Original Article

Effects of Combined Aerobic Dance Exercise and Honey Supplementation on Bone Turnover Markers in Young Females

Foong Kiew Ooi
Noorsuzanawati bt Ismail
Malisa Yoong bt Abdullah
Universiti Sains Malaysia

Abstract: This study investigates the effects of combined aerobic dance exercise and honey supplementation on bone turnover markers in young females. Forty young female subjects were divided into four groups, with 10 subjects per group: sedentary without honey supplementation control (C), sedentary with honey supplementation (H), aerobic dance exercise without honey supplementation (Ex), aerobic dance exercise with honey supplementation (HEx) groups. Aerobic dance exercise consisted of 1 hour per session, 3 sessions per week for 6 weeks. H and HEx groups consumed honey drink, in a dosage of 20g of Gelam honey diluted in 300ml of plain water, at 7 days per week for a total of 6 weeks. Subjects in HEx group consumed honey drink 30 min before performing exercise on the exercise days. Concentrations of serum alkaline phosphatase (ALP) as bone formation markers, C-terminal telopeptide of type 1 collagen (1CTP) as bone resorption marker were measured. After 6 weeks of study, HEx group exhibited highest percentage increment in serum ALP (+31.79%), among the groups. There was no statistical difference in 1CTP between pretests and posttests in all the groups. The present study suggests that combination of aerobic dance exercise and honey supplement may elicit more beneficial effects on increasing bone formation marker compared to aerobic dance exercise or honey supplementation alone.

Keywords: *Exercise, honey drink, serum alkaline phosphatase, serum C-terminal telopeptide of type 1 collagen, young females*

Foong Kiew Ooi is in the Sports Science Unit, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia. Noorsuzanawati bt Ismail and Malisa Yoong bt Abdullah are in the Exercise and Sports Science Programme, School of Health Sciences, Universiti Sains Malaysia, Kelantan, Malaysia.

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Corresponding author: Dr. Ooi Foong Kiew, Sports Science Unit, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia, E-mail address: fkooi@kb.usm.my; fkooi9@yahoo.com, Tel: +609-767 6931, Fax: +609-764 1945.

Introduction

The recognition of osteoporosis as a major health problem among the growing number of elderly people around the world has resulted in widespread efforts to determine the etiology of the disease and how it might be delayed or prevented. One strategy is to increase bone mass during the formative years of life and then subsequently either try to maintain the gain or reduce the rate of bone loss. These could be achieved through adequate nutritional intake and regular weight-bearing exercises.

It has been suggested that weight-bearing exercises such as walking, running, dancing, and jumping are particularly necessary to help develop and maintain strong bones (Dalsky, 1987; Kanis, 1996; Riggs & Melton, 1992; Sinaki, 1996). Among all types of weight-bearing exercises, high-impact exercise that produces peak forces greater than or equal to two times the body weight (Grove & Londeree, 1992) seems to be effective in producing great osteogenic effects (Fuchs, Bauer, & Snow, 2001; MacKelvie, McKay, Khan, & Crocker, 2001; MacKelvie, McKay, Petit, Moran, & Khan, 2002; McKay et al., 2000; Petit et al., 2002; Petterson, Nordstrőm, Alfredson, Henriksson-Larsén, & Lorentzon, 2000; Witzke & Snow, 2000). Dancing provides an ideal osteogenic stimulus because the various stepping, jumping, leg lifting, and landing activities during dancing elicit unusual loads and loads with impact ranging from low to high on the skeleton (Khan et al., 1998; Matthews et al., 2006). Thus aerobic dance exercise comprised of low-, moderate-, and high-impact activities is believed to be one appropriate form of weight-bearing exercise for enhancing bone health (Heinonen et al., 1995).

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, 2007). The nutritional fact of honey is illustrated in Table 1 (Bogdanov, Jurendic, Sieber, & Gallmann, 2008). It was reported previously that taking honey appeared to enhance calcium absorption in rats, and could therefore play a role in boosting bone health (Ariefdjohan, Martin, Lachik, & Weaver, 2008). Additionally, in another study, it was found that in a group of young Sprague Dawley rats fed with honey for 52 weeks, their bone mineral density was significantly greater than the sugar free diet-fed controls (Chepulis & Starkey, 2008); this again indicates that honey may enhance bone health. In a recent study carried out by the present research team, it was found that there were beneficial bone effects elicited by combined jumping exercise and honey supplementation with increases in femoral and tibial maximum diameter and strength, and reduction in serum bone resorption marker in female rats (Ooi et al., 2010; Tavafzadeh, 2009; Tavafzadeh, Ooi, Krasilshchikov, & Sulaiman, 2011).

