10.61186/aassjournal.1453

Ann Appl Sport Sci InPress(InPress): e1453.

e-ISSN: 2322-4479; p-ISSN: 2476-4981

ORIGINAL ARTICLE

L-Carnitine Supplementation and Moderate-Intensity Exercise Enhanced Bone Metabolism Markers and Muscular Performance in Overweight and Obese Individuals

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Submitted August 06, 2024;
Accepted October 31, 2024.

KEYWORDS
Exercise,
Performance,
Physical Activity,
Body Weight.

ABSTRACT

Background. Optimisation of bone health from exercise interventions and nutritional supplements was recommended. **Objectives.** This study aimed to determine whether L-carnitine supplementation combined with interval exercise training influenced bone metabolism markers and muscular strength and power in individuals with overweight and obesity. **Methods.** This randomized controlled trial study involved sixty-eight participants in four groups, i.e., control (C, n=17), L-carnitine supplementation alone (Lc, n=17), exercise alone (Ex, n=17) and combined L-carnitine supplement and exercise (LcEx, n=17) groups. The participants in Ex and LcEx groups participated in a 60-minute exercise program per day, 3 times a week, for 12 weeks at 50% heart rate reserved for brisk walking and 40-60% for interval exercise training. The participants in the Lc and LcEx groups consumed 1000 mg of L-carnitine supplement daily. The blood measurements for serum alkaline phosphatase (ALP), total calcium, and serum C-terminal telopeptide of type 1 procollagen (1CTP) were measured at weeks 0 and 12. **Results.** The result showed that the LcEx group showed the most remarkable increment in ALP ($p<0.05$) and total calcium ($p<0.05$); however, it showed a reduction in serum 1CTP ($p=0.003$). Regarding muscular performance, the LcEx group ($p<0.05$) also demonstrated a more significant increase in overall measured parameters of dominant knee extension and flexion peak torque and average power at $60o.s^{-1}$ and $300o.s^{-1}$ compared to other groups after 12 weeks. **Conclusion.** Combining L-carnitine supplementation with brisk walking and interval training can enhance bone formation and muscle performance in people with overweight and obesity.

INTRODUCTION

Optimizing peak bone mass early is one of the most essential preventive interventions. Peak bone mass is the higher bone mass attained at a specific skeletal region during life. Many techniques have been developed to prevent bone loss to date. Participation in exercise interventions and appropriate nutritional intake are two ways to combat this issue (1). Physical activities that exert mechanical stress on bone are well-established to benefit skeletal strength and development. According to Karlsson & Rosengren (2), during the developing years, bone mineral content (BMC) and bone mineral density (BMD) can be maximized by embracing weight-bearing physical exercise in childhood and adolescence. Exercise that involves bearing weight is a force-generating activity that applies loads to skeletal regions through routine tasks (3, 4). Weight-bearing exercises such as aerobic, resistance training, interval training, jumping, brisk walking, and other activities can generate a skeletal impact on the bone. Those activities are believed to enhance bone mass when the bone cells react to mechanical load activities during impact landing, resulting in a balanced development of bone resorption and formation (5). Bone turnover has been an effective parameter and specific biochemical indicator in measuring changes in bone metabolism and can be used to quantify the metabolic process of bone (6). The mechanical connection of bones and skeletal muscles has been simplified, with muscles serving as load providers and bones serving as attachment sites. Muscle mass increases tension on the periosteum and collagen fibers at the interface, stimulating local bone formation (7).

Therefore, this study was proposed to evaluate the effects of moderately intense exercise, including brisk walking and interval training, on bone turnover markers in selected population groups. Brisk walking is the type of physical activity that is advised for people who are overweight or obese (8). This exercise is one of the aerobic exercises that improve body fitness and general health, precisely the efficiency and function of human biochemical activities (9). Besides, brisk walking also involves repetitive movement that could promote bone health. Brisk walking is a very easy exercise because it is free and can be done anywhere, especially outside the house. Interval exercise training consists of low-intensity recovery periods intersected between repeated moderate exercise sessions. The traditional 20-second all-out effort followed by a 10-second rest period has given way to several modalities and activities that comprise interval exercise training (10). It is well known that interval training requires repeated jumping motions, high-intensity loading forces, and accelerating and decelerating movements that improve bone health status (11).

Besides regular exercise, enhancing and preserving bone health is also significantly influenced by nutrition. L-carnitine contains an essential amino acid of lysine, which is necessary for bone tissue maintenance and growth. Bone comprises 35% protein and requires a steady supply of amino acids for protein turnover (12). According to Tomé et al. (13), protein and amino acids enhance muscle development and are essential for bone health. L-active carnitine's compound of amino acids aids in the reduction of bone resorption and improves bone formation. Thus, this present study proposed 12 weeks of brisk walking and interval exercise training at 3 times per week, approximately 60 minutes per session, combined with a daily intake of 1000mg of L-carnitine in order to determine whether overweight and obese people have adverse effects on bone health and muscle strength and power. This study aimed to determine whether L-carnitine supplementation combined with interval exercise training influenced bone metabolism markers and muscular strength and power in overweight and obese individuals.

MATERIALS AND METHODS

Study Design. This randomized controlled trial study randomly assigned four groups with pre-test and post-test measurements. Randomization was done by block randomization using computer-based random numbers. A random allocation website (www.randomization.com) was used to allocate randomization among the participants. The manual randomization of the participants was based on gender matching to divide the participants equally in each group. An opportunistic sampling method was used, and each group had an equal number of participants.

The study was conducted under the Declaration of Helsinki and approved by the ethical researcher committee of the Universiti

Sains Malaysia, which granted approval to the study (USM/JEPeM/19100617) for studies involving humans. This study also has been registered for clinical trial registration and the number was TCTR20220830002. Being physically inactive, or not exercising more than twice a week was one of the additional inclusion criteria.

The participants who have health problems such as asthma, stroke, diabetes, heart disease, hypertension, and kidney disease were the exclusion criteria in this study. The participants who fulfilled the requirements for inclusion criteria provided their written approval.

Participants. Sixty-eight overweight and obese (class 1) (BMI between 23-30.0 $kg.m^{-2}$) male and female adults aged 18 to 40 were recruited through posters and social media advertisements in this study. The range of BMI uses in this study is based on Asia Pasific cut off point. In the present study, 20 males and 48 females were divided into four groups equally. Each group was with 5 males and 12 females. The participants were randomly assigned into four groups: control (C), L-carnitine supplementation alone (Lc), exercise alone (Ex), and combined L-carnitine supplement and exercise (LcEx) groups, with 17 participants for each group. Participants in the C and Lc groups did not perform brisk walking and interval exercise training and continued with their daily activities (e.g., walking to work, doing house chores, or gardening). The Ex and LcEx groups participated in brisk walking and interval exercise training throughout the 12-week intervention period. Participants in the Lc and LcEx groups consumed an L-carnitine supplement (1000mg) for 12 weeks (14). During the 12-week study period, all the participants were advised to avoid taking any food, supplement, or product containing antioxidants, carnitine, and vitamin complex.

