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EFFECTS OF PROBIOTICS CONSUMPTION AND RESISTANCE EXERCISE ON BONE METABOLISM MARKERS IN PHYSICALLY INACTIVE YOUNG MALES

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Abstract

This study aims to investigate the effects of probiotics supplementation and resistance exercise on bone metabolism markers and parathyroid hormones in physically inactive young men. Fourty-one healthy participants completed the study. They were randomized into sedentary placebo control (C: n=10), probiotics (P: n=10), resistance exercise with placebo (E: n=12), and probiotics consumption with resistance exercise (PE: n=9) groups. Participants in the P and PE consumed multi-strain probiotics containing $3x10^{10}$ CFU of L. acidophilus BCMC® 12130, L. casei BCMC® 12313, L. lactis BCMC® 12451, B. bifidum BCMC® 02290, B. infantis BCMC® 02129 and B. longum BCMC® 02120 twice daily for 12 weeks. Participants in the E and PbE performed circuit training comprised of 10 resistance exercises 3 times/week. Body height and weight, blood pressure, resting heart rate and blood samples were collected at pre and post-tests. Blood samples were analysed for the concentration of bone formation markers [alkaline phosphatase (ALP) and osteocalcin (OC)], bone resorption marker [cross-linked carboxyterminal telopeptide of type 1 collagen (1CTP)] and parathyroid hormones (PTH). There were no significant differences ($p>0.05$) in all measured parameters between groups and within each group ($p>0.05$). Nevertheless, ALP and OC concentration exhibited increasing trends in PE group. There was a decreasing trend in PTH concentration in P and PE groups. As a conclusion, probiotics consumption with resistance exercise resulted in increased trends of bone formation markers and a small reduction in the bone resorption marker implying that this combination may elicit beneficial effects in improving bone formation in young physically inactive males.

Keywords: *Probiotics, bone health, resistance exercise, physical activity, young males*

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INTRODUCTION

Bone is a metabolically active tissue where it undergoes continuous remodeling which involves bone resorption and formation throughout life (Kular et al., 2012). The state of bone is always close to equilibrium between bone formation and bone resorption (Seibel, 2005). This is important for allowing adaptation of the bones towards the changes in load and to repair damage caused by recurrent micro-traumas (Ooi et al., 2009).

Generally, weight-bearing exercise was found to result in better bone health compared with non-weight bearing exercise. The impact forces caused by weight-bearing exercise is due to gravitational force. Previous studies shown that exercise could reduce fracture risk and have a positive effect on bone metabolism (Duncan et al., 2002; Kohrt et al., 2009). It is believed that the strong forces generated by the muscles contraction which imposes on the bone tissue during the performance of an exercise or training can increase bone metabolism and promote osteogenesis (Lau and Ooi, 2014; Montgomery et al., 2016).

In the recent studies, gut microbiota was also found to give a beneficial effect on bone metabolism (Parvaneh et al., 2015) and bone mass (Weaver, 2015). Most probiotics strain study conducted that resulted an advantageous effect on bone were Lactobacillus and Bifidobacterium species bacteria (McCabe et al., 2013; Parvaneh et al., 2014; Sjogren et al., 2012). The potential mechanisms of action related to probiotics and bone health is due to increment in the mineral solubility that was caused by the production of short chain fatty acids. Production of short chain fatty acids leads to the production phytase enzyme by the bacteria in order to lower the effect of mineral reduction by phytate. In turn, this process will lower the intestinal inflammation, hydrolyzing glycoside bond food in the intestines and eventually increasing the bone mass density (Parvaneh et al., 2014).

Nevertheless, to our knowledge to date, there was no longitudinal study conducted particularly on the combined effect of probiotics with resistance exercise on bone metabolism markers and parathyroid hormone (PTH). Therefore, the objective of this study was to investigate the effects of probiotics supplementation and resistance exercise on bone formation markers [serum alkaline phosphatase (ALP) and osteocalcin (OC)], bone resorption marker [cross-linked carboxyterminal telopeptide of type 1 collagen (1CTP)], and parathyroid hormones (PTH) in young males following 12 weeks of the intervention period.

