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Research

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Changes of Bone Metabolism Markers and Muscular Performance with Combined Aerobic Dance Exercise and Honey Supplementation in Adult Women

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ABSTRACT

OBJECTIVE: This study investigated the effects of combined aerobic dance exercise and honey supplementation on blood bone metabolism markers and muscular power in adult women.
METHODS: Forty-four healthy sedentary women (25-40 year-old) were age and weight matched, and subsequently being assigned into four groups with n=11 per group: Control (C), honey supplementation (H), aerobic dance exercise (Ex) and combined aerobic dance exercise with honey supplementation (HEx) groups. Aerobic dance exercise was carried out for one hour per session, three times per week for eight weeks. Blood samples were taken to determine the concentrations of serum total calcium, osteocalcin (bone formation marker), serum C-terminal telopeptide of type 1 collagen (1CTP) (bone resorption marker), and parathyroid hormone (PTH). Meanwhile, subjects' lower limb muscular power was measured.
RESULTS: At the end of 8-weeks of experimental period, serum 1CTP concentration was significant greater in post-test than pre-test in Ex group. The percentage increment in 1CTP was the highest in Ex group. Meanwhile, the percentages of increment in 1CTP and PTH concentrations in HEx group were the lowest compared to the other experimental groups. Regarding muscular performance, Ex and HEx exhibited more discernable beneficial effects on lower limb average power compared to the H and C groups.
CONCLUSION: Combination of aerobic dance exercise and honey supplementation has potential to reduce the increment in bone resorption resulting from exercise, and this combination could enhance lower limb muscular power in sedentary women.

KEYWORDS: Aerobic dance; Honey supplementation; Bone metabolism; Muscular power; Women.

INTRODUCTION

Osteoporosis is a disease characterized by a loss of bone mass and the structure deterioration of bone tissue, resulting in bone fragility and an increase in susceptibility to fractures. This disease imposes major burden on the health economy and being recognized as one of the major public health problems worldwide. To date, many strategies have been developed with the aim of preventing bone loss. These include involvement in physical activity programs[1-4] and through adequate nutritional intake.[5-9]

Bone is a dynamic tissue that serves both mechanical and metabolic functions. It is in a continuous dynamic remodeling process: maintaining a tightly coupled balance between

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resorption of old bone and formation of new bone.[10,11] The balance between bone resorption and formation is influenced by age and level of strain on the bone generated by muscle contraction during movements. It is generally known that specific biochemical markers of bone turnover allows for an estimate of bone metabolic process and they have been established as useful parameters in assessing changes in bone turnover.[12-15]

Mechanical strain generated by exercise constitutes one of the most important stimuli to bone formation,[16-18] and 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.[19-22] It is known that bone tissue responds better to dynamic loading rather than static loading.[1,23,24] According to Khan et al[25] and Matthews et al[26], dance may provide an ideal osteogenic stimulus due to its various stepping, jumping, leg lifting and landing movements which elicit unusual and moderate to high impact dynamic loads on the skeleton. These loads are experienced by an individual primarily in the lower limbs, and the upper limbs may serve as a quasi-control site.

In general, muscle contraction forces act directly or indirectly on bone and are responsible for overloading bone tissue to produce an osteogenic stimulus. It was estimated that more than 70% of the bending moment on a bone is transmitted by muscle force rather than by body weight, supporting the idea that muscle strength places greater loads on bones than do gravitational forces associated with weight.[27] It is believed that the strong forces generated by the muscle contraction which impose on bone tissue during the performance of an exercise or training can increase bone metabolism and promote osteogenesis.[1] Therefore, it is speculated that strong muscle can generate high force which subsequently enable to produce strong bone, and there is a close relationship between muscular performance, bone metabolism and exercise.

Besides regular weight-bearing exercise, nutrition also plays an important role in enhancing and maintaining bone health. Honey contains carbohydrates such as glucose, fructose, sucrose and raffinose, enzymes, flavonoids, antioxidants, minerals, organic acids, proteins, phenolic acids, and vitamins such as vitamin C and vitamin E.[28] Some of these components are believed to be important for enhancing bone health. The nutritional fact of honey is illustrated in Table 1.[29]

Honey has been reported to have the potential to boost calcium absorption and increase bone mineral density in rats,[30,31] implying that honey may elicit beneficial effects on bone in animals. Since the combined effect of honey and exercise in

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.[29]

Table 1: Nutrition fact of honey.