Since the combined effects of an aerobic dance exercise regimen with honey supplementation on bone in humans have not been determined, the present study was proposed for determining the effect of 6 weeks of combined aerobic dance exercise and honey supplementation on bone metabolism in young females. The measurable changes in bone mineral density by using bone densitometry such as Dual Energy X-ray Absorptionmetry scanning are expected not be observed in a short duration of 6 weeks; therefore, the present study focused on changes in blood parameters, where changes in blood bone turnover markers such as serum alkaline phosphatase as bone formation marker, and serum C-terminal telopeptide of type 1collagen (1CTP) as bone resorption marker were observed. It is hoped that

Table 1

Nutritional Facts 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 (B1)	0.00-0.01
Riboflavin (B2)	0.01-0.02
Niacin2 (B3)	0.10-0.20
Panthothenic acid (B5)	0.02-0.11
Pyridoxin (B6)	0.01-0.32
Folic acid (B9)	0.01-0.7
Ascorbic acid (C)	2.2-2.5

(Adapted from Bogdanov et al., 2008)

results obtained from this can be used for developing age-specific exercise and nutritional programmes, by formulating guidelines for the maintenance of bone health. This will be useful in planning exercise and nutritional promotion programmes for young females.

Methods

Experimental Design

Subjects grouping. Forty young Malaysian female subjects aged 19 to 28 were recruited in this study. The subjects were matched in age, body weight, and percentage of body fat for assigning into the control and intervention groups. The subjects were divided into four groups with 10 subjects per group: 6 weeks of sedentary without honey supplementation control (C), 6 weeks of aerobic dance exercise (Ex), 6 weeks of honey supplementation (H), and 6 weeks of combined aerobic dance exercise and honey supplementation (HEx) groups. Subjects in the control group did not perform either exercises and did not take honey supplementation. Meanwhile, aerobic dance exercise group performed 1 hour of aerobic dance exercise per session, 3 times per week for 6 weeks. Subjects of honey group consumed honey drink containing 20g of Gelam honey, 7 days per week for a total of 6 weeks duration. Subjects in combined aerobic dance exercise with honey supplementation group performed aerobic dance exercises 1 hour per session, 3 times per week for 6 weeks and consumed honey drink 7 days per week for 6 weeks with dosage same as honey group. The subjects of HEx were required to consume the honey drink 30 minutes before performing aerobic dance exercise on the exercise days.

Aerobic dance exercise programme. The subjects of aerobic dance exercise group (Ex) and combined honey supplementation with aerobic dance exercise group (HEx) were required to have aerobic dance sessions for 3 sessions per week, 1 hour per session (from 5:30 p.m. to 6:30 p.m.) for 6 weeks. The aerobic dance exercise programme of this study consisted of two sessions of "high impact and low impact" and one session of "step board" aerobic dance exercises in a week. The1-hour session started with 10-15 minutes of warm up period, 30-35 minutes of aerobic dance activities, and ended with 5-7 minutes of cool down. In the high-impact and low-impact exercise sessions, subjects were required to do upper and lower limb movements according to the beats of the music played, which ranged from slow to fast. In the step board exercise sessions, subjects were required to step up and step down the step board while dancing. Two of the aerobic dance sessions in a week were conducted by instructors either in the Exercise and Sport Science Laboratory, campus of Universiti Sains Malaysia, or in a fitness centre off campus (i.e., Fit Fun Fitness Centre). The third session in each week was a home-based session. The subjects were given prerecorded CDs containing aerobic dance workout, and they were required to perform aerobic dance sessions on their own without supervision by following the workout in the prerecorded CD in the home-based sessions. The subjects were reminded to have the home-based sessions by the researcher through telephone calls.

