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Research

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Effects of Chocolate Malt Drink Consumption Combined with Aerobic Dance Exercise on Blood Bone Metabolism Markers, Antioxidant Enzymes and Aerobic Capacity in Young Females

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ABSTRACT

Purpose: The aim of this study was to investigate the effects of combined aerobic dance exercise and chocolate malt drink consumption on bone metabolism markers, antioxidant enzymes and aerobic capacity in young females.
Methods: Forty four physically healthy females (19-25 years old) were age and body mass matched and subsequently being assigned into four groups with n=11 per group: Control group (C), Chocolate malt drink group (Cmd), aerobic dance exercise group (Ex) and combined aerobic dance exercise and chocolate malt drink group (CmdEx). Participants' anthropometry and aerobic capacity were measured. Meanwhile, blood samples were taken in order to determine the concentrations of serum total calcium, serum osteocalcin and serum alkaline phosphatase (ALP) (bone formation marker), serum C-terminal telopeptide of type 1 collagen (1CTP) (bone resorption marker) and antioxidant enzyme activities, i.e. Glutathione peroxidase (GPx) and Superoxide dismutase (SOD).
Results: At the end of 8 weeks of the intervention period, the percentage of increment in serum osteocalcin was the highest in CmdEx group compared to the other experimental groups. Meanwhile, significant increased in glutathione peroxidase were observed in Ex ($p<0.05$) and CmdEx ($p<0.01$) groups after 8 weeks of intervention period. Additionally, the percentage changes in glutathione peroxidase and superoxide dismutase activity were the highest in CmdEx group compared to other groups. There were also significant increases in aerobic capacity in Ex and CmdEx groups.
Conclusions: The present study found that generally aerobic dance exercise alone and aerobic dance exercise combined with the consumption of chocolate malt drink elicited more beneficial effects on bone turnover, antioxidant enzyme activities and aerobic capacity compared to chocolate malt drink consumption alone or sedentary without chocolate malt drink consumption in young females.

KEYWORDS: Aerobic fitness; Antioxidant; Bone; Chocolate malt; Exercise.

INTRODUCTION

Physical activity or exercise is believed to be important for prevention and treatment of bone loss and osteoporosis.[1] Besides exercise, bone health can also be affected by nutritional status of an individual. Chocolate malt powder (MILO®) is mainly made up of cocoa and malt. It also contains protomalt, actigen-E, protein, carbohydrate and fat. Protomalt is a special malt extract from barley, which provides carbohydrate and supplies energy to body needs. ActigenE

is a combination of 8 vitamins and 4 minerals, which helps to release energy from carbohydrate in the diet. Vitamins and minerals that contained in chocolate malt powder (MILO®) are vitamin B complex and vitamin C, calcium, phosphorus, iron and magnesium.[2] Some of these vitamins and minerals are believed to play important roles in maintaining bone health.

Chocolate malt powder (MILO®) contains cocoa, which is a good source of minerals including flavanoids, phosphorus and magnesium.[3] Flavonoids are reported to influence bone mass density and protect against osteoporosis in older women.[4] Phosphorus is important for bone formation,[5] and impairment of serum phosphate can lead to impair bone mineralization and osteoblast function. Magnesium plays a role in bone growth and stabilization and involved in bone and mineral homeostasis.[5] Several previous studies reported that magnesium intake has positive correlation with bone mineral density and bone resorption markers in middle-aged women.[6] Chocolate malt powder (MILO®) also contains milk. Milk consists of carbohydrates, proteins, fats, vitamins and minerals, which are important to prevent osteoporosis.[3] It was reported that protein can influence bone growth and bone mass in children and adolescents.[7]

Regarding chocolate malt powder (MILO®) and antioxidant properties, the cocoa contained in chocolate malt powder has flavonoids, which are the polyphenolic compound that have antioxidant effects.[8] Cocoa has been reported can increase antioxidant defense system.[9] Cocoa supplementation was reported can increase superoxide dismutase activity.[10] Cocoa was also reported can enhance antioxidant enzymes activities in the liver and heart tissues of the rat.[11]

The present research group has carried out a few previous studies to investigate the effects of exercise alone and also combined effects of exercise with nutritional supplementation on bone health. For instance, Ooi, et al.,[12] found that bone mass and strength were significantly higher in jumping rats compared to controls. These findings imply that exercise can enhance bone mass and strength. Additionally, it was found by Ooi, et al.,[13] that serum alkaline phosphatase (a bone formation marker) increased in jumping exercise group and serum 1CTP (a bone resorption marker) decreased in jumping exercise group and authors concluded that jumping exercise could increase bone formation and decrease bone resorption.

Regarding animal study on combination effect of exercise and nutritional supplementation, Somayeh, et al.,[14] found that tibial mass and tibial and femoral strength in combined honey supplementation and jumping rats were significantly increased, implying that combination of jumping exercise and honey supplementation give more beneficial effects on tibia and femur bones compared to either jumping alone or honey supplementation alone.

