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BONE METABOLISM MARKERS IN RESPONSE TO THE CIRCUIT TRAINING AND HONEY SUPPLEMENTATION IN YOUNG MALES

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ABSTRACT

This study investigated the effects of 6 weeks combined circuit training programme and honey supplementation on bone metabolism markers in young males. Forty male participants were divided into four groups (n=10 per group): sedentary without honey supplementation control (C), sedentary with honey supplementation (H), circuit training without honey supplementation (Ex), circuit training with honey supplementation (HEx) groups. Circuit training was carried out one hour/session, 3 times/week. Participants in H and HEx consumed 300 mL of honey drink containing 20g of Tualang honey for 7 days/week. Immediately before and after six weeks of experimental period, blood samples were taken for measuring concentrations of serum total calcium, serum alkaline phosphatase as bone formation marker and serum C-terminal telopeptide of type 1 collagen (1CTP) as bone resorption marker. There was significantly (p<0.05) greater serum alkaline phosphatase concentration in HEx at post test compared to pre test value. Meanwhile, significant (p<0.05) reduction in serum 1CTP were found in both H and HEx groups at post test compared to their pre test value respectively. Combination of circuit training and honey supplementation elicited greater effects on bone turnover markers generally compared to circuit training alone, honey supplementation alone and sedentary without honey supplementation.

Keywords: Bone formation marker, Bone resorption marker, Circuit training, Honey

INTRODUCTION

Bone is an organ that supports body weight, protects vital organs and facilitates locomotion by providing attachments for muscles to act as levers. It also acts as a reservoir for ions, especially calcium and phosphate, the homeostasis of which is essential to life. The strength of a bone and its ability to perform physical functions depend on its structure and the intrinsic properties of the materials of which it is composed. The amount of bone, i.e. bone size, mass and density, its spatial arrangement, i.e. shape, geometry and micro architecture, its composition, i.e. intrinsic properties of bone materials and its turnover, i.e. rate and balance of formation and resorption are all such determinants of its ability to perform mechanical functions and to resist fracture (Woolf & Akesson, 2008).

It has been suggested that weight-bearing exercises are particularly necessary to help develop and maintain strong bones (Woolf & Akesson, 2008; Dalsky, 1987; Riggs & Melton, 1992; Kanis, 1996). Among all type of exercises, circuit training programs has been suggested enables to increase muscular strength, endurance, power and cardio respiratory endurance (Gettman & Pallock, 1998). A circuit weight training program usually consists of 6 to 15 stations per circuit. The circuit can be repeated two to three times so that the total time of continuous exercise is 20-30 minutes. In the present study, a circuit training program consisted of 2 circuits of 10 different activities was prescribed, and the prescribed activities are believed to be beneficial for increasing bone health in arms, legs and trunk of the participants.

Besides regular weight-bearing exercise, nutrition also plays an important role in enhancing and maintaining bone health. Honey contains mainly carbohydrates, vitamins and some minerals such as calcium, phosphorus and magnesium, which are believed to be important for enhancing bone health (National Honey Board Honey, 2007). The nutritional fact of honey is illustrated in Table 1 (Bogdanov *et al*., 2008).

Table 1: Nutritional fact of honey

Ingredient		**Amount in 100 g**
Carbohydrates	kcal	300
Proteins	g	0.5
Fats	g	0
Minerals	mg	
Sodium (Na)		1.6-17
Calcium (Ca)		3-31
Potassium (K)		40-3500
Magnesium (Mg)		0.7-13
Phosphorus (P)		2-15
Zinc (Zn)		0.05-2
Copper (Cu)		0.02-0.6
Iron (Fe)		0.03-4
Manganese (Mn)		0.02-2
Chromium (Cr)		0.01-0.3
Selenium (Se)		0.002-0.01
Vitamins	mg	
Phyllochinon (K)		0.025
Thiamin (B_1)		0.00-0.01
Riboflavin (B_2)		0.01-0.02
Niacin[2] (B_3)		0.10-0.20
Panthothenic acid (B_5)		0.02-0.11
Pyridoxin (B_6)		0.01-0.32
Folic acid (B_9)		0.01-0.7
Ascorbic acid (C)		2.2-2.5