Honey supplementation. A honey drink was consumed by the subjects in the honey (H) group and combined aerobic dance exercise and honey supplementation (HEx) group in the dose of 20g (Sulaiman et al., 2011) of Malaysian local Gelam honey diluted in 300ml of plain water (Gisolfi & Duchman, 1992), for 7 days per week for a total of 6 weeks duration. On the exercising days, the subjects of the combined aerobic dance exercise and honey supplementation (HEx) group were required to consume the honey drink 30 minutes before performing aerobic dance exercises.

Anthropometric measurements. Before the 6-week experimental period, subjects' anthropometric measurements such as body height, weight, and percentage of body fat were carried out. The subjects' body heights were measured by using a stadiometer (Seca 220,

Germany), the body weight and percentage body fat were measured by using a digital hand to foot body composition measuring device (Kadaca Scan, Japan).

Blood sample collection and analysis. Before and after the 6-week experimental period, subjects were seated and a 4ml of venous blood sample was taken from an antecubital vein after a 12-hour overnight fast (drinking plain water was allowed). The blood was withdrawn by the laboratory technologist in the Exercise and Sports Science Laboratory, School of Health Sciences, Health Campus, Universiti Sains Malaysia to determine the bone turnover markers. Blood taking for subjects in Ex and HEx were carried out 18-20 hr (8:00 a.m.–10:00 a.m.) after performing aerobic dance exercise. Serum was obtained from the blood sample using a centrifuge (Hettich-Rotina 46RS, Germany) for 10 minutes with 4000 rpm in 4 °C temperature. The serum was then divided into equal portions and stored at -80 °C in a freezer (ThermoForma, Model 705, USA) for subsequent analysis. In this study, serum alkaline phosphatase (bone formation marker) and C-terminal telopeptide of type 1 collagen/1CTP (bone resorption marker) were estimated in the blood. Serum alkaline phosphatase was analyzed colorimetrically by an analyzer (Hitachi Automatic Analyzer 912, Bohringer Mannheim, Germany) using commercially available reagent kits (Randox, UK, Germany). Serum C-terminal telopeptide of type 1 collagen (1CTP) was analysed using a commercially available enzyme immunoassay kit (Orion Diagnostica UniQ 1CTP, EIA, Finland), and the concentration was determined by a photometric microplate reader (Molecular Devices; VersaMax tunable microplate reader, USA). This study was approved by the Human Ethical Committee of Universiti Sains Malaysia.

Statistical analysis. Statistical tests contained in the Statistical Package for Social Sciences (SPSS) Version 18.0 were used for the statistical analysis. A repeated measure analysis of variance (ANOVA) was performed to determine the significance of the differences between and within groups. All data are reported as means ± standard deviation (SD). Difference was considered significant at $p < 0.05$.

Results

Anthropometric Characteristics of the Subjects

A total of 37 young female subjects with mean age 21.9 ± 2.4 years completed the study (Table 2). One subject from aerobic dance exercise group (Ex) and another two subjects from aerobic dance exercise with honey supplementation group (HEx) discontinued the programme due to other commitments. There were no significant differences between the groups in age, body height, weight, and percentage of body fat at the beginning of the experimental period.

Bone Turnover Markers

Bone formation marker: Serum alkaline phosphatase. The bone formation marker of serum alkaline phosphatase (ALP) concentrations in all the groups at pretest and posttests are presented in Table 3. At pretests and posttests, there were no significant differences in ALP among the groups. After 6 weeks of experimental period, there were significant ($p < 0.05$) increases in ALP in H (+15.91%) and HEx (+31.79%) groups at posttest compared to pretest values.

Bone resorption marker: Serum C-terminal telopeptide of type 1 collagen (1CTP). Results of serum C-terminal telopeptide of type 1 collagen (1CTP), a bone resorption

Table 2

Mean Age, Body Height, Weight and Percentage of Body Fat of the Subjects (Means ± SD)

	Control (C)	Honey (H)	Aerobic dance exercise (Ex)	Honey with aerobic dance exercise (HEx)
Number of subjects	10	10	9	8
Age (years)	21.6 ± 2.7	20.7 ± 2.3	22.2 ± 2.0	23.5 ± 1.7
Body Height (cm)	156.9 ± 6.7	155.5 ± 6.7	156.7 ± 6.7	157.9 ± 2.6
Body Weight (kg)	52.3 ± 8.2	53.5 ± 9.9	53.9 ± 11.2	50.5 ± 8.2
Percentage of body fat (%)	27.2 ± 6.2	27.3 ± 4.3	27.9 ± 4.5	27.5 ± 4.4