Blood Sampling and Research Tool.

a) Measurement of Bone Metabolism Markers (ALP, 1CTP, and Total Calcium). At week 0 (baseline) and week 12 (post-test) of the study, 6 mL of blood was withdrawn following 10 hours of overnight fasting (only plain water was allowed). Lab technologist assistants in Sports Science Laboratory conducted blood withdrawal sessions. Blood samples were drawn from participants' medical cubical veins by venepuncture with a 23-gauge needle, and a tourniquet was used on the upper selected arms to make the vein prominent and released as soon as blood flowed into the syringe. Then, blood samples were separated into SST (gel) tubes for all blood parameters. Next, the blood samples were centrifuged using Hettich Rotina RS Centrifuge (Health-Ratina 46RS, Germany) at 4000 revolutions per minute for 10 min at 4oC. The serum obtained after centrifuge was divided into equal portions in a 1.5 mL Eppendorf bullet tube using a disposable plastic Pasteur pipette and then stored at -80 degree celcius in the freezer for analysis.

Blood samples were collected to determine serum ALP (bone formation marker), serum 1CTP (bone resorption marker), and serum total calcium. To determine the level of ALP and 1CTP in samples, the reagent kits of ALP (Human ALP ELISA kit, Shanghai) and 1CTP (Human 1CTP ELISA kit, Shanghai) use a double-antibody sandwich enzyme-linked im-immunosorbent one-step procedure test. Using a pre-coated enzyme well that had already been coated with catalase antibody, the standard, test sample, and HRP-labeled catalase antibodies were applied to the enzyme wells, after which the incubation was completed, and the uncombined enzyme was rinsed away.

The color of the liquid was altered to blue after adding Chromogen Solution A and B before it turned yellow because of an acid reaction. A positive association was found between the concentration of catalase and the depth of color.

The serum total calcium was analyzed using a calorimetric method using commercially available reagent kits (Elabscience, China). The dilute 2.5 mmol/L calcium standard with deionized water was added to a serial concentration. Ten µL of standard solution was taken to the corresponding wells with different concentrations. Subsequently, 10µL of the sample was added to the corresponding wells. Next, 250 µL of working solution 1 was added to each well. The 10 mL MTB reagent and 20 mL alkali reagent were thoroughly mixed at 1 2 as working solution 1. The microplate reader was thoroughly mixed for 30 seconds and stood for 5 minutes at room temperature. The OD value was measured at 610 nm with a photometric microplate reader (Thermo Scientific, Varioskan Flash, Finland, Europe). All the tests were performed according to the manufacturer's guidelines.

b) Isokinetic Muscular Peak Torque (Strength) and Power Measurements. Using an isokinetic

dynamometer (BIODEX multi-joint system 3 pro, New York), the data from the dominant legs' knee flexion and extension joints was collected. The operation instructions of the BIODEX isokinetic dynamometer were followed (15).

Training Protocol. The workout regimen was proposed to consist of 3 sessions per week, lasting approximately 60 minutes each, for 12 weeks of training. The activity began with a 5-minute warm-up that involved stretching. Next, participants walked briskly at a 50% heart rate reserve for 30 minutes. Finally, participants engaged in interval exercise training for 20 minutes. The participant's heart rates (40–60% heart rate reserve) were used to regulate the intensity of the exercise, which was accomplished by switching between the upper and lower limbs (16). The exercise includes donkey kicks, inchworms, alternate touchdown, slide skaters, jump rope, seated knee tucks, basic crunches, shoulder taps, burpees, Russian twist, squats, lunges, mountain climbers, leg raises, and basic crunches (17).

The interval exercise training consisted of four 4-minute "segments". During exercise, participants were required to perform the type of activity as many times as possible in 20 seconds, followed by a 10-second rest period. The interval between each segment was one minute. Thus, the total number of minutes for each segment was 5 minutes. The duration of exercise progressively increased every 4 weeks. The exercise started with two segments equal to 10 minutes for the first 4 weeks and then was increased to three segments equal to 15 minutes. In the last 4 weeks, the participants were instructed to repeat the intervention until four segments; the total duration was 20 minutes. Figure 1 shows that exercise included in the 20-minute interval training exercise.

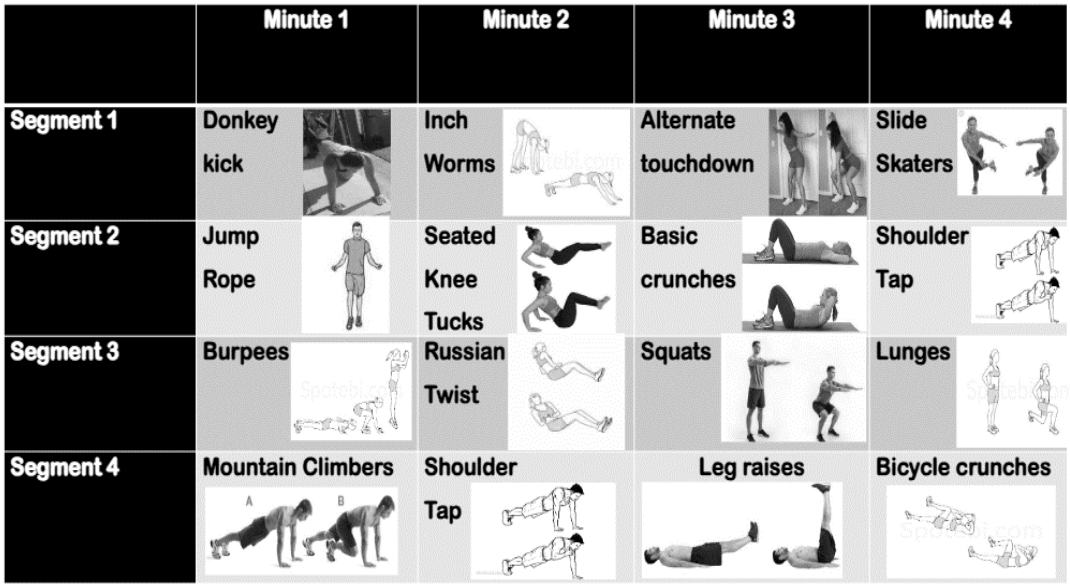

Figure 1. Exercise included in the 20-minute interval training exercise modified from Emberts et al., 2013 study (17).

Data Collection. Sixty-eight participants (males and females, between 18-40 years old) were required to fill out the study information sheet and receive explanations about the study purpose, procedures such as the experimental protocol, and possible risks before being given the consent form. The twelve (12) weeks exercise program was conducted at Sport Complex 2, Universiti Sains Malaysia. Participants' anthropometric, blood pressure, body composition, and waist and hip ratio were measured, and blood samples were taken at weeks 0 and 12.

Statistical Analysis. Statistical Package for Social Science (SPSS) Version 26.0 was utilized for statistical analysis. The normality of the data distribution was analyzed using the Shapiro-Wilk test. Two-way mixed ANOVA was used to analyze all measured parameters to find significant differences between and within the groups. Then, the post-hoc Bonferroni test was employed, and significant differences in the two-way mixed

ANOVA were examined. Furthermore, the mean differences between pre-and post-tests were compared across all groups using one-way ANOVA analysis. The results display all blood parameters as mean ± standard error (SE), while other values are presented as mean ± standard deviations (SD). A statistically significant p-value <0.05.

RESULTS

Anthropometry and Physiological Characteristics of the Participants. Table 1 shows the mean of anthropometric and physiological characteristics between pre-and post-tests of all the groups. Compared with the C group, the Lc, Ex, and LcEx groups showed significant changes in all measured parameters. Lc, Ex, and LcEx groups showed a significant reduction of body weight, body mass index, body fat percentage, fat mass, waist-to-hip ratio, and increment of fat-free mass except for waist-to-hip ratio in the Lc group after 12 weeks of intervention.

Table 1. Anthropometric and physiological characteristics of all the groups.