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animal has not been confirmed, thus the present research team has conducted a study to investigate the effect of this combination on bone in rats. Interestingly, we found that there were beneficial bone effects elicited by combined jumping exercise and honey supplementation on bone mineral density, geometry, mechanical properties, and bone metabolism in female rats.[32-34] As an extension work of the above mentioned animal study, in a recent human study carried out by the present research team, it was found that 6 weeks of combined aerobic dance exercise and honey drink supplementation elicited more beneficial effects on bone health by increasing blood bone formation marker in 19 to 29 years old young females compared to honey supplementation alone or exercise alone.[35]

This recent study indicated that six weeks of aerobic dance exercise at three times per week, one hour per session combined with daily consumption of 20 g of honey diluted in 300 ml of plain water, may enhance bone health in the young female subjects. It was speculated that bone response varies with age, thus bone metabolism may be different in older population compared to young females with the combination of exercise and honey supplementation. Therefore the present study was proposed for determining the effectiveness of combination of aerobic dance exercise and honey supplementation on bone metabolism markers and muscular performance in adult women with age ranging from 25 to 40 years old. It is hoped that results of the present study can be used for developing age-specific exercise and nutritional programs, by formulating guidelines for the maintenance of bone health in adult female population.

MATERIALS AND METHODS

Participants

Forty-four physically healthy sedentary adult female subjects, age between 25 to 40 years old from Kelantan region, Malaysia were recruited in the present study. The inclusion criteria of the subjects were: No health problems, non-smoker, not habitual consumer of honey daily, did not engage in any training program and did not exercise more than once per week. They were required to answer questionnaires to ensure their eligibility. Subjects were matched in age, body mass, height and percent of body fat before they were randomly assigned into the experimental groups.

All participants were fully informed by the researcher about the nature of the experiments, purpose of the study, procedures, benefits, risks of feeling discomforts experienced in this present study before giving their written and signed formal consent. The present study was approved by the Human Research Ethics Committee of Universiti Sains Malaysia. During the experimental period, the participants were provided checklists, and they were required to record their participation and honey consumption rates in the checklists for ensuring their compliance and commitment to the present study.

Experimental design

Subjects' grouping: The participants were divided into four groups with 11 participants per group (n=11): 8 weeks of sedentary without supplementation control (C), 8 weeks of aerobic dance exercise (Ex), 8 weeks of honey supplementation (H), and 8 weeks of combined aerobic dance exercise and honey supplementation (HEx) groups. Participants in the control group (C) did not perform exercises nor taking honey supplementation. Meanwhile, participants in aerobic dance exercise group (Ex) performed one hour aerobic dance exercise per session, 3 times per week for 8 weeks. Participants of honey group (H) consumed honey drink for 7 days per week for a total of 8 weeks duration. Participants in combined aerobic dance exercise with honey supplementation group (HEx) performed aerobic dance exercises one hour per session, 3 times per week for 8 weeks and consumed honey drink 7 days/week for 8 week with dosage same as honey group (H). The participants in HEx group were required to consume honey drink 30 minutes before performing aerobic dance exercise on the exercised days.

Blood sample taking: Before and after the 8 weeks of exercise/ supplementation/combined/ sedentary without supplementation periods, participants were seated and 8 ml of venous blood sample was taken from an antecubital vein after an 8 hours overnight fast. Drinking plain water was allowed during the fasting period. The blood was withdrawn by the laboratory technologist in the Sport Science Laboratory, Universiti Sains Malaysia to determine the concentrations of bone metabolism markers. Blood taking sessions for subjects in Ex and HEx in post-test were carried out at 8.30 the next morning after performing aerobic dance exercise, i.e. 14 hours post exercise. Serum from the clotted blood in the plain tube was used for determining serum bone metabolism markers. Serum was obtained by centrifuged the blood sample using a centrifuge (Hettich-Rotina 46RS, Germany) for 10 minutes with 4000 rpm, before being divided into equal portions and stored at -80 ˚C in a freezer (ThermoForma, Model 705, USA) for subsequent analysis.

Blood biochemical analysis: Serum total calcium was analyzed calorimetrically using an automatic analyzer (Hitachi Automatic Analyzer 912, Bohringer Mannheim, Germany) with commercially available reagent kits (Randox, UK). Serum osteocalcin, a bone formation marker, was analyzed using a commercially available enzyme immunological test kit (N-MID® Osteocalcin ELISA, UK), and the concentration was determined using a photometric microplate reader (Molecular Devices; Versamax tubable microplate reader, USA). Serum C-terminal telopeptide of type 1 collagen (1CTP), a bone resorption marker, was analyzed by a quantitative enzyme immunoassay kit (Orion Diagnostica UniQ 1CTP EIA, Finland), and the concentration was determined by a photometric microplate reader (Molecular Devices; Versamax tubable microplate reader, USA) Serum parathyroid hormone (PTH) was analyzed by using electrochemiluminescence immunoassay kit (ECLIA,

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Mannheim, Germany) and an analyzer (Cobas e 411, USA).