(Adapted from Bogdanov et al., 2008)

The present research team has carried out several studies to investigate the combined effects of honey and jumping exercise on bone in animals (Tavafzadeh *et al*., 2011; Ooi, 2014; Mosavat, Ooi & Mohamed, 2014; Tavafzadeh, 2015a; Tavafzadeh, 2015b), and also the combined effects of honey and aerobic dance on bone metabolism markers in young females (Ooi, Ismail & Abdullah, 2011) and adult women (Rahim, Ooi & Hamid, 2016). Past studies showed that combination of honey supplementation and aerobic dance elicited beneficial effect on bone formation marker, i.e. serum alkaline phosphatase in young females (Ooi, Ismail & Abdullah, 2011). Meanwhile, Rahim *et al*. (2016) found that there was lowest percentage of increment in bone resorption marker, i.e. serum C-terminal telopeptide of type 1 collagen (1CTP) in combined aerobic dance exercise with honey supplementation group compared honey supplementation alone, aerobic exercise alone and sedentary without honey supplementation control groups. Findings of these two previous human studies imply that combined honey with exercise may affect bone metabolism in female population with difference age.

To date, no studies have been undertaken to determine the combined effects of a 6 weeks circuit training programme and honey supplementation on bone metabolism markers in young males. Thus, the present study was proposed. If the present study can show that circuit training programme and honey supplementation can give positive effects on bone metabolism markers, it can be used for formulating guidelines in young males to plan their exercise and nutritional promotion program for maintaining bone health in young males.

METHODS

Participants

Forty young Malaysian male participants with age ranging from 19 to 25 years old were recruited in this study. Potential participants were recruited by advertisement within Universiti Sains Malaysia and the local area. The potential subjects were required to contact the investigators if they were interested to participate in the study. The inclusion criteria of the participants including the participants have to be free from any health problems and they did not have the habit of taking honey as daily supplementation prior to the experiment. Participants were matched in age and body mass before they were assigned randomly into the experimental groups.The participants were assigned into four groups, with each group consisting of ten participants (n=10): six weeks of sedentary without honey supplementation control (C), six weeks of sedentary with honey supplementation (H), six weeks of circuit training without honey supplementation (Ex), six weeks of circuit training with honey supplementation (HEx) groups. Each participant was given a detail explanation about the objectives, procedures, benefits, risks and possible discomforts experienced in this study. All participants were required to fill up participants' information sheets and sign on the consent forms.The experimental protocol was approved by Human Research Ethics Committee, Universiti Sains Malaysia.

Blood sample taking and anthropometric measurement

Before and after six weeks of experimental period, blood

samples were withdrawn from the participants, from an antecubital vein after a 12 hours overnight fast (drinking plain water was allowed) at 8.00 am. After the blood taking, participants' anthropometric measurements such as body height, body weight, and percentage of body fat were carried out on the same day. The participants' body heights were measured by a stadiometer (Seca 220, Germany), meanwhile the body weight and percentage body fat were measured by using a body composition analyser (TANITA, Model TBF-410, Japan). The blood taking and anthropometry measurement sessions were carried out in the Exercise and Sport Science Laboratory, School of Health Sciences, Health Campus, Universiti Sains Malaysia.

Circuit training programme

The participants in both the exercise without supplementation group (Ex) and honey supplementation with exercise group (HEx) were required to carry out circuit training sessions, 45 minutes per session (5.30 pm to 6.15 pm), three times per week for six weeks. The sessions started with 10 minutes of warm-up and ended with 5 minutes of cooling down activities. The exercise sessions started with 10 minutes of warm-up and ended with 5 minutes of cooling down activities. The circuit training programme consisted of two circuits. In each circuit, participants performed 10 different exercises in 10 different stations (one type of exercise per station, each participant spent 30 seconds in one particular station). The work rest ratio was 1:2, where participants exercised for 30 seconds for one activity, and rested for one minute before continued with the next activities. Resting time between circuits was five minutes. The activities that involved in circuit training were hand elastic bend exercise, leg elastic bend elastic, free-weight dumbbell triceps extension, rope skipping, free-weight dumbbell concentration curl, sit-up, back extension, burpee, push-up and split squat.

