of training (LPG=14 and UPG=13), and there were no missed sessions.

The participants were instructed not to practice any physical activity other than those in the training program. They were also asked not to change their feeding habits so that this variable would not affect the results.

The feeding habits, as evaluated by the recording of a 3-day food intake (energy, protein, carbohydrate, calcium, phosphorus, and magnesium), showed that daily calcium and magnesium intakes were below the recommended ones (1,000 mg day^{-1} of calcium and 310–400 mg day^{-1} of magnesium). There was no effect of time or time–group interaction, whereas, the effect of group was significant for energy, phosphorus, and magnesium, where the LPG ingested less of these than the UPG.

Dynamic muscular strength

Table 3 shows that there was a significant increase of maximal (1-RM) and submaximal (20-RM) strength in both groups for all exercises, with no significant difference between them (LPG and UPG). The results are expressed as percentages (%Δ).

Table 3 Percentage of increase (%Δ) of maximal strength (1-RM) and submaximal strength (20-RM) per group

Exercises	%Δ (Mean±SE)	
	LPG	UPG
1-RM leg press	48.2±6.07	51.8±8.04
1-RM hack	37.8±7.75	42.4±7.35
1-RM adductor	73.7±9.16	60.7±9.96
1-RM abductor	48.5±6.16	40.5±5.67
1-RM waist	49.0±8.36	55.2±7.64
1-RM abdominal	72.6±7.61	70.1±7.49
1-RM bench press	27.03±4.7	45.9±8.4
1-RM rowing	12.3±1.9	14.32±3.22
20-RM leg press	107±17.3	96.3±12.5
20-RM hack	92.9±8.47	85.5±9.14
20-RM adductor	93.8±9.40	75.0±6.71
20-RM abductor	88.3±7.01	70.0±9.49
20-RM waist	82.3±7.19	72.9±8.23
20-RM abdominal	114±16.36	102±12.14
20-RM bench press	118.1±15.64	85.34±7.6
20-RM rowing	113.1±8.6	78.1±7.5

SE standard error, *LPG* linear periodization group, *UPG* undulating periodization group, *1-RM* one maximal repetition, *20-RM* 20 maximal repetitions

Bone mineral density

Table 4 shows the BMD results before and after the training in each group. The effects of group, time (28 weeks), and time–group interaction were not significant in the analyzed regions.

Anthropometrical parameters

There was no change in body mass for both the LPG (from 58.9±1.7 to 60±1.93 kg) and UPG (from 58.2±1.41 to 59.4±1.6 kg). Also, sum of three skinfolds (triceps, suprailiac, and abdomen) and the circumferences (waist, abdomen, hip, and proximal, mesofemoral, and distal thigh) were similar across the groups and had no effect of the training model, time, and time–group; except for the perimeter of the distal thigh in the effect of time, which increased both for the LPG (from 39.7±0.67 to 41.6±0.67 cm) and for the UPG (from 39.4±0.77 to 40.9±0.77 cm).

Muscle damage parameters

Figure 1 shows the results of serum CK. The effect of group was not significant, but there was a significant effect

Table 4 Pre- and posttraining BMD (in grams per square centimeter) at L1–L4 and neck by group

BMD	Group	Pre Mean±SE	Post	Group *P*	Time	Time×group
L1–L4	LPG	1.207±0.031	1.208±0.031	0.3291	0.8479	0.8221
	UPG	1.241±0.025	1.230±0.025			
Neck	LPG	1.011±0.023	0.999±0.025	0.2909	0.6115	0.9625
	UPG	1.039±0.024	1.025±0.026			

SE standard error, *LPG* linear periodization group, *UPG* undulating periodization group, *BMD* bone mineral density

of the mesocycle and period of collection ($p<0.05$). Bonferroni's test indicated that CK of the first mesocycle (LPG: pre 107±20, post 24 h 152±35, post 48 h 173±43; UPG: pre 120±29, post 24 h 160±45, post 48 h 165±48 U L^{-1}) was greater ($p<0.05$) than in those of the other three mesocycles, regardless of group and collection. Also, the premesocycle mean was smaller ($p<0.05$) than the post 24 h and post 48 h means whatever the mesocycle and the group.