Measurement of lower limb muscular strength and power: The participants were allowed to have light meals before performing muscular strength and power test for right and left lower limbs. Participants' muscular peak torque (indicator of strength) and power of both lower limbs were measured by using isokinetic dynamometer (Biodex System 3 Pro, New York, USA). In the present study, 180°.sec^{-1} and 300°.sec^{-1} angular velocities for knee flexion and extension were used to evaluate the status of muscular strength and power of the subjects prior and post 8 weeks of experimental period. Before testing, each participant was required to do a standard quadriceps and hamstring stretching exercises to prevent injuries. Then, the participants were familiarized with the use of the dynamometer and testing procedures to reduce the possible influences of test habituation on muscular performance. They were asked to do 5 repetitions for the 180°.sec^{-1} angular velocity, and 10 repetitions for the 300°.sec^{-1} angular velocity for each lower limb. Sixty seconds of rest was given to the subjects between each angular velocity.

Aerobic dance exercise program: The participants of aerobic dance exercise group (Ex) and combined honey supplementation with aerobic dance exercise group (HEx) were required to attend aerobic dance classes for 3 sessions per week, one hour per session (from 5.30pm to 6.30pm) for 8 weeks. The one hour session started with 10-15 minutes of warming up period and ended with 5-7 minutes of cooling down activities. The activities prescribed in the present aerobic dance exercise program involved continuous, controlled movement of legs and trunk, and intermittent movement of arms. The movements involved were such as side stepping, fast walking, forward and backward stepping, stepping up and down a step board, leg lifts, placing foot to the front, side and behind, knee bends, forward and side-lunging, heel rises and jumping. The intensity of aerobic dance exercise was estimated by using heart rate monitor (polar watch, S710, USA) wore by subjects throughout the dancing sessions. Besides, the subjects were given pre-recorded CD containing aerobic dance workout, and they were required to follow the workout in the CD given at home if they missed any of the aerobic dance sessions.

Honey supplementation: Honey drink was consumed by the participants in the honey (H) group, and combined aerobic dance exercise and honey supplementation (HEx) group in a dose of 20 g of honey[36,37] diluted in 300 ml of plain water,[38] for 7 days per week for a total of 8 weeks duration. Gelam honey, which is a local Malaysian honey contributed by Federal Agriculture Marketing Authority, Malaysia was used in this study. In the combined aerobic dance exercise and honey supplementation (HEx) group, the subjects were required to consume honey drink 30 minutes before performing aerobic dance exercise on the exercised days.

Statistical Analysis

Statistical analysis was done by using Statistical Package for Social Science (SPSS) version 18.0. Mean and standard deviation (SD) of the experimental data was calculated, and data was reported as mean±SD. Repeated measure analysis of variance (ANOVA) was performed to determine the significance of the differences within and between groups. Difference was considered significant at $p<0.05$. Confounding variables such as subjects' age, body mass, height and body fat were considered before the commencement of the study. The participants were matched in age, body mass, body height and body fat before they were randomly assigned into the experimental groups. One-way analysis of variance (ANOVA) was performed to ensure that there were no significant differences in the aforementioned confounding variables among the groups at the beginning of the study.

RESULTS

A total of forty healthy sedentary adult women (mean age 29.7±5.3 years) completed the present study. Two participants from honey supplementation group (H) and two participants from combined aerobic dance exercise with honey supplementation group (HEx) were unable to continue the program due to pregnancy and personal reason during the experimental period. Participants' mean body height in C, H, Ex and HEx was 154.2±5.6 cm, 153.8±4.8 cm, 154.6±6.1 cm, and 156.4±6.0 cm respectively. The mean body weight and percentage of body fat of the subjects were 56.0±9.9 kg and 32.5±9.8% in C group, 54.5±7.8 kg and 33.0±7.2% in H group, 55.3±5.0 kg and 32.7±5.0% in Ex group, and 53.4±7.7 kg and 30.0±7.4% in HEx group respectively. There were no significant differences ($p>0.05$) between groups in means age, body height, body weight and percentage body fat at the beginning of the experimental period. The participants' mean heart rates recorded during exercise were ranging from 120 to 140 beats/min^{-1}.