Honey Supplementation

Three hundred ml of honey drink which containing 20g of Tualang honey was consumed by the participants of honey supplementation group (H) and honey supplementation with exercise group (HEx) per day, seven days per week for six weeks. Participants in HEx consumed 300 mL of honey drink 30 minutes before performing circuit training. The mixture of honey was prepared by mixing 20g of honey with 300 mL of plain water. In the present study, a Malaysian local product, i.e. 'Tualang' honey was used.

Blood Biochemical Analysis

Serum total calcium was analysed colorimetrically (Hitachi Automatic Analyser 912, Bohringer Mannheim, Germany) using commercially available reagent kits (Roche Diagnostic GmbH, Germany). Serum ALP is a bone formation marker, which was analysed colorimetrically by using a chemistry analyser (Architec C 8000, USA) with commercially available reagent kits (Randox, UK). Serum C-terminal telopeptide of type 1 collagen (1CTP) was analysed using a commercially available enzyme immunoassay kit (Orion Diagnostic UniQ 1CTP, EIA, Finland), and the concentration was determined by a photometric microplate reader (Molecular Devices; Versamax tunable microplate reader, U.S.A).

Statistical Analysis

Statistical software in the Statistical Package for Social Sciences (SPSS) Version 18.0 was used for the statistical analysis. All data are expressed as mean ± standard deviation (SD). Repeated measures ANOVA were performed to determine the significance of the difference between and within groups. Statistical significance was accepted at $p<0.05$.

RESULTS

Participants' Anthropometric Data

A total of 37 young male participants with mean age 21.65 ± 1.51 years old completed the study. One participant from exercise with honey supplementation group (HEx), honey supplementation group (H) and control group (C) respectively discontinued the programme due to personal reasons. The mean anthropometric values (mean ± SD) obtained were as follows: Body height: 169.8 ± 6.1 cm, body weight: 68.1 ± 13.4 kg, body mass index: 23.55± 4.06 kg m^2 and percentage of body fat: 18.9±6.0%.

Bone Metabolism Markers

Mean serum total calcium concentrations of all the groups at pre and post tests are presented in Table 2.

Table 2: Mean serum total calcium concentration at pre- and post tests (Mean ± SD)

Serum total calcium concentration ($mmol.L^{-1}$)			
Groups	**Pre test**	**Post test**	**% difference between pre - and post tests**
Control (C)	2.40 ± 0.07	2.36 ± 0.06	-0.84
Honey (H)	2.37 ± 0.07	2.34 ± 0.08	-4.17
Exercise (Ex)	2.41 ± 0.09	2.36 ± 0.13	-2.07
Honey with exercise (HEx)	2.34 ± 0.06	2.36 ± 0.08	0.85

After 6 weeks of experimental period, there were no significant differences of serum total calcium in post test compared to their respective pre test value in all the groups. Figure 1 illustrates results of serum alkaline phosphates (ALP) concentrations.

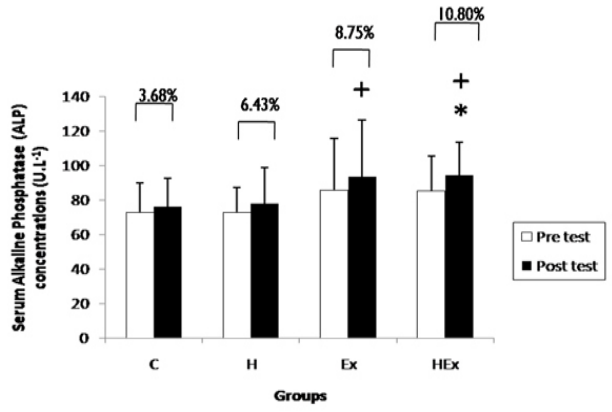

Figure 1: **Mean serum alkaline phosphatase concentrations at pre and post tests (Mean ± SD).**

*, significantly different from pre test ($p<0.05$)
+, significantly different from respective control group ($p< 0.05$)

At pre test, no significant differences were evident in serum alkaline phosphatase concentrations between C, H, Ex and HEx groups. At post test, mean serum alkaline phosphatase concentration in Ex and HEx were significantly higher than C respectively ($p<0.05$). After 6 weeks of experimental period, there was significant ($p<0.05$) increase in serum alkaline phosphatase concentration in HEx groups at post test compared to pre test value, with percentage increment of +10.80%. Mean serum C-terminal telopeptide of type 1 collagen (1CTP) concentrations of all groups are presented in Figure 2.