Variables	Groups			
	C	Lc	Ex	LcEx
Body weight (kg)				
Pre-test	68.3±11.4	71.1±7.7	67.0±10.0	73.7±12.5
Post-test	69.5±11.5[a]	69.3±7.9[a]	65.2±9.5[a]	71.7±12.0[a]
p-value	0.000	0.000	0.000	0.000
Body mass index (kg.m^{-2})				
Pre-test	26.7±2.4	27.7±1.9	26.0±2.4	27.4±2.4
Post-test	27.2±2.4[a]	27.0±2.0[a]	25.3±2.4[a]	26.8±2.3[a]
p-value	0.000	0.000	0.000	0.000
Body fat percentage (%)				
Pre-test	38.5±5.9	39.2±8.5	36.6±7.8	37.4±7.1
Post-test	40.4±5.0[a]	37.6±8.5[a]	33.9±7.7[a]	33.8±7.1[a]
p-value	0.002	0.002	0.000	0.000
Fat mass (kg)				
Pre-test	26.2±6.1	28.3±7.4	24.5±6.3	27.5±6.5
Post-test	27.5±5.5	26.6±7.2[a]	23.1±6.2[a]	24.1±6.1[a]
p-value	0.088	0.002	0.000	0.000
Fat-free mass (kg)				
Pre-test	41.9±8.5	43.5±7.2	42.7±9.2	46.6±10.9
Post-test	40.8±7.8[a]	44.2±7.3[a]	44.4±9.1[a]	49.7±10.7[a]
p-value	0.002	0.000	0.000	0.000
Waist-to-hip ratio				
Pre-test	0.7±0.1	0.8±0.1	0.8±0.1	0.8±0.1
Post-test	0.7±0.1	0.8±0.1	0.7±0.1[a]	0.7±0.0[a]
p-value	0.108	0.425	0.01	0.002

Values are expressed as means ± standard deviations (SD) and means ± standard error (SE) for blood parameters; C: Control group; Lc: L-carnitine supplement alone group; Ex: Exercise alone group; LcEx: Combined L-carnitine supplement with exercise; a: Significantly different from pre-test (p<0.05).

Bone Formation Marker: Serum Alkaline Phosphatase (ALP). Table 2 shows the serum ALP concentration for all the groups. This analysis indicated that the level of serum ALP at post-test was increased significantly statistically in Lc (p=0.000), Ex (p=0.000), and LcEx (p=0.000) groups but was statistically significantly decreased (p=0.017) in C group at post-test.

Meanwhile, Figure 2 displayed the mean difference in serum ALP concentration between the groups' pre-and post-tests. Lc, Ex, and LcEx groups showed a more significant increase value (p=0.000) in serum ALP compared to the C group. Overall, the LcEx group elicited the most remarkable increment (257.6±15.2 pg.ml^{-1}) compared to the Lc (p=0.000) and Ex (p=0.016) groups in serum ALP after 12 weeks of intervention.

Bone Resorption Marker: Serum C-Terminal Telopeptide of Type 1 Procollagen (1CTP). The results of serum 1CTP concentration in all the groups are presented in Table 2.

At the end of the study, there was a significant difference in all the groups after a 12-week intervention period. 1CTP concentration had increased (p=0.000) significantly statistically in the C group and decreased significantly

statistically in Lc (p=0.000), Ex (p=0.000), and LcEx (p=0.003) groups, as shown in Table 2. The mean difference of the serum 1CTP concentration between pre-and post-tests of all the groups was illustrated in Figure 3, and in comparison to the C group, Lc, Ex, and LcEx groups demonstrated higher values of reduction (p=0.000) in serum 1CTP.

Table 2. Bone metabolism markers concentration of all the groups.

Variables	Groups			
	C	Lc	Ex	LcEx
ALP (pg.ml^{-1})				
Pre-test	924.9±57.0	904.8±127.5	802.7±147.6	848.2±195.8
Post-test	852.5±119.7[a]	978.4±149.8[a]	1001.9±190.0[a]	1045.9±173.3[a]
p-value	0.017	0.000	0.000	0.000
1CTP (ng.ml^{-1})				
Pre-test	104.2±22.3	128.7±21.0	131.9±20.9	136.4±14.9
Post-test	132.6±20.0[a]	113.1±16.1[a]	117.9±20.4[a]	119.4±18.5[a]
p-value	0.000	0.000	0.000	0.003
Total calcium (mg.dl^{-1})				
Pre-test	1.9±0.2	1.2±0.1	1.2±0.1	1.2±0.1
Post-test	1.3±0.2[a]	1.6±0.1[a]	2.0±0.1[a]	2.2±0.1[a]
p-value	0.000	0.000	0.000	0.000

Values are expressed as means ± standard deviations (SD); ALP: Alkaline phosphatase; 1CTP: C-terminal telopeptide of type 1 procollagen; C: Control group; Lc: L-carnitine supplement alone group; Ex: Exercise alone group; LcEx: Combined L-carnitine supplement with exercise; a: significantly different from the pre-test (p<0.05).

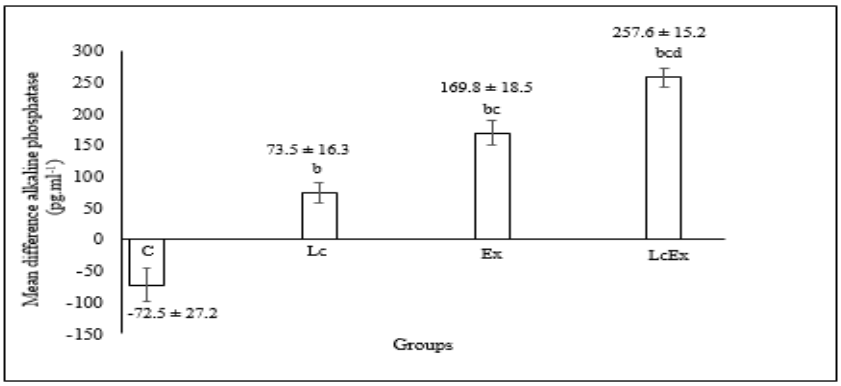

Figure 2. Mean difference of serum bone alkaline phosphatase concentration between pre and post-tests of all the groups. Data presented in mean difference and expressed as means ± standard error (SE); [b]: Significantly different from the sedentary control group (p<0.05); [c]: Significantly different from the L-carnitine supplement alone group (p<0.05); [d]: Significantly different from exercise alone group (p<0.05); C: Control group; Lc: L-carnitine supplement alone group; Ex: Exercise alone group; LcEx: Combined L-carnitine supplement with exercise.

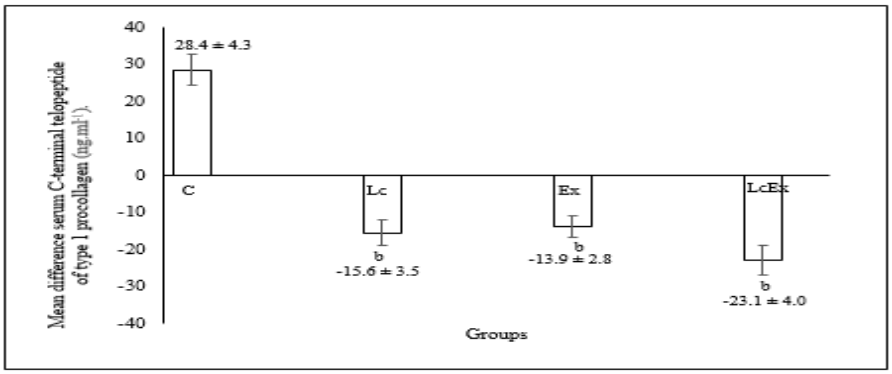

Figure 3. Mean difference of serum C-terminal telopeptide of type 1 procollagen (1CTP) between pre and post-tests of all the groups. Data presented in mean difference and expressed as means ± standard error (SE). [b]: Significantly different from the sedentary control group (p<0.05); C: Control group; Lc: L-carnitine supplement alone group; Ex: Exercise alone group; LcEx: Combined L-carnitine supplement with exercise.