The study results showed that there was statistically significant greater post-test value of serum total calcium than pre-test value in H group (Table 2). The percentage increment in osteocalcin, a bone formation marker in Ex group was the highest (+19.85%) compared to the other experimental groups (Table 2). Serum 1CTP concentration was significant greater in post- test than pre-test in Ex group. The percentage increment of serum 1CTP concentration was the highest (+40.51%) in Ex group, and the percentage increment of this parameter was the lowest (+14.75%) in HEx group among all the experimental groups (Table 2). The present study results also exhibited that the percentage increment of PTH hormone concentration was the lowest in HEx group among all the groups (Table 2).

Results of participants' right and left leg muscular peak torque and average power in HEx, Ex, H and C groups are illustrated in Table 3, 4, 5 and 6. In HEx group, out of 16 measured muscular performance parameters, 11 parameters showed significantly ($p<0.05$) increases, and the increases were in right knee extension peak torque at 180°.sec^{-1}, right knee extension average power at 180°.sec^{-1}, right knee flexion average power at 180°.sec^{-1}, right knee extension peak torque at 300°.

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Groups	Serum total calcium concentration (mmo/L) (Mean ± SD)			Percent difference compared to pre-test (%)	Serum parathyroid hormone (PTH) concentration (pmol/L) (Mean ± SD)			Percent difference compared to pre- test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test		Pre Test	Post Test	Mean difference between pre- and post-test	
Control (C)	2.32±0.09	2.35±0.05	0.03±0.08	1.29	3.18±0.88	3.86±0.64*	0.68±0.80	21.38
Honey (H)	2.30±0.05	2.40±0.11*	0.10±0.10	4.35	3.96±0.76	4.42±1.62	0.45±1.33	11.36
Exercise (Ex)	2.38±0.18	2.34±0.10	-0.05±0.14	-2.10	3.92±1.03	4.53±1.16	0.61±1.00	15.56
Combined (HEx)	2.29±0.14	2.33±0.07	0.04±0.11	1.75	3.76±1.45	3.91±1.44	0.15±1.20	3.99
Groups	Serum osteocalcin (µg/mL) (Mean ± SD)			Percent difference compared to pre-test (%)	Serum 1CTP concentration (µg/ml) (Mean ± SD)			Percent difference compared to pre- test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test		Pre Test	Post Test	Mean difference between pre- and post-test	
Control (C)	13.11±4.80	11.43±4.86	-1.68±2.92	-12.81	2.33±0.93	3.05±1.30	0.73±1.19	30.90
Honey (H)	10.51±4.69	11.44±2.94	0.94±3.91	8.85	2.60±0.92	3.10±1.25	0.50±1.12	19.23
Exercise (Ex)	9.22±3.28	11.05±5.25	1.83±5.28	19.85	1.95±0.85	2.74±1.06*	0.79±1.06	40.51
Combined (HEx)	10.53±3.33	10.65±3.18	0.12±4.42	1.14	2.44±0.94	2.80±1.07	0.36±1.17	14.75

*p<0.05 significantly different from pre test.

Table 2: Mean of serum total calcium, serum parathyroid hormone (PTH), serum osteocalcin, and serum 1CTP concentrations.

Groups	Right knee extension peak torque at 180°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)	Right knee extension average power at 180°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test (±SD)		Pre Test	Post Test	Mean difference between pre- and post-test (±SD)	
Control (C)	77.11±10.65	79.46±11.42	2.35±7.68	3.05	129.83±20.97	136.52±5.33	6.69±14.70	5.15
Honey (H)	73.49±10.71	78.06±11.81*	4.57±4.44	6.22	123.83±21.00	134.28±25.25*	10.44±11.88	8.44
Exercise (Ex)	77.60±7.44	81.18±9.30*	3.58±4.00	4.61	135.75±15.37	144.82±21.16*	9.07±9.34	6.68
Combined (HEx)	72.86±14.55	82.41±16.58*	9.56±7.07	13.11	126.27±31.46	141.49±33.01*	15.22±17.59	12.05
Groups	Left knee extension peak torque at 180°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)	Left knee extension average power at 180°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test (±SD)		Pre Test	Post Test	Mean difference between pre- and post-test (±SD)	
Control (C)	78.66±10.26	78.72±12.86	0.05±5.17	0.08	128.96±19.64	136.91±23.52	7.95±12.44	6.16
Honey (H)	74.16±8.76	77.08±9.16	2.92±4.36	3.94	133.33±18.69	133.81±12.75	0.48±10.03	0.36
Exercise (Ex)	79.47±8.46	82.92±12.68	3.45±5.95	4.34	140.04±18.03	147.62±19.59*	7.58±8.89	5.41
Combined (HEx)	75.31±15.29	83.26±13.42**	7.94±4.95	10.56	132.62±33.47	144.41±32.28*	11.79±11.78	8.89

*p<0.05 significantly different from pre test.
**p<0.01 significantly different from pre test.