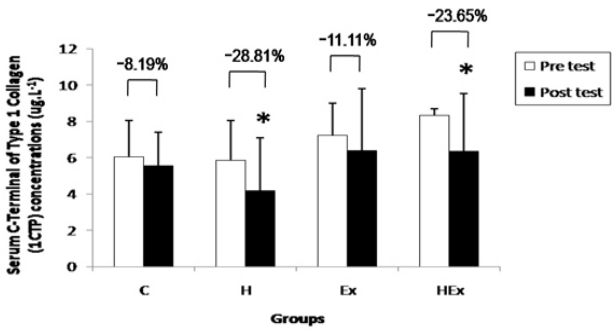

Figure 2: Mean serum C-terminal telopeptide of type 1 collagen (1CTP) concentrations at pre and post test (Mean ± SD)

*significantly different from pre test ($p<0.05$)

DISCUSSION

In the present study, we investigated whether exercise alone, honey supplementation alone and combined of exercise and honey supplementation could cause observable changes in levels of serum calcium, which could be associated with bone turnover as indicated by serum biochemical bone turnover markers of formation and resorption.

In human, ninety nine percent of calcium is stored in bone as hydroxyapatite crystal, while 1% is present as ionized calcium in the intracellular and extracellular fluid. Effective calcium homeostasis is essential for most of the biological processes, including bone metabolism, cell proliferation, blood coagulation, hormonal signaling transduction and neuromuscular functions (Narattaphol, 2007). The present findings showed that six weeks of circuit training programme, honey supplementation alone and combined circuit training programme with honey supplementation did not elicit any significant changes in serum total calcium concentration in all the groups (Table 2). As observed in the present study, two previous studies carried out by Holy and Zerath (2000) and Kim and Park (2005) also reported that there were no significant changes in serum total calcium concentration as results of exercise in rats. Nevertheless, a recent animal study carried out by the present research team as reported in Tavafzadeh *et al.* (2015a) that, 8 weeks of jumping exercise at 40 jumps per day, 5 days per week combined with daily honey supplementation could increase serum total calcium in the rats. Additionally, in our another recent animal study as reported in Mosavat *et al.* (2014), it was also found that combined jumping exercise at 80 jumps per day, 5 days per week with daily honey supplementation for a total of 8 weeks resulted increase in this serum

parameter in the rats. The discrepancy between the findings of the present human study compared to our previous animal studies (Mosavat, Ooi & Mohamed, 2014; Tavafzadeh *et al.*, 2015a) reflects that responses of serum total calcium to combined exercise and honey supplementation varied between human and animal studies.

The notable findings in the present study are that there was significantly (p<0.05) greater serum alkaline phosphatase concentration in HEx at post test compared to pre test value. Meanwhile, significant (p<0.05) reduction in serum 1CTP were found in both H and HEx groups at post test compared to their pre test value respectively. These findings imply that the combination of a circuit training programme and honey supplementation may elicit beneficial effects on bone health by increasing serum alkaline phosphate, a bone formation marker. In addition, combination of circuit training programme and honey supplementation and also honey supplementation alone may elicit significant effect on reducing bone resorption in young males. Collectively, these findings showed that honey supplementation alone may reduce bone resorption, and combination of circuit training and honey supplementation may increase bone formation and reduce bone resorption.

It is known that honey contains vitamin K and mineral such as calcium, phosphorus, iron and magnesium as sown in Table 1, which are vital for increasing bone health. In the present study, it was observed that honey supplementation alone was able to reduce bone resorption marker in young males and his have confirm the beneficial effects of honey supplementation on bone health in human beings. It was speculated that the nutrients contained in honey may have played crucial roles in reducing bone resorption in young male participants.