Total Calcium Concentration. The total calcium concentration for all the groups is shown in Table 2. This finding indicated a significant increase (p=0.000) in all the groups at the post-test compared with pre-test except for the C group, which showed significantly decreased (p=0.000) serum total calcium. Figure 4 shows the mean difference in total calcium concentration between the groups' pre- and post-tests. Lc, Ex, and LcEx groups (p=0.000) showed significantly greater increment than the C group displayed in Figure 4. Moreover, the LcEx group also demonstrated the most significant value of increment (1.1±0.2 mg.dl^{-1}) in total calcium concentration compared to the Lc (p=0.004) and Ex (p=0.017) groups.

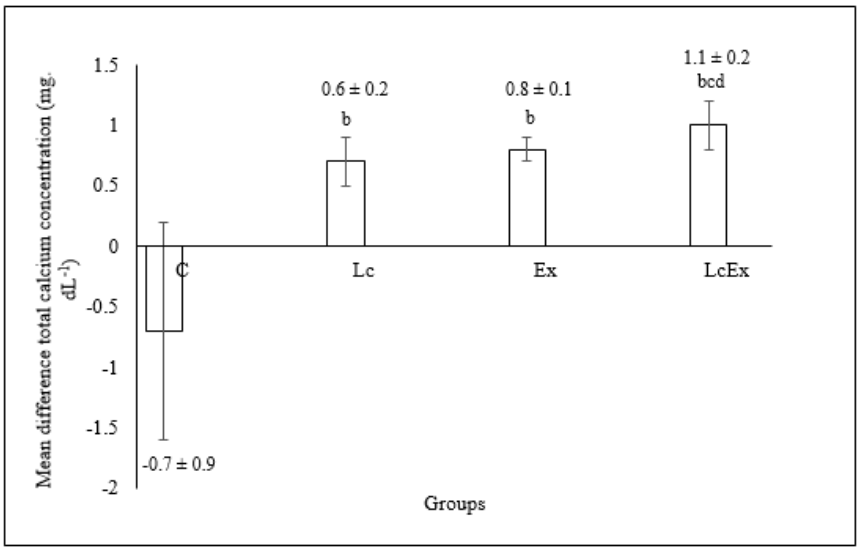

Figure 4. Mean difference in total calcium concentration between pre-and post-tests in all the groups. Data presented in mean difference and expressed as means ± standard error (SE). [b]: Significantly different from the control group (p<0.05); [c]: Significantly different from the L-carnitine supplement alone group (p<0.05); [d]: Significantly different from exercise alone group (p<0.05); C: Control group; Lc: L-carnitine supplement alone group; Ex: Exercise alone group; LcEx: Combined L-carnitine supplement with exercise.

Isokinetic Muscular Peak Torque and Power of Dominant Leg.

Knee extension peak torque at 60os^{-1}: Table 3 shows the means dominant knee extension peak torque at 60o.s^{-1} of the dominant of the participants between pre- and post-tests overtime points. Further analysis showed a significant increment (p=0.002) in Ex and LcEx groups at post-test. Moreover, there was significantly greater peak torque in the Lc (p=0.005), Ex (p=0.000), and LcEx (p=0.000) groups compared to the C group. In addition, the LcEx group showed the most significant peak torque compared to other groups (p=0.000), especially the Lc and Ex groups.

Knee extension average power at 60o.s^{-1}: Table 3 shows the means dominant knee extension average power at 60o.s^{-1} of the dominant leg between pre and post-tests overtime points. There were no significant differences within and between groups after 12 weeks of the intervention.

Knee flexion peak torque at 60os^{-1}: The means knee flexion peak torque at 60o.s^{-1} of the dominant leg of the participants for all the groups is shown in Table 3. After the intervention, Lc, Ex, and LcEx groups displayed changes after 12 weeks of intervention compared to the C group. Further analysis indicated that there were significant increases in Lc (p=0.005), Ex (p=0.001), and LcEx groups (p=0.004) compared to the C group.

Knee flexion average power at 60o.s^{-1}: Table 3 demonstrated the means dominant knee flexion average power at 60o.s^{-1} of the dominant leg between pre-and post-tests of all the groups. Further analysis showed that there were significantly greater values in Lc (p=0.006), Ex (p=0.001), and LcEx group (p=0.001) compared to the C group at the post-test.

Moreover, it was observed that there was a significantly greater value of means dominant knee flexion average power at 60o.s^{-1} of the dominant leg in the Lc, Ex, and LcEx groups (p=0.000) compared to the C group. LcEx group (p=0.000) showed a more significant improvement than Lc and Ex groups.

Knee extension peak torque at 300os^{-1}: This means that the dominant knee extension peak torque at 300o.s^{-1} of the dominant leg of the participants in all the groups is expressed in Table 3. Following up, this interaction shows significantly greater values in the Lc, Ex, and LcEx groups (p=0.01) at the post-test compared to the C group.

Meanwhile, Lc, Ex, and LcEx groups showed greater peak torque at 300o.s^{-1} (p=0.000) compared to the C group.

Table 3. Dominant knee extension and flexion peak torque of dominant legs at pre and post-tests for all the groups.

Variables	Groups			
	C	Lc	Ex	LcEx
Peak Torque Extension 60º.s^{-1}				
Pre-test	115.9±38.4	120.7±43.5	133.0±54.3	140.3±53.3
Post-test	90.6±27.1	126.1±34.8	176.0±31.8^{a}	232.1±28.1^{a}
Mean difference	-25.3±52.2	6.6±59.1^{b}	43.0±48.6^{b}	81.8±56.1^{bcd}
Average Power Extension 60º.s^{1}				
Pre-test	79.9±29.2	64.9±22.2	72.9±28.4	85.6±30.9
Post-test	74.5±21.2	75.2±20.0	92.6±32.9	106.6±151.1
Mean difference	-5.4±40.0	10.2±10.5	19.8±38.8	21.0±159.0
Peak Torque Flexion 60º.s^{-1}				
Pre-test	35.2±3.2	47.6±14.2	55.4±24.7	50.5±16.3
Post-test	33.3±4.3	55.8±16.6^{a}	64.8±24.6^{a}	65.4±22.4^{a}
Mean difference	-1.9±5.5	8.2±8.4^{b}	9.4±13.0^{b}	14.9±9.6^{b}
Average Power Flexion 60º.s^{-1}				
Pre-test	31.6±5.5	34.5±11.5	38.7±16.2	37.1±12.2
Post-test	31.4±6.3	45.5±8.2^{a}	51.3±13.4 ^{a}	65.4±7.0^{a}
Mean difference	-0.2±6.6	11.0±14.2^{b}	12.6±10.7^{b}	28.3±13.7^{bcd}
Peak Torque Extension 300º.s^{-1}				
Pre-test	35.3±3.1	56.4±16.6	53.4±36.7	51.1±30.4
Post-test	35.5±2.9	72.4±31.3^{a}	81.1±26.5^{a}	84.9±26.4 ^{a}
Mean difference	0.1±3.5	15.9±23.8^{b}	27.7±19.2^{b}	33.8±18.1^{b}
Average Power Extension 300º.s^{-1}				
Pre-test	36.1±3.6	95.6±40.1	132.2±71.0	139.8±61.0
Post-test	36.3±3.2	122.2±32.0^{a}	151.7±54.5^{a}	169.5±65.0^{b}
Mean difference	0.2±4.3	26.7±31.4^{b}	19.5±43.3^{b}	29.7±29.5^{bc}
Peak Torque Flexion 300º.s^{-1}				
Pre-test	40.5±7.5	50.5±15.7	56.1±22.7	55.7±20.2
Post-test	33.7±4.7	52.4±17.2	62.5±18.0	64.7±20.8
Mean difference	-6.7±8.9	1.9±11.1^{b}	6.5±19.3^{b}	9.0±14.8^{b}
Average power Flexion 300º.s^{-1}				
Pre-test	34.3±9.7	45.4±21.2	64.4±49.5	51.1±34.9
Post-test	33.0±4.6	55.1±28.4	78.2±37.1^{a}	72.6±30.8^{a}
Mean difference	-24.5±20.4	10.9±21.5	13.8±16.1^{b}	21.5±28.9^{b}