Table 3: Right and left knee extension peak torque and average power at 180°.sec^{-1}.

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Groups	Right knee flexion Peak torque at 180°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)	Right knee flexion Average power at 180°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test (±SD)		Pre Test	Post Test	Mean difference between pre- and post-test (±SD)	
Control (C)	47.87±14.44	49.97±7.83	2.10±12.27	4.39	72.32±21.37	76.88±18.11	4.56±11.23	6.31
Honey (H)	39.79±6.96	41.76±9.13	1.97±6.11	4.95	63.38±10.34	66.51±15.64	3.13±8.89	4.94
Exercise (Ex)	46.72±7.77	44.14±9.43	-2.58±8.99	-5.52	70.06±16.89	75.98±16.27*	5.92±6.75	8.45
Combined (HEx)	48.92±12.50	52.10±11.81	3.18±5.40	6.50	78.91±18.75	83.21±15.49*	4.30±4.95	5.45
Groups	Left knee flexion Peak torque at 180°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)	Left knee flexion Average power at 180°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test (±SD)		Pre Test	Post Test	Mean difference between pre- and post-test (±SD)	
Control (C)	45.46±10.37	45.92±11.53	0.46±9.73	1.01	66.36±17.34	70.66±17.97	4.29±8.88	6.46
Honey (H)	45.69±10.55	41.62±7.09	-4.07±8.33	-8.91	60.58±10.76	58.57±8.20	-2.01±7.08	-3.32
Exercise (Ex)	50.85±11.30	47.52±8.56	-3.33±13.02	-6.55	63.34±15.19	70.59±11.27	7.25±11.50	11.45
Combined (HEx)	45.38±11.63	50.82±9.99	5.44±7.67	11.98	73.80±17.66	82.97±16.29**	9.17±6.40	12.43

*p<0.05 significantly different from pre test.
**p<0.01 significantly different from pre test.

Table 4: Right and left knee flexion peak torque and average power at 180°.sec^{-1}.

Groups	Right knee extension Peak torque at 300°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)	Right knee extension Average power at 300°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test (±SD)		Pre Test	Post Test	Mean difference between pre- and post-test (±SD)	
Control (C)	64.56±8.92	67.61±12.24	3.05±9.04	4.72	139.98±19.01	139.06±15.98	-0.92±13.87	-0.66
Honey (H)	59.01±5.93	62.54±8.38	3.53±5.58	5.98	133.24±13.40	142.41±20.61*	9.17±9.85	6.88
Exercise (Ex)	67.11±9.19	66.11±6.64	-1.00±7.89	-1.49	146.42±17.14	158.86±16.75***	12.45±7.15	8.50
Combined (HEx)	63.46±11.48	73.39±15.85*	9.93±10.69	15.65	135.22±34.07	151.78±36.89*	16.56±18.70	12.25
Groups	Left knee extension Peak torque at 300°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)	Left knee extension Average power at 300°.sec^{-1} (Mean±SD)			Percent difference compared to pre- test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test (±SD)		Pre Test	Post Test	Mean difference between pre- and post-test (±SD)	
Control (C)	72.89±8.33	64.70±9.65*	-8.19±9.43	-11.24	141.18±16.20	141.90±32.55	0.72±18.25	0.51
Honey (H)	66.99±8.30	65.53±5.94	-1.46±8.68	-2.18	132.34±15.90	143.69±19.63**	11.34±8.02	8.57
Exercise (Ex)	69.25±8.20	72.61±10.10	3.36±8.73	4.85	147.77±18.00	161.53±20.43***	13.75±8.61	9.31
Combined (HEx)	67.42±13.64	71.73±13.65	4.31±6.86	6.39	131.86±33.02	155.56±38.08**	23.70±17.79	17.97

*p<0.05, **p<0.01, ***p<0.001 significantly different from pre test.

Table 5: Right and left knee extension peak torque and average power at 300°.sec^{-1}.