MATERIALS & METHODS

Research Design

A randomised, parallel, placebo-controlled study design was employed for the present study. Measurements were conducted at pre- and post-tests of 12 weeks intervention period. Exercise sessions and data collections were conducted at the Health Campus Universiti Sains Malaysia

(USM). The procedures for this study has been approved by the Human Research Ethics Committee, Health Campus, USM, Kelantan (JEPeM Code: USM/JEPeM/15040132) that was accordance with the Declaration of Helsinki.

Participants

The calculated sample size was 9 participants per group, which was equivalent to at least 36 participants for 4 groups. The calculation was done by using G-power Software 3.1.9.2. The power of the study was set at 80% with 95% confident interval and 30% of effect size. Participants were recruited among USM students via snowball sampling technique. The inclusion criteria included healthy young males who are physically inactive (exercise less than twice per week), age between 19 to 26 years old, do not consume probiotics supplementation at least 6 months prior to the study, and do not engage in any physical training programme. The exclusion criteria include smoking/vaping, on medication and having chronic diseases. Throughout the study period, participants were required to abstain from taking any supplements that are known to affect bone health e.g. vitamin C, vitamin D and calcium.

RESEARCH PROCEDURES

Before participating, participants were given a health questionnaire form to assess their overall health status. Participants were also given the participants' information sheet and were asked to fill in the consent form when they agreed to participate. Participation in this study was on a voluntary basis, thus participants have the right to withdraw at any time during the course of this study.

After recruitment of participants, pre-test measurements which included measurements of body height and weight (body composition analyzer and stadiometer: TANITA, TBF-410 Model, Japan and SECA, UK), blood pressure and resting heart rate (OMRON, Japan) were carried out. In addition, 4 mL of blood were collected in the morning at 8:30 am after an overnight fast (drinking plain water was permitted). Serum was obtained by centrifuging the blood sample using a centrifuge (Hettich-Rotins 46RS, Germany) for 10 minutes at 4000 rpm in 4ºC temperature, before being divided into equal portions and stored at -80ºC in a freezer (ThermoForma, Model 705, USA) for subsequent analysis.

Then, participants were randomly divided into four groups: sedentary with placebo group (C), sedentary with probiotics supplementation group (P), resistance exercise with placebo group (E), probiotics supplementation combined with resistance exercise group (PE). This study was double-blinded where, both researchers and participants did not know which supplements they were taking. Hence, the randomization of the groups was carried out by a laboratory assistant who was not involved with data collection. Each group was assigned with a number and inserted in an opaque, sealed envelopes. Participants were asked to take one envelope each and the number inside the envelope will determine which group he or she was allocated to. This information was

kept confidential until the end of the 12 weeks intervention period. This procedure guaranteed that randomization concealment was adequate and double blinded is employed.

After randomization into groups, participants involved in 12 weeks of intervention according to the group allocated. Those in the P and PE groups consumed probiotics twice per day for 12 weeks while those in the C and E groups took placebo twice daily for 12 weeks. In addition, participants in the PE and E groups performed resistance exercise 3 times per week for 12 weeks while those in the C and P groups did not involve in any exercise programme. During this intervention period, participants were given a checklist and form to record their weekly activity and to assess their adherence to the supplementation regimen. Following the 12 weeks of the intervention period, post-test measurements which were similar to the pre-test measurement were carried out.

The blood samples were analysed for bone metabolism markers. Serum alkaline phosphate (ALP) was analysed calorimetrically by using a chemistry analyser (Architec C 8000, USA) with a commercially available reagent kit (Randox, UK). Serum osteocalcin (OC) was analysed using an enzyme immunological test kit (N-MID® Osteocalcin ELISA, UK). Serum cross-linked carboxyterminal telopeptide of type 1 collagen (1CTP) was analysed by a quantitative enzyme immunoassay kit (ELISA kit 1CTP EIA, China) and serum parathyroid hormone (PTH) was analysed by using an enzyme immunological test kit (Calbiotech PTH ELISA, USA).

Resistance Exercise

The participants in both E and PE groups were required to attend the resistance exercise sessions 3 times per week for 12 weeks. This prescribed exercise regimen was based on the previous study carried out by Lau and Ooi (2014). It was commenced with 5 minutes of warming up and ended with 5 minutes of cooling down. In each circuit, participants were required to perform a different type of resistance exercises in 10 different stations (one type of resistance exercise per station).