Values are expressed as means ± standard deviations (SD); C: Sedentary control group; Lc: L-carnitine supplement alone group; Ex: Exercise alone group; LcEx: Combined L-carnitine supplement with exercise group. ^{a}: Significantly different from pre-test (p<0.05); ^{b}: Significantly different from C group (p<0.05); ^{c}: Significantly different from Lc group (p<0.05); ^{d}: Significantly different from Ex group (p<0.05).

Knee extension average power at 300o.s^{-1}: The mean dominant knee extension average power at 300o.s^{-1} of the dominant leg of all groups is shown in Table 3. Following up on the significant difference, there was a significant increase after 12 weeks of intervention in Lc (p=0.003), Ex (p=0.003), and LcEx (p=0.001) groups compared to the C group. Regarding the significant difference between groups, there was also a significantly higher value in the Lc group (p=0.000), Ex group (p=0.000), and LcEx group (p=0.000) compared to the C group. The LcEx group exhibited more significant improvement (p=0.021) than the Lc group after 12 weeks of intervention.

Knee flexion peak torque at 300os^{-1}: The means knee flexion peak torque at 300o.s^{-1} of the dominant leg of the participants over time are shown in Table 3. LcEx (p=0.000) group showed the highest increment after 12 weeks of intervention, followed by Ex (p=0.000) and Lc (p=0.009) groups compared to the C group.

Knee flexion average power at 300o.s^{-1}: Table 3 shows the means dominant knee flexion average power of the dominant leg at 300o.s^{-1} of all the groups. Within-group analysis demonstrated a significantly increased (p=0.01) of the measured parameter in the Ex and LcEx groups at the post-test. Moreover, there were significantly greater values in the Ex group (p=0.000) and LcEx groups (p=0.001) than in the C group.

DISCUSSION

Bone Metabolism Markers. The main findings of this study were high increments in serum ALP concentration, a biomarker of bone production, and a reduction in serum 1CTP, a biomarker of bone resorption in the LcEx group compared to other groups. Meanwhile, the Lc, Ex, and LcEx groups increased serum ALP and reduced serum 1CTP compared to the C group after 12 weeks. These findings collectively implied that the combination of L-carnitine supplementation and moderately intense exercise, which included brisk walking and interval training, elicited beneficial impacts on bone metabolism markers by enhancing serum ALP, a bone formation marker, and has a significant effect on reducing 1CTP, a bone resorption marker.

It has been recommended that mechanical loading imposed on specific bone sites creates high stresses in unique patterns over short periods that are frequently repeated to get the best osteogenic impact (18). Brisk walking is a weight-bearing exercise involving repetitive movement of the lower limbs and is considered an intermediate-intensity exercise. Interval exercise combines weight-bearing sports with strength training that requires multi-joint movement alternating between upper and lower limbs and involves repeated jumping motions, high-impact loading forces, and dynamic strength and power. Previous research has found that this type of training benefits bone mass in humans (19, 20). Hart et al. (21) describe bone tissue as a highly adaptable tissue that responds to strain demands posed by physical activity. The intense strains created by muscular contractions imposed on bone tissue during the training enhance bone metabolism and promote osteogenesis. As a result, this exercise will build solid muscles and produce strong bones.

The rhythmic nature of high-impact loading exercise enhances blood supply to working muscles, positively affecting bone mass (22). The authors, Alghadir et al. (23), reported a substantial increase in all bone metabolism indicators after 12 weeks of moderate aerobic exercise, including serum ALP, serum osteocalcin, serum-free calcium, and BMD in both males and females. Our findings support previous research, which shows that brisk walking combined with interval exercise training three times per week for 12 weeks can improve bone mass in overweight and obese individuals.

Bone comprises 35% protein and requires a supply of amino acids for protein turnover (12). Tomé et al. (13) stated that protein and amino acids aid muscle development and are vital to bone health. In the current investigation, L-carnitine supplementation alone was found to promote bone formation and lower bone resorption markers in overweight and obese participants, confirming the positive benefits of L-carnitine supplementation on human bone health.

It is known that L-carnitine facilitates the beta-oxidation of fatty acids, acts as a shuttle for acetyl groups across the mitochondrial membrane, and increases human osteoblast activity and intracellular calcium signaling (24). As bone remodeling, particularly osteoblast differentiation, necessitates significant energy, effective mitochondria are critical for bone production and bone mass maintenance. In previous studies mentioned by Terruzzi et al. (24), the significance of mitochondrial activity in maintaining the effectiveness of cellular metabolic processes, including the generation of ROS, calcium homeostasis, oxidative phosphorylation, and the electron transport chain (ETC) to produce adenosine triphosphate (ATP), and regulation of cellular apoptosis. In addition, L-carnitine plays a vibrant cofactor for fatty acid conversion, which plays a vital role in energy transfer in the human body for bone and muscle development (25).

Fatty acid oxidation has been reported to influence the amount of energy available for

protein synthesis in osteoblasts (25). Furthermore, Shen et al. (26) discovered that fatty acid oxidation provides 40% to 80% of the energy required for osteoblasts. As a result, L-carnitine may influence bone density, slow bone turnover, and directly affect human osteoblasts.

Regarding serum 1CTP, a statistically reduced concentration was measured after 12 weeks compared to the baseline in the LcEx group among other groups. The findings of this study implied that the LcEx group might have the potential to reduce bone resorption after 12 weeks of intervention in overweight and obese participants. A high blood volume could be supplied to the working muscles during interval training and brisk walking. This may increase blood flow to the muscles and bones (27). The absorption of the active compounds in L-carnitine into the blood and subsequently into the muscles and bones may increase bone formation and reduce bone resorption markers.

This study found that moderate brisk walking and Interval exercise training elevates serum calcium, consistent with findings from previous research (24, 28). Our study hypothesized that L-carnitine could improve calcium absorption, resulting in skeletal advantages with acute ingestion. It is due to the considerable rise in serum total calcium in the L-carnitine supplementation alone group after 12 weeks of research. It was discovered that L-carnitine supplementation can significantly increase total serum calcium compared to the baseline value. We are implying that L-carnitine, which contains calcium, amino acids, and other nutritional elements, can improve circulating calcium levels in blood vessels. Unfortunately, the calcium absorption efficiency was not determined in this study. Therefore, further research is needed to incorporate absorption efficiency analysis to clarify the physiological mechanism of calcium metabolism produced by L-carnitine supplementation.