The occurrence of DOMS was greater in mesocycle 1 than in the subsequent ones (Fig. 2). The percentage of the presence of DOMS in the LPG was 64.3% in the 1st mesocycle; 14.3% in the 2nd mesocycle; 26.2% in the 3rd mesocycle; and 21.4% in the 4th mesocycle. In the UPG, the respective results were similar, 66.7%, 10.3%, 15.4%, and 2.6%.

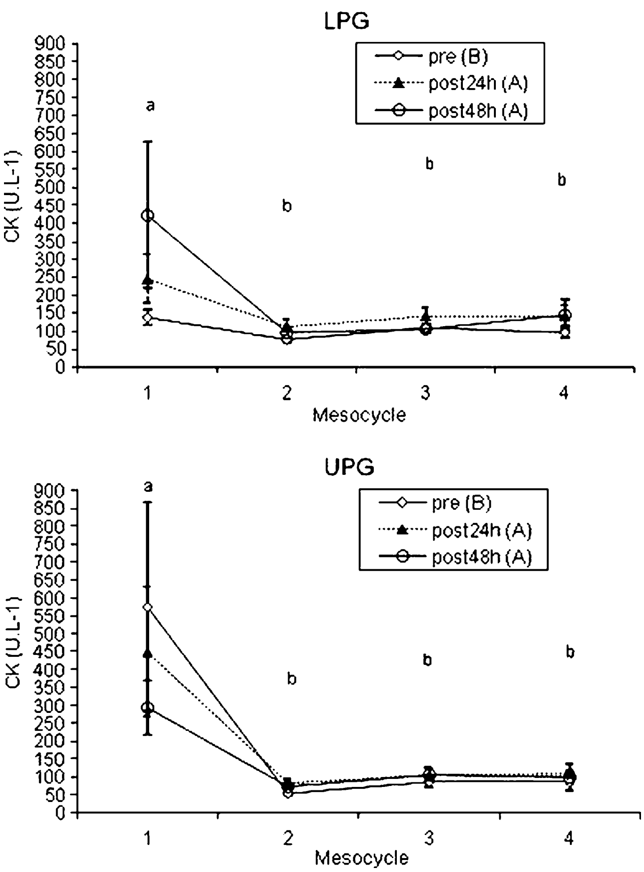

Fig. 1 Means and standard errors of creatine kinase (CK) as a function of mesocycle and collection for each group. Mesocycles *beside distinct lower case letters* differ significantly ($p<0.05$). Collections *beside distinct capital letters* differ significantly ($p<0.05$)

Discussion

This study compared the effect of resistance training for 28 weeks in the models of linear and undulated periodization on BMD, dynamic muscle strength, anthropometrical variables, and muscle damage parameters of premenopausal women. Neither model increased BMD ($BMD_{L1–L4}$ e BMD_{Neck}), despite a significant increase in muscle strength. In both groups, there was an increase in the circumference of the distal thigh (LPG 4.8% and UPG 3.8%). The pattern of muscle damage responses was also similar across the groups. It is difficult to generalize our results because so far most of the studies included postmenopausal women [6, 9] who trained for more than 28 weeks [33, 34] and used calcium supplementation.

At the present training, the exercises focused the regions evaluated in BMD (lumbar spine and proximal femur). In a study with postmenopausal women [35], the BMD was unchanged in a 24-week resistance training prioritizing exercises for the hip and lumbar region. In fact, there was a fall of 1.1% in the total BMD in the high load regimen as compared to the lower load one with more repetitions. This occurred despite calcium supplementation (1,500 mg dia^{-1}), which was not a variable in our study.