The type of resistance exercises prescribed in one circuit training programme from station 1 to station 10 were heel raise with dumbbell, side lateral raise with elastic band, leg abduction with elastic band, shoulder extension and flexion using elastic band, rope skipping, triceps extension with dumbbell, half squat with elastic band, standing chest fly with dumbbell, leg curl with elastic band and biceps curl with dumbbell respectively. Participants performed 2 circuits of resistance exercises from week 1 to week 8 followed by 3 circuits of resistance exercises from week 9 to week 12. The work to rest ratio was 1:2 (participants exercised for 30 seconds in one particular station, and rested for one minute before continuing with the next station) while the resting period between the circuits was 5 minutes.

The elasticity of the resistance band used was reduced from week 5 to week 12 (the resistance was increased). In addition, the weight of the dumbbells weight used was increased 1-2.5 kg; depending on individuals' capacity from week 5 to week 12. This was done to increase

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the intensity of the training. The intensity of resistance exercise was estimated by referring to the post-exercise heart rates of the participants as measured by heart rate monitors (Polar watch, S710, USA) worn by the participants throughout the training session.

Probiotics and Placebo Supplementation

Participants in the C and E groups consumed placebo while participants in the P and PE groups consumed probiotics with a dosage of 2 sachets per day (1 sachet in the morning and 1 sachet in the afternoon) for 12 weeks. All the supplements were prepared, packaged, and labelled by the supplier. Probiotics used in this study is called Hexbio© granule. Each sachet has a concentration of 3 x 10^{10} colony forming unit (CFU) of probiotics at the time of manufacturing with 6 different microorganism strains (L. acidophilus BCMC® 12130, L. casei BCMC® 12313, L. lactis BCMC® 12451, B. bifidum BCMC® 02290, B. infantis BCMC® 02129 and B. longum BCMC® 02120). It is an orange flavoured, cream colour granular powder. The nutritional composition contains in each sachet is tabulated in Table 1. As for placebo, it was identical in shape, taste and colour to the probiotics but contains no bacteria.

Table 1: Nutritional Composition in Each Probiotics Sachet (3g)

Nutritional compositions	Value per sachet
Carbohydrate	2.70 g
Lactose	1.28 g
Sugar	0.12 g
Protein	0.16 g
Fat	0.01 g
Fibre	0.01 g
Energy Value	11.55 kcal

Statistical Analysis

Statistical analysis was performed by using Statistical Package for Social Sciences (SPSS) version 25.0 (IBM Corporation, Armonk, NY, USA). Descriptive statistics and mixed-factorial analysis of variance (ANOVA) were used to analyse the data. Differences considered significant at $p<0.05$. Results are reported as the mean ± standard deviation (SD).

RESULTS

Participants

Forty-one participants completed the study; control group (C, n=10), probiotic group (P, n=10), exercise group (E, n=12) and probiotic combined with exercise group (PE, n=9). The average attendance of the participants during training sessions was good with 90%. Similarly,

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participants' adherence to the supplementation regimen was also good with 92%. The physical characteristics of the participants were tabulated in Table 2. There were no significant differences on age, body height and weight, BMI and body fat percentage among all groups and between pre and post-tests in each group.

Table 2: Physiological Characteristics of Participants

Variables		**C (n=10)**	**P (n=10)**	**E (n=12)**	**PE (n=9)**
Age (years old)		22 ± 2.0	23 ± 1.0	21 ± 2.0	22 ± 3.0
Body height (cm)		170.4 ± 7.2	169.8 ± 5.0	169.3 ± 7.0	170.2 ± 5.1
Body weight (kg)	Pre-test	61.4 ± 10.4	63.0 ± 10.3	60.6 ± 9.9	64.1 ± 10.2
	Post-test	61.8 ± 10.5	62.4 ± 11.5	60.9 ± 10.0	63.7 ± 11.4
Body mass index (kg/m^2)	Pre-test	21.1 ± 2.8	21.8 ± 3.4	21.1 ± 2.7	22.1 ± 3.4
	Post-test	21.3 ± 2.9	21.5 ± 3.8	21.2 ± 2.8	21.9 ± 3.9
Body fat percentage (%)	Pre-test	18.6 ± 7.3	20.5 ± 6.4	18.3 ± 6.8	21.1 ± 6.4
	Post-test	19.8 ± 7.2	20.1 ± 6.7	19.3 ± 6.7	20.7 ± 6.8