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Groups	Right knee flexion Peak torque at 300°.sec-1 (Mean±SD)			Percent difference compared to pre- test (%)	Right knee flexion Average power at 300°.sec-1 (Mean±SD)			Percent difference compared to pre-test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test (±SD)		Pre Test	Post Test	Mean difference between pre- and post-test (±SD)	
Control (C)	50.11±10.27	54.23±8.93	4.12±11.22	8.22	79.46±25.28	78.35±20.99	-1.12±11.58	-1.41
Honey (H)	49.98±8.44	54.10±9.09	4.12±9.56	8.24	64.59±11.36	69.13±17.62	4.54±13.78	7.03
Exercise (Ex)	58.45±9.42	59.46±9.25	1.02±6.26	1.75	78.90±22.87	85.26±23.13*	6.36±8.16	8.06
Combined (HEx)	60.67±18.82	56.77±14.82	-3.90±8.12	-6.43	85.22±19.29	93.50±19.30*	8.28±9.59	9.72
Groups	Left knee flexion Peak torque at 300°.sec-1 (Mean±SD)			Percent difference compared to pre- test (%)	Left knee flexion Average power at 300°.sec-1 (Mean±SD)			Percent difference compared to pre-test (%)
	Pre Test	Post Test	Mean difference between pre- and post-test (±SD)		Pre Test	Post Test	Mean difference between pre- and post-test (±SD)	
Control (C)	51.30±12.35	50.66±13.98	-0.64±14.26	-1.25	70.74±21.65	67.79±26.13	-2.95±7.89	-4.17
Honey (H)	48.27±9.13	45.71±8.83	-2.56±10.90	-5.30	60.52±8.48	62.58±11.06	2.06±9.32	3.40
Exercise (Ex)	49.61±9.01	59.83±9.43**	10.22±7.88	20.60	68.86±15.98	81.25±15.06*	12.38±14.97	17.98
Combined (HEx)	54.96±8.62	58.78±16.34	3.82±10.41	6.95	76.97±20.16	89.20±19.59*	12.23±11.81	15.89

*p<0.05,**p<0.01 significantly different from pre test.

Table 6: Right and left knee flexion peak torque and average power at 300°.sec-1.

sec-1, right knee extension average power at 300°.sec-1, right knee flexion average power at 300°.sec-1, left knee extension peak torque at 180°.sec-1, left knee extension average power at 180°.sec-1, left knee flexion average power at 180°.sec-1, left knee extension average power at 300°.sec-1, and left knee flexion average power at 300°.sec-1. In Ex group, out of 16 measured muscular performance parameters, 9 parameters showed significantly (p<0.05) increases, and the increases were in right knee extension peak torque at 180°.sec-1, right knee extension average power at 180°.sec-1, left knee extension average power at 180°.sec-1, right knee flexion average power at 180°.sec-1, right knee extension average power at 300°.sec-1, left knee extension average power at 300°.sec-1, right knee flexion average power at 300°.sec-1, left knee flexion peak torque at 300°.sec-1, and left knee flexion average power at 300°.sec-1. Meanwhile in H alone group, out of 16 measured muscular performance parameters, 4 parameters showed significantly (p<0.05) increases, and the increases were in right knee extension peak torque at 180°.sec-1, right knee extension average power at 180°.sec-1, right knee extension average power at 300°.sec-1, and left knee extension average power at 300°.sec-1. In C group, with no honey supplementation and without aerobic dance exercise, none of the measured muscular performance parameter increased significantly.

DISCUSSION

In the present study, we observed that there was significant increase in serum total calcium after 8 weeks of experimental period in H group. Serum 1CTP, a bone resorption marker was significantly higher in post-test compared to pre-test value in Ex alone group. Meanwhile, the percentages increment in serum 1CTP and parathyroid hormones (PTH) in HEx group were the lowest among all the experimental groups. In general, the present findings showed that honey supplementation alone could significantly elevate serum total calcium level, whereas aerobic dance sessions alone could significantly elevate concentration of bone resorption marker. It is interestingly to observe that combined aerobic dance exercise and honey supplementation showed its potential in reducing bone resorption induced by exercise.

Since changes in bone mineral density are expected cannot be observed in a short duration of 8 weeks, therefore the present study focused on changes in blood bone metabolism parameters, where changes in blood bone turnover markers such as serum osteocalcin as bone formation marker, and serum C-terminal telopeptide of type 1collagen (1CTP) as bone resorption marker were observed. Additionally, serum total calcium and parathyroid hormone were used to reflect bone related metabolic changes.