Regarding effects of exercise on bone formation marker, it was observed that serum alkaline phosphatase concentration increased in rats given work loads of 40 jumps per week and above for a total of 24 weeks (Ooi, Singh & Singh, 2012). However, discernable effects on bone formation marker were not observed in the present study with circuit training alone which consisted of difference activities and shorter exercise duration compared to the mentioned jumping animal study. Nevertheless, when circuit training was combined with honey supplementation, beneficial effects on increasing bone formation marker was evident in young males. This observation confirmed our hypothesis that combined regular weight-bearing exercise and nutritional supplementation such as honey play an important role in enhancing bone health by affecting bone metabolism. Consistent with the present finding, positive findings on serum formation marker have also been reported by Mosavat *et al.*, (2014), in which serum alkaline phosphatase increased with jumping exercise at 40 jumps per day, 5 days per week combined with daily honey supplementation for 8 weeks in young female rats.

Regarding serum C-terminal telopeptide of type 1 collagen (1CTP), a bone resorption marker, it was found that there was statistically lower concentration of this measured parameter in post test compared to pre test in HEx group (Figure 2). In the study of Ooi, Singh & Singh, 2012, it was reported that serum 1CTP concentration decreased in rats given work loads of 40 jumps per week and above for a total of 24 weeks. Further study indicates the reduction in bone resorption marker with combined jumping exercise and honey supplementation was reported in Tavafzadeh *et al.* (2015a). The human study carried out Lau and Ooi (2014) also found that combined circuit training programme and chocolate malt drink supplementation elicited significant effect on reducing in 1CTP in young males. Similarly, in a previous animal study carried out by Gala *et al.* (2001) which used another type of bone resorption marker, i.e. tarteate-resistance acid phosphatase (TRAP), it was found that that a TRAP reduced with treadmill running exercise and calcium supplementation in ovarectomised rat. The consistent results between the present study, animal study of Tavafzadeh *et al.* (2015a), human studies of Lau and Ooi (2014) and Gala *et al.* (2001) may indicate that even though there were differences in the types of exercise and nutritional supplementation prescribed in the animal and human studies, bone resorption marker still respond the same with the intervention of combined exercise and nutritional supplementation.

The speculation of the reason of the increase in bone formation marker and reduction in bone resorption marker with combined circuit training and honey supplementation in young males is that, rhythmic nature of dynamic loading have been elicited by physical activities while participants performing circuit training. This can cause enhancement of the blood flow to the muscle and subsequently to the bone (Ooi, Singh & Sing, 2009). Increased bone formation marker and reduced bone resorption marker with this combination may be resulted from the absorption of the nutrients contained in honey into the blood, and subsequently transported into the muscle and bone, caused by the dynamic loading elicited by physical activities.

Strain rate, strain distribution and strain magnitude play major roles in determining bone response to loading, in which physical activities can act as a type of loading on bone (Ooi, Singh & Sing, 2009). Bone tissue responds better to dynamic exercises i.e. dynamic loading compared to static loading. This is because dynamic loading can create higher hydrostatic pressure gradients within bone's fluid-filled lacunar-canalicular network compared to static load which produce less stresses or strains for initiating osteogenesis (Turner & Robling, 2003). It is speculated that the present observation of increased serum bone formation marker and reduction in bone resorption marker in the combined group may imply that the strain rate, strain distribution, strain magnitude and the dynamic nature of the physical activities prescribed in the circuit training are appropriate in influencing bone metabolism markers when combined with honey supplementation in young males.

CONCLUSION

The most notable findings of the present study are that there was significant increase of serum alkaline phosphatase, a bone formation marker, and significant reduction in serum 1CTP, a resorption marker in the young male participants when circuit training programme were carried out 30 minutes after consumption of honey drink by the participants. Findings of the present study reflect that the six weeks of circuit training programme at three times per week, one hour per session combined with daily consumption of 20g of honey supplementation diluted in 300 mL of plain water may affect bone metabolism positively.

In conclusion, combination of circuit training and honey supplementation and honey alone has potential to be proposed for formulating guidelines in planning exercise and nutritional promotion programs for the maintenance bone health in young males. Nevertheless, further study with longer duration is needed to confirm the present findings.

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CONFLICT OF INTEREST

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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