Bone Formation Markers

The mean of serum ALP and OC concentrations are shown in Figure 1 and Figure 2 respectively. There were no significant differences in ALP ($F = 13.750$; $df = 1$; $p = 0.441$) and OC ($F = 10.547$; $df = 1$; $p = 0.665$) among all the groups based on time. There were also no significant differences in ALP ($F = 7.475$; $df = 1$; $p = 0.441$) and OC ($F = 0.338$; $df = 1$; $p = 0.152$) within each group based on time. Nevertheless, serum ALP and OC concentration exhibited increasing trends in PE group.

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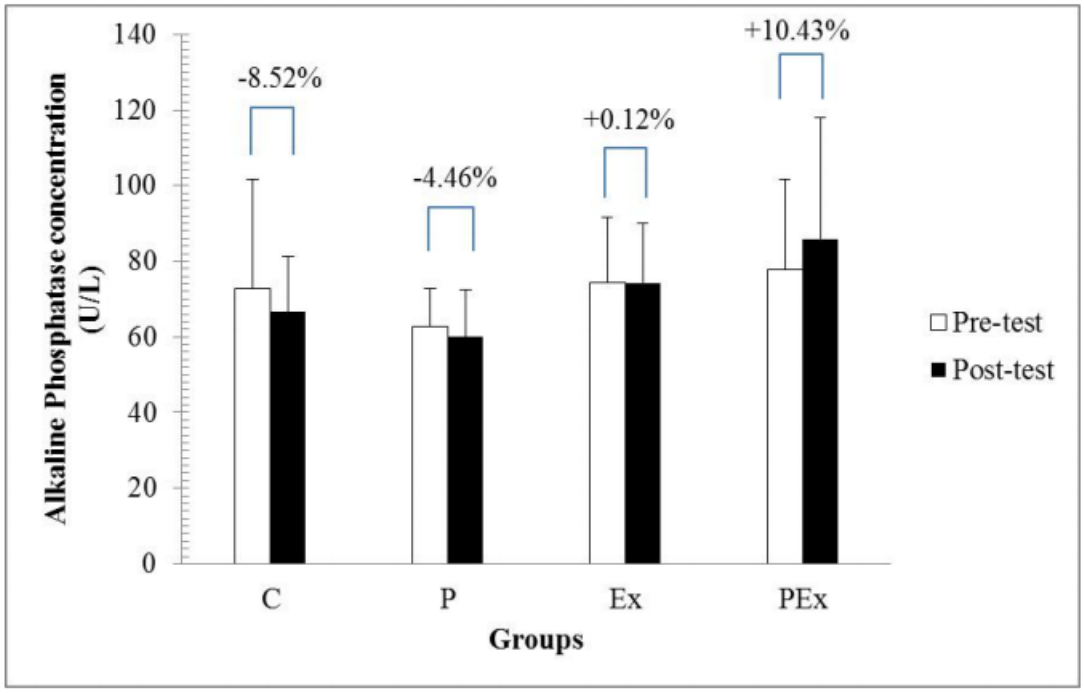