The mean difference in serum total calcium concentration was the highest in the combination of L-carnitine supplementation and moderately intense exercise (brisk walking and interval training). In our hypothesis, this combination might increase calcium absorption induced by exercise. Thus, it was hypothesized that L-carnitine would improve calcium availability in the blood, as indicated by this study's L-carnitine supplementation alone group. Blood will be transported to the working muscles when the participants perform brisk walking and interval exercises. Thus, the increase in calcium levels in the peripheral blood caused by L-carnitine consumption, brisk walking, and interval exercise may reflect calcium mobilization from the bone, as seen in the current study.

Furthermore, L-carnitine containing lysine can enhance intestinal calcium absorption and improve the renal conservation of the absorbed calcium (29). The abovementioned phenomenon could have caused the combined effects of brisk walking and interval exercise with L-carnitine supplementation on serum total calcium in the present study. The combined effects may contribute to a positive calcium balance. Thus, it is suggested that L-carnitine supplementation has the potential for preventive and therapeutic interventions in osteoporosis.

The interplay is complex, always involving the interaction between the skeletal system, kidneys, and gastrointestinal tract to regulate the level of calcium within the body. Most physiological alterations in calcium are due to alterations in the rate of bone resorption and deposition, renal reabsorption, and intestinal absorption. PTH and vitamin D are the primary regulators of this process: increased intestinal calcium absorption, renal tubular reabsorption, and bony resorption are balanced by normal renal excretion to maintain the steady state of blood calcium. Weight-bearing physical activity, such as brisk walking and interval exercise, can stimulate bone mineralization and calcium retention, enhancing bone density. Conversely, any disruption to these processes, such as reduced physical activity or hormonal changes, releases calcium from the bones, predisposing one to disorders such as osteoporosis (30).

However, in this present study, even without direct intervention, changes in the control group became significant in markers of bone metabolism due to several factors. Bone metabolism represents a continuous dynamic process between bone formation and resorption. With time, oscillations in natural bone metabolism markers can occur due to hormonal changes, activity level, diet, and age, which may occur even without any particular intervention on a person. Examples include changes in hormonal factors in adulthood that influence but are not limited to, the metabolism of vitamin D and calcium, thus influencing bone turnover independently from physical activity or nutrition (31).

Even though the control group did not experience a systematic intervention, physical activities incidentally might have occurred in daily life routines such as walking, standing, and climbing stairs. It has been shown in several studies that even at low to moderate levels, physical activity itself, independent of any exercise intervention, can improve bone turnover by applying mechanical loading to the skeleton itself (32). Thus, these unmeasured daily activities may improve bone metabolism markers in the control group. Diet has excellent effects on bone health; a slight change in calcium, vitamin D, and protein intake would result in remarkable changes in bone metabolism markers. The research indicated that an increase in dietary calcium and vitamin D through food may improve the markers of bone resorption, even in the placebo group (33). The significant changes might have been attributed to the unmonitored diet that had changed in the control group.

Muscular Performance. Regarding muscular performance, LcEx groups performed greater knee flexion and extension of the dominant leg, peak torque, and power measurement than supplement and exercise alone groups. It appears that the brisk walking and Interval exercise training designed in this study has increased the muscle strength and power of the participants. Brisk walking is a whole-body movement that involves the legs, resulting in repetitive movements of the lower limb. During training, interval exercise training enables the participants to use their entire upper and lower extremities. As a result, we discovered that brisk walking and interval exercise training could improve knee muscular strength on the dominant side of the legs, enhancing the muscular balance on the dominant knees. It would be reasonable to assume that numerous joint movements were emphasized in this interval training regimen, which allowed for the simultaneous training of multiple muscle groups and the use of heavier loads for better physical strength improvement (34).

Schmidt et al. (35) found similar findings in their investigation. Women who did low-intensity circuit training combined with high-resistance exercises for 12 weeks improved their bench press, knee extension, and muscular endurance scores considerably. In addition, Kumar (36) found that participants who did six-station circuit training significantly increased their leg muscle strength and agility. The circuit training involved exercising for 25 to 35 seconds with 20 to 30 seconds of rest at each station, for 2 to 3 minutes between each set (in a total of 2 to 4 sets) for eight weeks. Therefore, strength and power were expected to increase since the exercise focused on the core, upper limb, and lower strength.

Furthermore, as evidenced by the evolution of workload in performing Interval exercise training, the duration of training will progressively increase by 4 minutes from week 1 to week 12, with a total of 20 minutes. Increased overload training produces significant tensional stress. This progression of exercise training could avoid injury and benefit the human body. For example, encouraging muscle growth and enhancing intramuscular anabolic signaling promotes a greater rate of muscle protein synthesis than breakdown and maximizes the response to muscle fiber recruitment, time under tension, and metabolic load (37). Increased myofibril size and number contribute to the most significant adaptations, such as a larger cross-sectional area of the whole muscle and myofibrils within individual muscles (37).

According to a prior study, the adaptations of training are influenced by the level of training. Those with lower fitness levels may gain more from brisk walking and Interval exercise training. However, those with higher strength levels may require a particular stimulus (38). Other factors influencing muscle mass gains after exercising include frequency, intensity, and volume (39, 40). It has been reported that two or three sessions per week with at least ten series per week are sufficient to improve strength, power, and hypertrophy improvements (41-44). This study's muscle strength and power enhancement may be due to a gradual increase in exercise intensity and repetition frequency and continuous moderate aerobic exercise. As for intensity, moderate-intensity resistance workouts with short rest intervals have been advised to target muscular growth with strength gains (41) primarily. The strength of skeletal muscles may be affected by both neurological stimulation and adaptation (42). Initial strength improvements have been linked to neurological modifications involving a more effective activation pattern of active muscles in previously

untrained individuals (43). As a result, any changes in muscle activation are almost certainly due to a change in muscle size. Previously, there were significant strength gains during the first weeks of training in resistance-trained athletes (39). Therefore, it was speculated that the above explanations could increase all the measured parameters of knee extension and flexion among participants who performed Interval exercise training 3 times per week for 12 weeks in overweight and obese participants.

L-carnitine contains lysine and methionine, essential in skeletal maintenance and help improve muscle strength and power. Lysine is required by the body to produce protein, which significantly contributes to muscle development and joint strengthening. Lysine is mainly generated by skeletal muscle. Therefore, it helps develop and maintain muscular tissue.

During exercise, it also interacts with numerous vitamins, minerals, and amino acids, such as vitamin C, to produce another amino acid, carnitine, which aids in fat-burning and oxygen delivery to active muscles.

Lysine raises human growth hormones in the blood that help gain muscle and improve performance. Significantly, lysine slows the rate of catabolism. Naturally, the body goes into a catabolic state after exercise, but lysine reverses that process, and with enough lysine reserves, the body returns to an anabolic state. For this reason, lysine absorption is thought to induce tissue development, recuperation, and muscle rebuilding (45).

As a result, in this study, whether L-carnitine alone or the combination of L-carnitine and exercise may significantly improve muscular strength and power in overweight and obese participants.

The limitation of this study is that there is no use of the Dual-Energy X-ray Absorptiometry (DEXA) scan, considered a gold standard for measuring body composition, including bone density, fat mass, and lean mass, with high precision. Although alternative methods for assessing body composition were employed, such as a bone sonometer that measures the ankle and wrist, they may not offer the same level of accuracy as DEXA. The lack of access to DEXA scanning could have affected the precision of body density results, particularly in detecting small changes over time. Future research should consider incorporating DEXA scans to improve the accuracy of body density measurements.