Table 3

Mean Serum Alkaline Phosphatase (ALP) Concentrations (U.L-1) at Pretests and Posttests (Mean ± SD)

Groups	Pretest	Posttest	P values	Percent difference compared to pre-test (%)
Control (C)	1.90±16.59	47.90±10.49	0.122	-7.70
Honey (H)	46.50±8.68	53.90±10.18*	0.001	+15.91
Aerobic dance exercise (Ex)	46.22±12.72	52.22±12.30	0.062	+12.98
Honey with aerobic dance exercise (HEx)	49.13±15.29	64.75±16.52*	0.002	+31.79

*$p<0.05$ significantly different from pretest value

marker, of all the groups at pretests and posttests are shown in Table 4. At the pretest, there were no significant differences in 1CTP among the groups. After 6 weeks of experimental period, no significant differences were observed in 1CTP among the groups at posttest. Additionally, there was no statistical difference between pretests and posttests in all the groups.

Discussion

In the present, the subjects did not show significant differences in age, body weight, and percentage of body fat at the beginning of the study (Table 2) indicating that the subjects were well matched in terms of age, body weight, and percentage body fat, and implying that any differences in all the other measured parameters were therefore consequences of either exercise, honey supplementation, or the combination of both exercise and honey supplementation.

As the bone is a highly active organ metabolically, it is always in a continuous dynamic remodeling process that is maintained in a tightly coupled balance between resorption of old bone and formation of new bone (Christenson, 1997). Therefore, specific biochemical markers of bone turnover allow for an estimate of bone metabolic process, and they have been established as useful parameters in assessing changes in bone turnover (Leo et al., 2000; Souberbielle, Cormie, & Kindermans, 1999). In the present study, serum alkaline phosphatase (ALP) was measured as biomarker of bone formation, and C-terminal telopeptide of type 1 collagen (1CTP) was measured as a biomarker of resorption.

The most notable finding of this study is that at the end of the 6-week study period, the combined aerobic dance exercise and honey supplement group (HEx) exhibited the highest percentage increment in serum alkaline phosphatase, a bone formation marker among the experimental groups (Table 3). The present study suggests that a combination of aerobic

Table 4

Mean Serum C-Terminal Telopeptide of Type 1 Collagen (1 CTP) Concentrations (μg.L-1) at Pretests and Posttests (Mean ± SD)

Groups	Pretest	Posttest	P values	Percent difference compared to pre-test (%)
Control (C)	7.22 ± 1.71	7.39 ± 2.29	0.750	+2.30
Honey (H)	6.71 ± 1.35	7.15 ± 1.88	0.546	+6.56
Aerobic dance exercise (Ex)	6.50 ± 1.20	6.67 ± 1.78	0.747	+2.62
Honey with aerobic dance exercise (HEx)	7.03 ± 2.32	6.71 ± 2.55	0.596	-4.55

dance exercise and honey supplement may elicit more beneficial effects on increasing bone formation marker compared to honey supplementation alone (H) or aerobic dance exercise (Ex) generally.

The present study also revealed that there was a statistically significant greater value of serum alkaline phosphatase in honey supplementation alone group (H) and combined honey and exercise group (HEx) after the 6-week experimental period compared to their pretest value respectively (Table 3). Nevertheless, the percentage increase of serum alkaline phosphatase was greater in HEx group (+31.79%) than H group (+15.91%). This implies that a combination of honey and exercise elicited greatest effects in increasing serum alkaline phosphatase, a bone formation marker among all the groups.

It is known that honey contains vitamin K, and minerals such as calcium, phosphorus, iron, and magnesium as illustrated in Table 1, which are important for increasing bone health (National Honey Board, 2007). Honey has been reported to have the potential to boost calcium absorption and increase bone mineral density in rats (Ariefdjohan et al., 2008; Chepulis & Starkey, 2008), implying that honey may elicit beneficial effects on bone in animals. In the present study, it was found that honey supplementation alone (H) was able to increase blood bone formation marker significantly in young females, and this has confirmed the beneficial effects of honey supplementation on bone health in human beings. The nutrients contained in the honey may have played crucial roles in boosting bone formation in the young female subjects.