In the present study, the significant increase in serum total calcium after 8 weeks of study period in the honey supplementation alone group reflects that the daily honey supplementation for a duration of 8 weeks enable to increase blood

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calcium level in adult women. In a previous animal study done by Ariefdjohan et al,[31] it was evidenced that consuming honey for 2 days appeared to enhance calcium absorption efficiency in rats, nevertheless, the calcium absorption enhancing effect of honey diminished with 8 weeks of chronic feeding. The authors speculated that carbohydrates found in honey such as glucose, fructose and raffinose may enhance the absorption of calcium that translates to skeletal benefits with acute feeding. Nevertheless, the calcium absorption enhancing effect did not persist with chronic long-term feeding in growing rats in this previous study. Our observation is inconsistent with this previous animal study which indicated that early nutritional benefits may not translate to long-term effects. In fact, the present human study found that consuming honey drink for 8 weeks has potential to significantly increase serum total calcium about 4.35% compared to pre-test value, and implying that honey which contained calcium, carbohydrates and other nutritional elements (Table 1) could enhance the level of circulating calcium in the blood vessels in women. Unfortunately, the calcium absorption efficiency was not measured in the present study; therefore, further study is needed to include analysis on absorption efficiency to clarify the detail physiological mechanism of the calcium metabolism induced by honey supplementation.

In the exercise alone group in the present study, significant increase in serum 1CTP was observed, and the percentage increment of 1CTP was the highest among the groups. Additionally, a non-statistically significant elevation of serum osteocalcin was observed in this exercise alone group, and the percentage increment of osteocalcin of this group was the highest among the groups. This observation reflects that aerobic dance sessions which were conducted three times per week for duration of 8 weeks may have potential to enhance both bone formation and resorption, i.e. one's bone turnover.

Several previous studies have been conducted to investigate the influence of exercise on bone turnover. For instances, Welsh et al[39] investigated the effects of acute exercise i.e. after 30 minutes of moderate brisk treadmill walking on bone remodeling in ten healthy young men, and they found that exercise have stimulated bone resorption within 32 hours of exercise, but there was no measureable effect on bone formation after 32 hours. The authors mentioned that acute and moderate exercise has caused a stimulation of osteoclasts and triggering of bone remodeling in response to exercise. In another previous study by Thorsen, Kristoffersson, and Lorentzon,[40] it was reported that a single bout of 90 minutes moderate intensity brisk walking i.e. 50% of VO_{2max} resulted an increase in the concentration of serum 1CTP at 72 hours post exercise. However, significant change in serum osteocalcin was not observed in this previous study. Meanwhile, Brahm et al[41] reported that running on a treadmill with 76% of VO_{2max} followed by a maximal effort until exhaustion resulted significant increases of 1CTP and osteocalcin levels during 24 hours of recovery. The authors speculated that during exercise, plasma content decreased and resulted diffusion of osteocalcin to the extravascular space. The increased amount of osteocalcin during recovery may indicate either a stimulation of the osteoblasts activity or a rediffusion of osteocalcin from extravascular space during plasma volume expansion. In our present study, statistically significant increment in bone resorption marker was also observed in the day following exercise as these aforementioned previous studies, implying that aerobic dance exercise alone may stimulated osteoclastic activity and have triggered bone turnover. Nevertheless, further study is needed to confirm the effects of exercise on bone formation marker based on the fact that merely non-statistically significant elevation of serum osteocalcin was observed in the present study.

In the combined aerobic dance exercise with honey supplementation group, the percentages increment of serum 1CTP and PTH concentration were the lowest among groups. We speculated that this combination may elicit beneficial effect in reducing the increment of bone resorption induced by exercise as mentioned earlier. The findings of this present human study were slightly different from our previous animal study[34] which found that 8 weeks of combination jumping exercise and honey supplementation could significantly reduce the bone resorption marker of 1CTP in rats. The inconsistent results between this previous animal study and the present study may indicate that animals and humans may respond differently in serum bone metabolism markers.