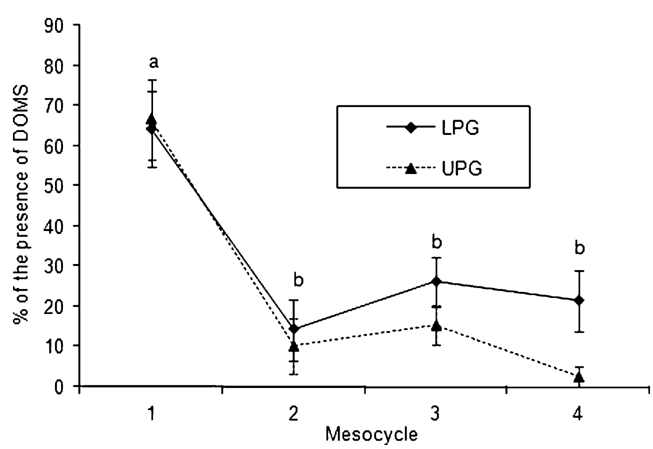

Fig. 2 Percentage of positive evaluations for DOMS as a function of groups and mesocycles. Mesocycles *beside distinct lower case letters* differ significantly ($p<0.05$)

An increase of BMD was found in premenopausal women under resistance training for 24 weeks [5] and 1 year [36]. In the former study [5], women supplemented their diets with calcium (500 mg day^{-1}) during the training period. In the latter [36], women performed not only resistance training but also jumping sequences (high-impact training).

In a review [12], it was suggested that high-intensity resistance training in premenopausal women promotes increase of lumbar BMD. From the several studies analyzed, only in one [37] women were not supplementing their diets with calcium and vitamin D, with the BMD not changing after 20 weeks of training. Women in the present study did not use calcium supplements and ingested much less than recommended: in the LPG, the ingestion of calcium was 61% and of magnesium, 64%; whereas, for the UPG, these were 73% and 81%, respectively.

In a similar magnitude, both training regimens in the present study increased maximal (1-RM) and submaximal (20-RM) strengths. We believed that the undulating periodization would result in a greater increase of muscular soreness. Trials comparing the same periodization models (linear versus undulating) among sedentary women (between 18 and 32 years of age) used shorter trainings (12 [38] and 6 [39] weeks) found no difference between the regimens. One of the trials [38] compared linear periodization training with an undulating one in women and the other [39] compared the two training models in both women and men. The results, still inconclusive, about the efficacy of the periodization models, whatever the gender, are due to the difficulty in equaling the volume and the intensity of the trainings [38]. In the present study, we equaled the groups' total training load.

In our study, the 1-RM for the LPG increased 12% in rowing and up to 73% in the hip adductor, while for the UPG, 14% in rowing and 70% in the abdominal. The increase of strength in the 20-RM for the LPG was 82% in the waist exercise and as much as 118% in the bench press, while for the UPG it was 70% in the hip adductor and as much as 102% in the abdominal. This significant increase may be due to a sedentary lifestyle and inexperience with resistance trainings of our sample. Inexperienced or training naive individuals show more expressive and rapid strength increases [3], even in regimens with various volumes of training [40]. A study comparing athletes and nonathletes taking part in a 21-week resistance training program found a significant increase in the isometric strength (21%) and maximal dynamic strength (19%) among nonathletes, while among athletes the increase was not significant (4% and 7%, respectively) [41]. Therefore, when the purpose of the resistance training was to increase the maximal and submaximal dynamic muscular strength, both models of periodization (linear and undulating) were effective for premenopausal women. Still, we have to consider muscle damage risks.

The hypothesis that the training with undulating periodization would have a greater effect on CK and BMD than the one with linear periodization was not supported. The lower CK at the prelevel of all mesocycles for both LPG and UPG indicates an alteration in the integrity of the sarcolemma caused by the alteration of the training volume and intensity [20]. This was also observed in studies using a diversity of protocols and sessions of resistance training to evaluate the extent of muscle damage through this indirect indicator [14, 18, 42].