CONCLUSION

In summary, the combination of L-carnitine supplement with exercise could significantly increase bone formation and serum total calcium levels but reduce bone resorption among overweight and obese individuals, subsequently enhancing muscular strength and power. Thus, the formulation of guidelines for implementing exercise and nutrition promotion programs to enhance bone health and muscle performance is likely to be beneficial with adding 1000mg of L-carnitine daily combined with three days of brisk walking per week and interval exercise training in overweight and obese people.

APPLICABLE REMARKS

- Current study supports the importance of exercise and L-carnitine supplements in preventing bone loss, either by taking supplements alone, exercising alone, or combining both.
- Interestingly, the combination of L-carnitine and exercise group expressed the highest peak torque and average power at $60o.s^{-1}$ and $300o.s^{-1}$.
- Therefore, we claim that the active compound in the L carnitine supplement positively affects bone and muscular performance in combination with brisk walking and interval exercise training.

ACKNOWLEDGMENTS

Acknowledgment to the Ministry of Higher Education Malaysia for the Fundamental Research Grant Scheme. The authors would like to thank all the participants and the staff of the Exercise and Sports Science Laboratory, School of Health Sciences, Universiti Sains Malaysia, for their assistance and technical support throughout the study.

AUTHORS' CONTRIBUTIONS

Study concept and design: Norsuriani Samsudin, Nur Syamsina Ahmad. Acquisition of data: Norsuriani Samsudin. Analysis and interpretation of data: Norsuriani Samsudin, Nur Syamsina Ahmad, Foong Kiew Ooi, Azidah Abdul Kadir. Drafting the manuscript: Norsuriani Samsudin. Critical revision of the manuscript for

important intellectual content: Foong Kiew Ooi, Azidah Abdul Kadir. Statistical analysis: Nur Syamsina Ahmad. Administrative, technical, and material support: Foong Kiew Ooi, Nur Karyatee Kassim. Study supervision: Nur Syamsina Ahmad, Nur Karyatee Kassim.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

FINANCIAL DISCLOSURE

This project received research grants from the Ministry of Higher Education Malaysia.

FUNDING/SUPPORT

The Ministry of Higher Education Malaysia funded this Fundamental Research Grant Scheme project with Project Code FRGS/1/2019/STG04/USM/03/3.

ETHICAL CONSIDERATION

The study was conducted under the Declaration of Helsinki and approved by the ethical researcher committee of the Universiti Sains Malaysia, which granted approval to the study (USM/JEPeM/19100617) for studies involving humans. Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the participants to publish this paper.

ROLE OF THE SPONSOR

The funding organizations are in the government sector and have no role in the design and conduct of the study, collection, management, and analysis of the data or preparation, review, and approval of the manuscript.

ARTIFICIAL INTELLIGENCE (AI) USE

The authors disclosed that they do not use AI and AI-assisted technologies in this manuscript.

REFERENCES

1. Robinson J, Nitschke E, Tovar A, Mattar L, Gottesman K, Hamlett P, Rozga M. Nutrition and physical activity interventions provided by nutrition and exercise practitioners for the general population: An evidence-based practice guideline from the Academy of Nutrition and Dietetics and American Council on Exercise. J Acad Nutr Diet. 2023;123(8):1215-1237. [doi:10.1016/j.jand.2023.04.004] [PMid:37061182]
2. Karlsson MK, Rosengren BE. Exercise and Peak Bone Mass. Curr Osteoporos Rep. 2020;18(3):285-290. [doi:10.1007/s11914-020-00588-1] [PMid:32249382]
3. Cartledge TJ, Murphy J, Foster CE, Tibbitts B. The effect of weight-bearing exercise on the mechanisms of bone health in young females: A systematic review. J Frailty Sarcopenia Falls. 2022; 7(4):231-250. [doi:10.22540/JFSF-07-231] [PMid:36531516]
4. Simões D, Craveiro V, Santos MP, Camões M, Pires B, Ramos E. The effect of impact exercise on bone mineral density: A lon-gitudinal study on non-athlete adolescents. Bone. 2021; 153:116151. [doi:10.1016/j.bone.2021.116151] [PMid:34391957]
5. Lanyon LE. Functional strain in bone tissue as an objective, and controlling stimulus for adaptive bone remodelling. J Biomech. 1987; 20(11-12):1083-93. [doi:10.1016/0021-9290(87)90026-1] [PMid:3323200]
6. Szulc P, Bauer DC, Eastell R. Biochemical markers of bone turnover in osteoporosis. In Marcus and Feldman's Osteoporosis. Academic Press. 2021; 1545-1588. [doi:10.1016/B978-0-12-813073-5.00065-4]
7. Burr DB. Bone morphology and organization. In Basic and applied bone biology. Academic Press. 2019; 3-26. [doi:10.1016/B978-0-12-813259-3.00001-4]
8. Sushma BJ, Thiruveedhula CS. Brisk Walking and Lipid Profile in Obese Subjects. Int. J. Health Sci. 2022; (III):2555-61. [doi:10.53730/ijhs.v6nS3.6086]
9. Watson KB, Frederick GM, Harris CD, Carlson SA, Fulton JE. US Adults' Participation in Specific Activities: Behavioral Risk Factor Surveillance System--2011. J Phys Act Health. 2015; 12 Suppl 1(01):S3-10. [doi:10.1123/jpah.2013-0521] [PMid:25157914]