Figure 1: Mean of Serum Alkaline Phosphatase (ALP) Concentration

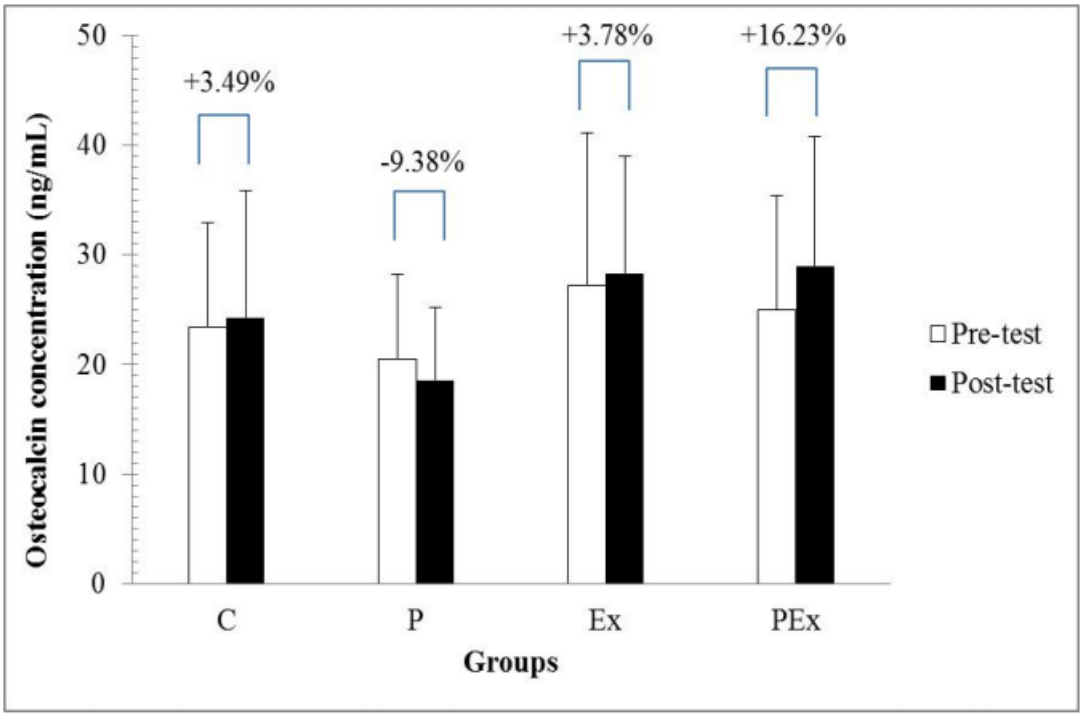

Figure 2: Mean of Serum Osteocalcin (OC) Concentration

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Bone Resorption Marker

There was no significant difference in 1CTP concentration among all the groups based on time (F = 9.182; df = 1; p = 0.166) (Figure 3). There was also no significant difference in 1CTP within each group based on time (F = 12.726; df = 1; p = 0.102).