It was speculated that consumption of honey may increase calcium availability in the blood as evidenced in the honey supplementation alone group of this present study, and high volume of blood would be delivered to the working muscles when the participants performed aerobic dance exercise. Thus, the enhancement of calcium level in the peripheral blood due to honey drink ingestion and involvement in aerobic dance exercise may have caused reduction in bone resorption which can be reflected by 1CTP level, and PTH hormone level which reflects mobilization of calcium from the bone as observed in the present study. Furthermore, according to Ososki and Kennelly,[42] phenolic compounds in plants which are termed as phytoestrogens can possess estrogenic activity, and it is present in honey. [32,43,44] The rise in estrogen levels at menarche in girls has been reported to be associated with a large reduction in bone turnover markers, and the effect of estrogen on bone remodeling is to decrease activation frequency and subsequent decrease the numbers of osteoclasts and osteoblasts.[45] The above mentioned phenomenon can be the reason which caused the combined effects of honey on bone turnover markers in the present study.

Regarding effects of combined exercise and nutritional supplementation, Evans et al[46] has investigated the combination effects of soy and exercise in postmenopausal women. They found that dietary supplementation with soy decreased bone resorption and formation, whereas moderate intensity endurance exercise training did not alter bone resorption, and there were no apparent additive or synergistic effects of soy and exercise on markers of bone turnover. The absence of beneficial effects

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of combined nutritional supplementation and exercise in their study when compared to the present findings could be due to differences in the type of nutritional supplementation and exercise prescribed, and the age of the subjects recruited.

Recently, the present research group has conducted two combined nutritional supplementation and exercise studies in young population. Ooi et al[35] found that 6 weeks of aerobic dance exercise combined with honey supplementation elicited additional effects in increasing bone formation marker concentration in 19 to 28 years old young females. In another study, Lau and Ooi[47] found that combined circuit training and chocolate malt drink elicited significant effect on reducing bone resorption marker in 19 to 25 years old young males. Comparison between the findings of the present study and these two previous studies showed that humans with different age may respond differently in serum metabolism markers, and responses of these markers may be dependent on the types of exercise and exercise prescribed.

One of the most notable findings in the present was that combined exercise and honey elicited the greatest beneficial effects on muscular strength (indicated by peak torque) and average power among the groups, and this combination exhibited greater beneficial effects than exercise alone in improving these two muscular performance parameters. Furthermore, discernable improvements were not observed in both muscular strength and power in honey alone group. As expected, in the control group with no honey supplementation and without aerobic dance exercise, none of the measured muscular performance parameter increased significantly. This showed that honey elicited the best effects on muscular strength and power in the participants when it was combined with aerobic dance exercise rather than consuming honey alone.

As reported in several previous studies, physical activities such as strength training,[48] resistance training,[49-51] endurance training,[48,52] and aerobic dance training[53,54] have shown their potential to increase muscular strength of the participants after interventions. As in the present study, improvement in muscular strength and power were also observed in participants in the aerobic dance group.

In a previous study carried out by Engels et al,[53] it was found that 10 weeks of aerobic dance training for 60 min per session at 3 days per week, with moderate intensity of 50-70% of participants maximal heart could elicit beneficial effects on participants' lower extremity muscle strength in older adults. Okura et al[54] mentioned that increase in leg muscular strength as results of aerobic exercise might be due to increases in both nerve impulse frequency i.e. action potential and synchronicity of motor unit activations. Similar physiological responses are believed may have happened as a result of aerobic dance exercise intervention in increasing leg muscular strength and power of the participants in the present study.

One of the notable finding in the present study is that combined honey and exercise has potential to increase muscular performance compared to other experimental groups. It is generally known that carbohydrate is the main source of energy during exercise by maintaining and increasing an individual blood glucose concentration.[55,56] It is speculated that there was increase in absorption of carbohydrates and vitamins contained in the honey into the muscles through the enhancement of blood flow caused by the rhythmic nature of dynamic loading elicited by the physical activities during aerobic dance sessions. In the presence of exogenous carbohydrate, working muscles of the participants were able to generate more energy and managed to carry out the exercise more efficiently. The above explanation can be the reason of the present observation of greater improvement of muscle force and power generation in combined exercise with honey group than other experimental groups.

CONCLUSION

In conclusion, the present study found that honey supplementation alone could significantly elevate serum total calcium level, whereas aerobic dance sessions alone could significantly elevate bone resorption. It was also found that combination of aerobic dance exercise and honey supplementation may elicit beneficial effects on reducing bone resorption induced by exercise, and enhancing muscular strength and power in sedentary women. Therefore, supplementation of honey drink with 20 g of honey diluted in 300 ml of plain water combined with 3 days per week of aerobic dance exercise has potential to be proposed for formulating guidelines in planning exercise and nutrition promotion programs for the enhancement of bone health and muscular performance in sedentary women.

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CONFLICTS OF INTEREST: None.

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