10. Tabata I, Nishimura K, Kouzaki M, Hirai Y, Ogita F, Miyachi M, Yamamoto K. Effects of moderate-intensity endurance and high-intensity intermittent training on anaerobic capacity and VO_2max. Med Sci Sports Exerc. 1996; 28(10):1327-30. [doi:10.1097/00005768-199610000-00018] [PMid:8897392]
11. Lu M, Li M, Yi L, Li F, Feng L, Ji T, Zang Y, Qiu J. Effects of 8-week high-intensity interval training and moderate-intensity continuous training on bone metabolism in sedentary young females. J Exerc Sci Fit. 2022; 20(2):77-83. [doi:10.1016/j.jesf.2022.01.001] [PMid:35096081]
12. Carbone JW, Pasiakos SM. Dietary Protein and Muscle Mass: Translating Science to Application and Health Benefit. Nutrients. 2019;11(5):1136. [doi:10.3390/nu11051136] [PMid:31121843]
13. Tomé D, Benoit S, Azzout-Marniche D. Protein metabolism and related body function: mechanistic approaches and health consequences. Proc Nutr Soc. 2021; 80(2):243-251. [doi:10.1017/S0029665120007880] [PMid:33050961]
14. Lee BJ, Lin JS, Lin YC, Lin PT. Anti-inflammatory effects of L-carnitine supplementation (1000 mg/d) in coronary artery disease patients. Nutr J. 2014;31(3):475-479. [doi:10.1016/j.nut.2014.10.001] [PMid:25701337]
15. Norsuriani S, Ooi FK. Bone health status, isokinetic muscular strength and power, and body composition of Malay adolescent female silat and taekwondo practitioners. Int. J. Public Health Clin. Sci. 2018; 5(2): 244-262.
16. Karvonen J, Vuorimaa T. Heart rate and exercise intensity during sports activities. Practical application. Sports Med. 1988; 5(5): 303-311. [doi:10.2165/00007256-198805050-00002] [PMid:3387734]
17. Emberts T, Porcari J, Dobers-Tein S, Steffen J, Foster C. Exercise intensity and energy expenditure of a tabata workout. J Sports Sci Med. 2013;12(3):612-3.
18. Hamilton BR, Staines KA, Kelley GA, Kelley KS, Kohrt WM, Pitsiladis Y, Guppy FM. The Effects of Exercise on Bone Mineral Density in Men: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. Calcif Tissue Int. 2022;110(1):41-56. [doi:10.1007/s00223-021-00893-6] [PMid:34382100]
19. Carter MI, Hinton PS. Physical activity and bone health. Mo Med. 2014; 111(1):59-64.
20. Moreira LD, Fronza FC, Dos Santos RN, Zach PL, Kunii IS, Hayashi LF, Teixeira LR, Kruel LF, Castro ML. The benefits of a high-intensity aquatic exercise program (HydrOS) for bone metabolism and bone mass of postmenopausal women. J Bone Miner Metab. 2014; 32(4):411-9. [doi:10.1007/s00774-013-0509-y] [PMid:24048909]
21. Hart NH, Nimphius S, Rantalainen T, Ireland A, Siafarikas A, Newton RU. Mechanical basis of bone strength: influence of bone material, bone structure and muscle action. J Musculoskelet Neuronal Interact. 2017; 17(3):114-139.
22. Mosavat M, Ooi FK, Mohamed M. Effects of honey supplementation combined with different jumping exercise intensities on bone mass, serum bone metabolism markers and gonadotropins in female rats. BMC Complement Altern Med. 2014; 14:126. [doi:10.1186/1472-6882-14-126] [PMid:24708608]
23. Alghadir AH, Gabr SA, Al-Eisa E. Physical activity and lifestyle effects on bone mineral density among young adults: socio-demographic and biochemical analysis. J Phys Ther Sci. 2015; 27(7):2261-70. [doi:10.1589/jpts.27.2261] [PMid:26311965]
24. Terruzzi I, Montesano A, Senesi P, Villa I, Ferraretto A, Bottani M, Vacante F, Spinello A, Bolamperti S, Luzi L, Rubinacci A. L-Carnitine reduces oxidative stress and promotes cells differentiation and bone matrix proteins expression in human osteoblast-like cells. Biomed Res Int. 2019; 2019:5678548. [doi:10.1155/2019/5678548] [PMid:30800672]
25. Dirckx N, Moorer MC, Clemens TL, Riddle RC. The role of osteoblasts in energy homeostasis. Nat Rev Endocrinol. 2019; 15(11):651-665. [doi:10.1038/s41574-019-0246-y] [PMid:31462768]
26. Shen L, Hu G, Karner CM. Bioenergetic Metabolism In Osteoblast Differentiation. Curr Osteoporos Rep. 2022 Feb;20(1):53-64. [doi:10.1007/s11914-022-00721-2] [PMid:35112289]
27. Ooi FK, Singh R, Singh HJ. Changes in bone turnover markers and bone mass with reducing levels of jumping exercise regimens in female rats. Asian J Sports Med. 2012; 3(4):225-32. [doi:10.5812/asjsm.34541]
28. Dolan E, Dumas A, Keane KM, Bestetti G, Freitas LHM, Gualano B, Kohrt W, Kelley GA, Pereira RMR, Sale C, Swinton P. The influence of acute exercise on bone biomarkers: protocol for a systematic

review with meta-analysis. Syst Rev. 2020; 9(1):291. [doi:10.1186/s13643-020-01551-y] [PMid:33308281]
29. Ferraretto A, Bottani M, Villa I, Giusto L, Signo M, Senesi P, Montesano A, Vacante F, Luzi L, Rubinacci A, Terruzzi I. L-Carnitine activates calcium signaling in human osteoblasts. J. Funct. Foods. 2018; 47:270-8. [doi:10.1016/j.jff.2018.05.068]
30. Jones MA, Smith RL, Carter HP. The regulation of calcium homeostasis in response to physical activity and aging: A review of physiological mechanisms. J. Bone Miner. Res. 2021; 36(2): 245-256.
31. Rondanelli M, Faliva MA, Monteferrario F, Perna S, Antoniello N, Peroni G. Nutritional strategies in preserving bone health. Int. J. Environ. Res. Public Health. 2020; 17(21): 7997.
32. Kanis JA, McCloskey EV, Harvey NC, Johansson H, Leslie WD, Reginster JY. Intervention thresholds and the diagnosis of osteoporosis. Bone. 2020; 134: 115293.
33. Mangels AR, Messina V, Messina M, Melina V. Bone health and plant-based diets: Impact of dietary calcium, vitamin D, and protein on bone mineral density and fracture risk. Nutrients. 2019; 11(1): 1-14.
34. Goncalves A, Gentil P, Steele J, Giessing J, Paoli A, Fisher JP. Comparison of single- and multi-joint lower body resistance training upon strength increases in recreationally active males and females: a within-participant unilateral training study. Eur J Transl Myol. 2019; 29(1):8052. [doi:10.4081/ejtm.2019.8052] [PMid:31019663]
35. Schmidt D, Anderson K, Graff M, Strutz V. The effect of high-intensity circuit training on physical fitness. J Sports Med Phys Fitness. 2016; 56(5):534-40.
36. Kumar, V. Effect of circuit training program on selected motor abilities among university male. Int. J. Phys. Educ. Sports Health. 2016; 3: 255-257.
37. Krzysztofik M, Wilk M, Wojdała G, Gołaś A. Maximizing Muscle Hypertrophy: A Systematic Review of Advanced Resistance Training Techniques and Methods. Int J Environ Res Public Health. 2019; 16(24):4897. [doi:10.3390/ijerph16244897] [PMid:31817252]
38. Lorenz D, Morrison S. Current concepts in periodization of strength and conditioning for the sports physical therapist. Int J Sports Phys Ther. 2015; 10(6):734-47.
39. Grgic J, Schoenfeld BJ, Davies TB, Lazinica B, Krieger JW, Pedisic Z. Effect of Resistance Training Frequency on Gains in Muscular Strength: A Systematic Review and Meta-Analysis. Sports Med. 2018; 48(5):1207-1220. [doi:10.1007/s40279-018-0872-x] [PMid:29470825]
40. Schoenfeld BJ, Ogborn D, Krieger JW. Effects of Resistance Training Frequency on Measures of Muscle Hypertrophy: A Systematic Review and Meta-Analysis. Sports Med. 2016; 46(11):1689-1697. [doi:10.1007/s40279-016-0543-8] [PMid:27102172]
41. Schoenfeld BJ, Ogborn DI, Krieger JW. Effect of repetition duration during resistance training on muscle hypertrophy: a systematic review and meta-analysis. Sports Med. 2015; 45(4):577-85. [doi:10.1007/s40279-015-0304-0] [PMid:25601394]
42. Iversen VM, Norum M, Schoenfeld BJ, Fimland MS. No Time to Lift? Designing Time-Efficient Training Programs for Strength and Hypertrophy: A Narrative Review. Sports Med. 2021; 51(10):2079-2095. [doi:10.1007/s40279-021-01490-1] [PMid:34125411]
43. Smith JAB, Murach KA, Dyar KA, Zierath JR. Exercise metabolism and adaptation in skeletal muscle. Nat Rev Mol Cell Biol. 2023; 24(9):607-632. [doi:10.1038/s41580-023-00606-x] [PMid:37225892]
44. Hedayatpour N, Falla D. Physiological and Neural Adaptations to Eccentric Exercise: Mechanisms and Considerations for Training. Biomed Res Int. 2015; 2015:193741. [doi:10.1155/2015/193741] [PMid:26543850]
45. Weinert DJ. Nutrition and muscle protein synthesis: A descriptive review. Journal of the Canadian Chiropractic Association. 2009; 53(3): 186-193.