Reference values for CK (26–155 U L^{-1}) were surpassed only in the post 24 h and post 48 h collections of the 1st mesocycle (LPG: post 24 h 152±35 and post 48 h 173±43; UPG: post 24 h 160±45 and post 48 h 165±48 U L^{-1}). Such increased serum CK as compared to the other mesocycles, whatever the time of collection (pre, post 24 h, or post 48 h) or the periodization model, shows an adaptation to the training in both groups. This was matched by a higher prevalence of DOMS in the first mesocycle than in the other ones. We do not know about studies evaluating serum CK and DOMS at different mesocycles after periodized models of resistance training. When eccentric [42] or concentric and eccentric exercises of elbow flexion [43] were used, there was a gradual serum CK decrease upon the repetition of the training sessions or after the repetition of the exercises. This adaptation to the training is likely to occur as a result of the strengthening of muscle fibers and activity and synchronism of motor units or increase of ATP supply.

A study [22] investigated the same variables as we did, but among younger women (18–28 years) and using progressive models of high and low intensity of eccentric strength training twice a week for a shorter period of time (16 weeks). The BMD was unchanged, but the bone mineral content of the lumbar region increased by 1.7% ($p<0.05$) in the low-intensity training group. Both groups increased the maximal dynamic strength from 20% to 40% as well as serum CK after 4 weeks of training. Serum CK in the high-intensity training group gradually decreased over the training and eventually reached baseline levels, while in the low intensity group it remained increased until the 8th week.

In the low intensity group, the DOMS was reported as fairly intense or intense in the first weeks but was reduced to moderate or low in the following weeks. In the high intensity group, DOMS was reported as more intense (very intense or extremely intense in the first 2 weeks) and then decreased and was reported as very low or absent. Thus, the eccentric strength training of high and low intensity promotes musculoskeletal adaptations, but since the training with submaximal loads (75% of 1-RM) poses less risk of damage and DOMS, it seems more advantageous.

We expected more significant effects on the anthropometrical parameters but only the circumference of the distal thigh increased in both groups. Despite an insignificant increase of body mass in both groups (~1 kg), the increase of the distal thigh circumference suggests an increment of the muscle mass in this region, since the weights used in the knee exercises were considerably high. Similarly, the circumference of the thighs of 26 males after 8 weeks of training involving high intensities of jumping and squatting was significantly increased as compared to the group that trained with low intensity [44].

The unchanged sum total of skinfolds is in agreement with other studies of females who trained for 24 weeks [2] and even 52 weeks [33] or of males who took part in a progressive training for 8 weeks [25]. On the other hand, Brentano et al. [6] observed a significant decrease in the sum total of seven skinfolds in women after 24 weeks of resistance training.

The maintenance of BMD, not necessarily its increase, may be considered as an optimistic result, as balance bone deposition and absorption. Thus, the resistance training models used in the present study can promote fitness and health of premenopausal women because it may promote musculoskeletal strength and stability. One limitation of the present study was that other measurements, such as cortical thickness and cross-sectional area by peripheral quantitative computed tomography, were not performed and exercise may improve these bone traits despite no change in BMD. Another limitation was that dynamic and static performance tests, such as figure-of-eight running and balance tests were not done. We also suggest that future investigations should follow longer trainings periods, with calcium and vitamin D supplementation, in addition to monitoring the intake of magnesium, phosphorus, and calcium.

Conclusions

- Both models of periodization of resistance training for 28 weeks did not affect the BMD of premenopausal women.
- The trainings with linear and undulating periodization resulted in similar increases in the maximal (1-RM) and submaximal (20-RM) dynamic muscle strength.
- The responses in muscle damage parameters (CK and DOMS) were similar in the two training models. Values above reference values for CK were observed only at the post 24 h and post 48 h collections of the 1st mesocycle. Over the training, the absence of DOMS increased, supporting the hypothesis of adaptation to the training.
- Except for the distal thigh parameter, which was increased in both groups, the two periodization models did not affect the other anthropometrical parameters analyzed.

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Conflicts of interest None.

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