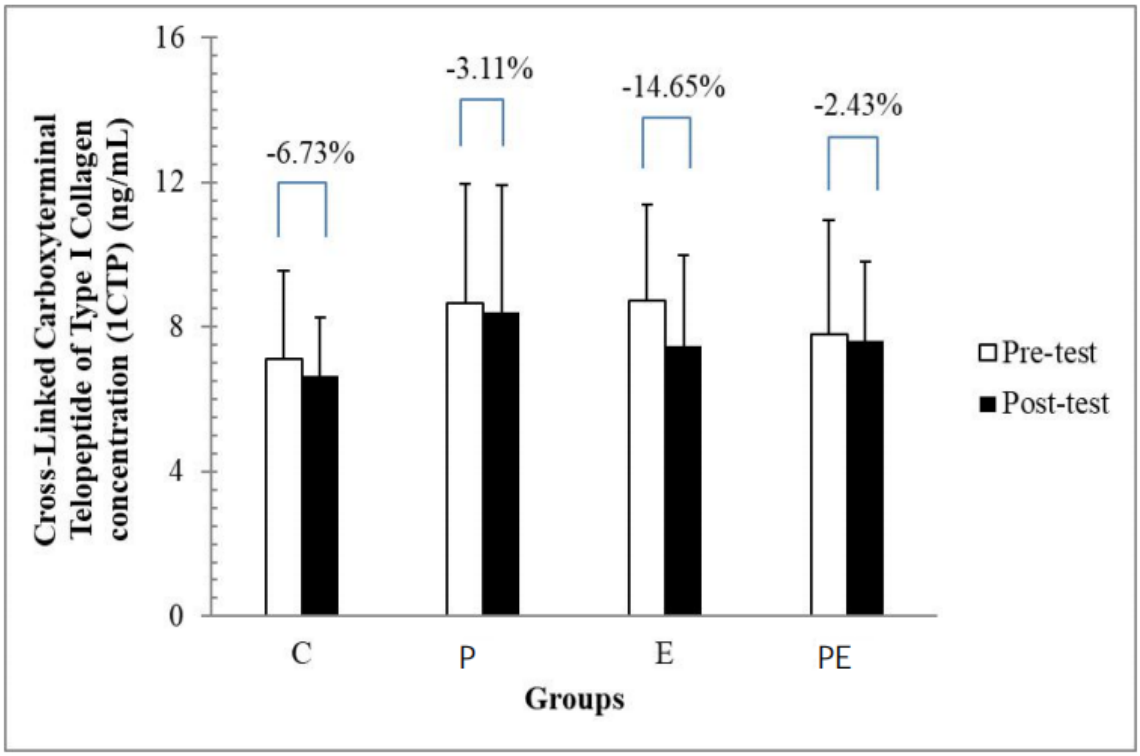

Figure 3: Mean of Serum Cross-linked Carboxyterminal Telopeptide of Type 1 Collagen (1CTP) Concentration

Parathyroid Hormone

Parathyroid hormone concentration was represented in Figure 4. Between groups based on time effect, there was no significant difference in all variables (F = 0.367; df = 22; p = 0.137). There was also no significant difference in all variables within each group based on time (F = 1.978; df = 1; p = 0.841). Despite no significant difference found, there was a decreasing trend in PTH concentration in P and PE groups respectively.

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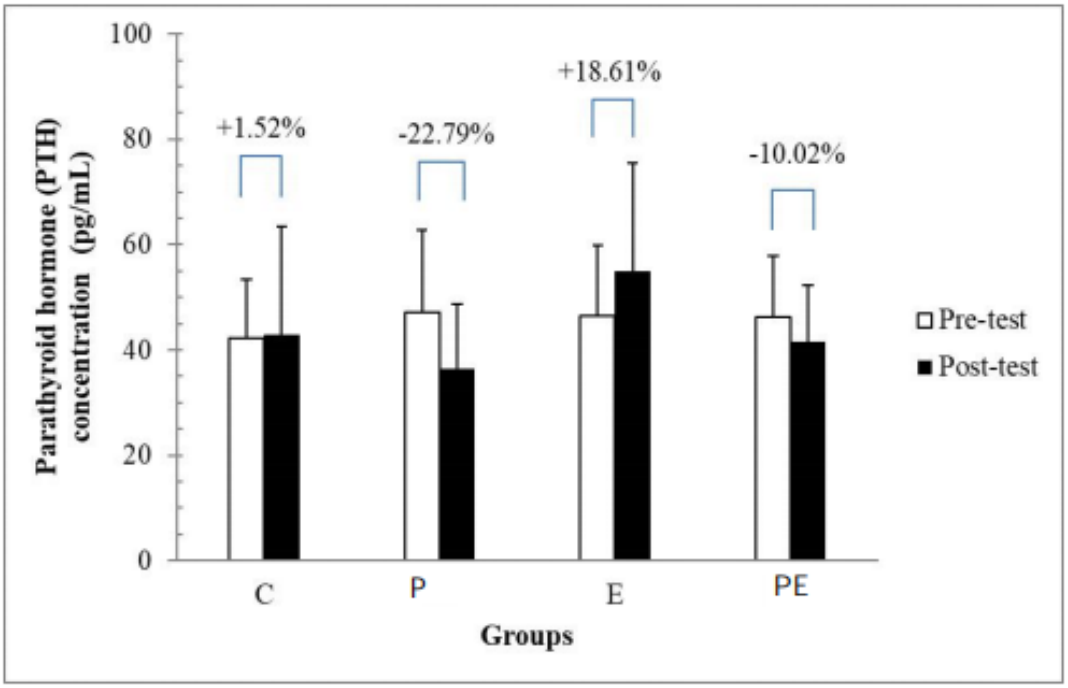

Figure 4: Mean of Serum Parathyroid Hormone (PTH) Concentration

DISCUSSION

The primary aim of this study was to investigate the effects of combined probiotics supplementation with resistance exercise on bone metabolism markers (blood bone formation and resorption markers) and PTH concentration in young males following 12 weeks of the intervention period. Overall, sedentary males recruited in this study were healthy with resting heart rate between 60 and 100 beats per minute (bpm), and mean blood pressure of 120/71 mmHg. The BMI was within normal range for Asian population which was between 18.5 and 22.9 kg/m^2. In addition, it was found that 12 weeks of resistance exercise did not elicited significant effects on body weight and fat loss among participants.