Regarding effects of exercise on bone formation marker, observation of increases in serum alkaline phosphatase in the rats have been reported by Ooi, Singh, and Singh (2009) with 24 weeks of jumping exercise in the rats. In this present 6-week human study that was prescribed with a different type of exercise and shorter exercise duration compared to the aforementioned jumping exercise animal study, statistically significant increase in serum alkaline phosphatase was not observed in aerobic dance exercise alone group (H). However, when aerobic dance exercise was combined with honey supplementation (HEx), additional effects of increasing in bone formation marker concentration was observed in young females. Similarly, in a previous combined exercise and supplementation animal study by Gala, Di'az-Curiel, de la Piedra, and Calero (2001), it was reported that combined treadmill running with calcium supplementation could produce a significant increase in serum alkaline phosphatase in ovariectomised rats. Specker and Brinkley (1996) have reported that calcium plays an important role in bone health—an increase in bone density was related to calcium intake when calcium supplementation accompanied a physical exercise programme that consisted of jumping, hopping, and skipping activities. Consistent with the findings of the previous studies mentioned above, when considering the effectiveness of combined exercise and supplementation on bone health, the combination of exercise and supplementation, such as honey as in the present study, appears to have potential in increasing bone formation marker.

It is speculated that the probable reasons of the present observation of increase in bone formation marker in combined aerobic dance exercise and honey might be due to aerobic dance exercise that increases absorption of nutrients contained in the honey into the bone, through the enhancement of blood flow to the muscle, and subsequently to the bone caused by the rhythmic nature of dynamic loading elicited by physical activities during aerobic dance sessions. The nutrients contained in honey may enhance bone formation in the young female subjects. To our knowledge, the present study is the first study that

reported the combination effects of aerobic dance exercise and honey supplementation on bone formation marker in humans.

Regarding serum C-terminal telopeptide of type 1 collagen (1CTP), a bone resorption marker, it was found that there was no statistical difference between pretests and posttests in all the groups (Table 4). Reduction in bone resorption marker with combined exercise and supplementation was reported in Tavafzadeh (2009), which showed a reduction in 1CTP with combined jumping and honey supplementation in the rats, and also in a previous animal study carried out by Gala et al. (2001), which used another type of bone resorption marker (i.e., tartrate-resistance acid phosphatase [TRAP]). It was reported in Gala et al. (2001) that a reduction of TRAP was observed with treadmill running exercise and calcium supplementation in ovariectomised rats. The inconsistent results between the animal studies of Tavafzadeh (2009), Gala et al. (2001), and the present aerobic dance exercise and honey supplementation human study may indicate that animals and humans may respond differently in serum bone resorption marker, and may be dependent on the types of exercise and supplementation prescribed.

In general, the present study showed that 6 weeks of combined aerobic dance exercise and honey supplementation has the potential to increase bone formation marker. Nevertheless, further studies are needed to elucidate the efficacy of the effect of combined aerobic dance exercise and honey supplementation on the bone metabolism markers by a prolonged period of intervention to confirm the present findings.

Conclusion

In summary, the most notable finding of the present study is that the combination of aerobic dance exercise and honey supplementation may elicit more beneficial effects on bone health by increasing bone formation marker in young females compared to honey or exercise alone. The present study findings reflect that the 6-week combination of aerobic dance exercise at 3 times per week, 1 hour per session with 20g of Gelam honey supplementation per day given for 7 days per week, may affect bone health positively in the young female subjects where aerobic dance exercises were carried out 30 min after consumption of honey.

Building and maintaining healthy bones throughout life is dependent on lifestyle factors. Our study results imply that engagement in regular physical activities by having a certain level of exercise such as aerobic dance exercise and implementing healthy nutritional habits by consuming optimal amount of honey supplementation may help in maintaining and enhancing bone health. In conclusion, combination of aerobic dance and honey supplementation has potential to be proposed for formulating guidelines in planning exercise and nutritional promotion programmes for the maintenance of bone health in young females.

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