Exercise, Probiotics and Bone Formation Markers

Based on the present study, although it was not significant, there was an increase trend of serum ALP (+10.43%) and OC (+16.23%) concentration in the PE group. This observation implying that combination of probiotics and resistance exercise may be beneficial in improving bone formation. This increasing trend observed in serum ALP and OC concentration might be possibly explained by the combined effect of mechanical loading induced by the circuit training and the interaction of probiotics in the body.

To date, there was no study conducted with regards to combination effect of resistance exercise and probiotics consumption on bone metabolism. Nevertheless, based on the previous study done by Khodkaran et al. (2014) resistance exercise and the mineral intake supported the increasing trend of ALP and OC concentration. Based on this previous study, the combination intake of calcium and phosphorus supplement along with resistance exercise resulted in an increment of bone serum ALP and OC concentrations compared with the resistance exercise alone.

In 2012, Lin et al. investigated the acute effects of plyometric jumping and intermittent running on serum bone markers in young males. Blood samples were collected during 5 and 15 minutes pre-exercise trial as well as 5 minutes, 1 hour, 3 hours, 6 hours, 24 hours, 48 hours, and 72 hours post-exercise trial. This study reported that the OC concentration only increased shortly after the exercise bout (at 5 minutes and 1-hour post-exercise). The authors speculated that the changes might occur due to the exercise-induced the mechanical impact rather than bone cellular activities. This might explain the finding of the study conducted by Pettersson et al. (2008) where, it was found that ALP concentration was not significantly changed one-hour after weight lifting programme. The trend of increasing in OC concentration was also observed in a previous study involved a single bout of resistance exercise by Ashizawa et al. (1998). The OC concentration was increased by 13% and 9% on day two and three respectively.

However, ALP and OC concentration was found decreased after a strenuous exercise among males' army recruits during 10 weeks of basic training as reported by Etherington et al. (1999). In addition, Hughes et al. (2014) reported not only the declined of ALP concentration, but also declining of OC concentrations after a physical training conducted among 22 military males. Nevertheless, the serum OC concentration returned to baseline, whereas the serum ALP remained suppressed 2–6 weeks post-training. The authors speculated that strenuous exercise training among physically active participants causes decrease production of ALP concentration in the blood. In contempt of the present study, the moderate intensity was maintained throughout the exercise training by using heart rate monitor. Hence might explain why ALP concentration was not significantly reduced in the present study compared with the previous studies (Etherington et al., 1999; Hughes et al., 2014).

In the present study, consumption of probiotics alone for 12 weeks did not significantly change the bone formation markers across all the groups. Nevertheless, it was mentioned that probiotics were associated with the synthesis of vitamins D, C, K and folate involved in the metabolism of calcium that would result in positive benefit on bone formation (Hancock and Viola, 2001; Villa et al., 1995). Even though the treatment of probiotics has already been shown to enhance bone mass in rodent models (Yousf et al., 2015), its effects in human is yet to be established. Bioavailability of the probiotics in human body, supplementation period and dosage of the supplement are among the factors that have to be considered when doing human research. Thus further study is warranted.

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Regarding bone resorption marker, serum 1CTP was not significantly different between groups and between both time points; pre and post-test. Hence it can be stipulated that probiotics and resistance exercise did not affect serum 1CTP concentration. It general, higher bone resorption with lower bone absorption resulted in bone loss and deterioration of bone architecture. The reduction in the ability of osteoblasts to content the resorption cavity leads to decreasing the thickness of the bone packets and thinning of the trabeculae (Appelman-Dijkstra and Papapoulos, 2015). Previous probiotics studies (animal studies) with L. reuteri and B. longum treatment were found to result in the suppression of bone resorption (Britton et al., 2014; Parvaneh et al., 2015). McCabe et al. (2013) also reported a reduction in bone resorption after examined healthy male mice with L. reuteri orally for 3 times weekly for 4 weeks. In addition, they also observed significant correlative increased in bone mass density, trabecular number and thickness during post-test. To date, human studies in this area is lacking.

Nevertheless, Narva et al. (2004) has investigated the effect of *Lactobacillus helveticus* fermented milk on acute changes in calcium metabolism in postmenopausal women where they found that probiotics consumption did not significantly affect changes in the serum 1CTP concentration as the present study observed. Some probiotics, for instance, L. helveticus, produces bioactive peptides that can affect the proline-containing peptides isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) production which may induce greater availability of minerals (Matar et al., 1996). Therefore, it could support the release of minerals from insoluble ion and enhance the mineral absorption in the body. The IPP and VPP may also inhibit the formation of Angiotensin II (Ang II) from Angiotensin I (Ang I) that help to stimulate the bone resorption (Hagiwara et al., 1998).

Moreover, exercise alone also did not significantly affect the 1CTP concentration in the present study, and this observation was consistent with a few other studies (Hinton et al., 2015; Maimoun and Sultan, 2011). According to Brahm et al. (1996), modification of serum 1CTP was believed to be temporary changed after being exposed to the mechanical loading and returns to its normal range with regards to time. Generally, exercise is known to cause positive effects on bone. This is due to the intrinsic “mechanostat” in the bone tissue that helps the bones to adapt to stresses (Frost, 1990). The physical loads induce dynamic changes to local mechanical conditions; therefore, stimulate activation of osteocytes through fluid shifts in the canalicular network. The osteocytes produced signaling molecules to regulate bone formation and absorption by osteoblast and osteoclasts (Price et al., 2011). In addition, a regular exercise causes changes in bone turnover resulted in decreased bone resorption and increased bone formation (Umemura et al., 2008) to ensure the balanced bone conservation effect towards exercises. Furthermore, it was reported that the 15-20% increase in bone formation markers was parallel with the decrease of 20-25% in bone resorption markers (Craciun et al., 1998; Pearson, 2007).

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Exercise, Probiotics and Parathyroid Hormone (PTH)

The PTH concentration in the present study was not significantly changed between groups as well as within each group after 12 weeks of intervention. The absence of significant changes in PTH concentration might be related to the nullity changes in bone absorption and resorption markers. PTH is one of the key factors which involved in controlling bone remodelling and act as a principal regulator of calcium homoeostasis. According to Poole and Reeve (2005), the increased levels of PTH cause an increase in bone turnover, further induces to either anabolic or catabolic effects on the skeleton regulated upon the pattern and duration of increment. This observation may also be caused by the higher increment in plasma ionised Ca^{+} which blocked the increment in serum PTH. In addition, insufficient increase in plasma catecholamines may also be the factor that causes lower stimulation in PTH secretion. It has been reported that disruption of calcium homoeostasis during exercise training might be the other possible reason that affects bone metabolism. A small reduction in calcium can trigger an increment in serum PTH levels as it was related to a potent stimulator of the bone resorption (Barry and Kohrt, 2007).

In previous studies, exercise can cause the bone threshold stimulation to increase PTH serum concentration and elicited by the anabolic action on bone turnover (Bouassida et al., 2003; Scott et al., 2011). It was speculated that PTH response was affected by the amount of exercise in which the moderate exercise suppressed PTH secretion, whereas strenuous exercise-induced the secretion of PTH (Takada et al., 1998). Exercise can also cause a series of physiological responses that involve the hypothalamus-hypophysis-adrenal and hypothalamus hypophysis-gonad axes that may promote the production of PTH hormones (Smith and Vale, 2006) and can stimulate the differentiation of mesenchymal stem cells (MSCs) into osteoblasts and subsequently affect bone metabolism (Boeloni et al., 2009).

In this study, although it was not statistically significant, there was a decreasing trend in PTH concentration in both probiotic groups; P (-22.7%) and PE (-10.02%) groups. Consistent with the present finding, a study conducted by Narva et al. (2004) which assessed the effects of probiotics on bone in 20 postmenopausal females reported that Lactobacillus from fermented milk caused reduction of PTH followed by increased serum calcium levels and consequently reduced bone resorption in post-menopausal females. Since to date, information on the combined effects of probiotics and exercise on serum PTH are lacking, further studies are warranted.

CONCLUSION

The present study found that combined probiotics with resistance exercise resulted in increased trends of bone formation markers and no changes in bone resorption marker, implying that this combination may elicit beneficial effects in improving bone health among young sedentary males. Nevertheless, more human studies in this field are warranted. Future studies, should consider to include measurement of bone mass density to determine the exact changes in the bone density following the intervention.

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