In humans, Ooi, et al.[15] found that combination of honey supplementation aerobic dance give beneficial effect on bone formation marker, i.e. serum alkaline phosphatase in young females.[15] Similarly, Ooi, et al. [16] found that there was decreased in 1CTP in combined honey with circuit training in young males, even though no significant changes in alkaline phosphatase and osteocalcin were observed. In year 2013, Marhasiyah, et al.[17] had conducted a study on "Effects of Combined Aerobic Dance Exercise and Honey Supplementation on Bone Metabolism in Women" (Malaysia). It was found that there was lowest percentage of increment in 1CTP in combined aerobic dance exercise with honey supplementation group compared to other groups, even though there were no discernable changes in alkaline phosphatase and osteocalcin were observed. The authors concluded that, combination of aerobic dance exercise and honey supplementation may elicit beneficial effect on bone health in adult women. Lau and Ooi.[18] found that serum 1CTP decreased in combined circuit training with chocolate malt supplementation group. Nevertheless, serum alkaline phosphatase and osteocalcin were not significantly affected by this combination. The authors concluded that circuit training combined with chocolate malt supplementation elicited greater effect on bone resorption in young males.

It is generally known that the type of exercise or physical activity which is necessary to improve and maintain bone density is weight bearing exercise. Weight bearing exercises include walking, running, dancing and jumping.[19] Dancing serves an ideal osteogenic stimulus because it has various jumps and landings which provide unusual impact and high-impact loads on the skeleton.[20] To date, no studies have been carried out to investigate the effects of chocolate malt drink consumption with aerobic dance exercise on blood bone metabolism markers, antioxidant enzymes and aerobic capacity in young females, thus the present was proposed. If the present study can show that chocolate malt drink combined with aerobic dance exercise can give positive effects on bone metabolism markers, antioxidant enzymes and aerobic capacity, it can be used for formulating guidelines in young females to plan their exercise and nutritional promotion program for maintaining bone health and reducing the risk of osteoporosis, enhancing antioxidant enzymes activities and increasing cardiorespiratory endurance.

METHODS AND MATERIALS

Participants

In this study, forty four physically healthy females, with age ranged from 19-25 years old were recruited. The participants should be leading sedentary lifestyles (not involved in regular physical activity for more than once per week) and do not have the habit of consuming chocolate malt drink (MILO®) as daily consumption prior to the experiment. Participants were matched in age and body mass before they were assigned randomly into the group.

All participants were fully informed by the researcher about the nature of the experiments, purpose of the study, pro-

cedures, benefits and risks of feeling discomforts experienced in this present study. All participants were required to fill up participants' information sheets and sign on the consent forms. The present study was approved by the Research Ethics Committee (Human) of Universiti Sains Malaysia.

Experimental Design

Participant's grouping: The participants were age and body mass matched and then being randomly divided into four groups, with 11 participants per group (n=11): 8 weeks of sedentary without chocolate malt consumption control (C), 8 weeks with chocolate malt drink consumption (Cmd), 8 weeks of aerobic dance exercise without chocolate malt drink consumption (Ex) and 8 weeks of chocolate malt drink consumption with aerobic dance exercise (CmdEx) groups. Participants in the control group did not perform neither exercises nor taking chocolate malt drink consumption. Participants of chocolate malt drink consumption group were required to consume 45 g of chocolate malt powder mixed with 300 ml of plain water, 7 days per week for 8 weeks duration. Meanwhile, aerobic dance exercise group were required to perform aerobic dance exercise for 1 hour per session, 3 times per week for 8 weeks. Participants in combined chocolate malt drink consumption with aerobic dance exercise were required to consume 45 g of chocolate malt powder mixed with 300 ml of plain water for 7 days per week for 8 weeks and performed aerobic dance exercise for 1 hour per session, 3 times per week for 8 weeks. The participants of CmdEx group were required to consume chocolate malt drink 30 minutes before performing aerobic dance exercise on the exercised days.

Blood sample taking: Before and after 8 weeks of intervention, blood samples were withdrawn from the participants, from an antecubital vein after a 12 hours overnight fast (drinking plain water was allowed) at 8.00-10.00 am. The blood was withdrawn by the laboratory technologist in the Sport Science Laboratory, School of Medical Science, Health Campus, Universiti Sains Malaysia to determine the concentrations of bone metabolism markers and antioxidant enzymes activities. Blood taking for participants in Ex and CmdEx were carried out 13 to 16 hours after performing aerobic dance exercise.

During each blood taking, 6 ml of resting venous blood sample was collected into a plain, without EDTA tube from each participant. Serum was obtained by centrifuging blood sample for 10 minutes of 3000 RPM at 4 ºC (Hettich Zentrifuger-Rotina 46RS, Germany). Then, the serum obtained was divided into equal portions in bullet tubes and stored at -80 ºC in a freezer (ThermoForma, Model 705, USA) until subsequent analysis of serum bone metabolism markers and antioxidant enzymes.

Anthropometric measurements: In this study, anthropometric measurements were recorded before the commencement of the study. Body mass and percentage of body fat were measured by using Body Composition Analyser (TANITA, Model TBF-410, Japan) to the nearest 0.1 kg and 0.1 % respectively. Meanwhile, body height was measured by using a scale (Seca 220, Gemany) to the nearest 0.1 cm.

Chocolate malt drink consumption: Chocolate malt drink was consumed by the participants in chocolate malt drink (Cmd) and combined chocolate malt drink and aerobic dance exercise (CmdEx) groups in the dosage of 45 g of chocolate malt powder (containing 4.5 g of fat, 5.3 g of protein, 30 g of carbohydrate, 234 mg of calcium, 248 mg of phosphorus, 76.5 mg of magnesium, 4.7 mg of iron) mixed with 300 ml of plain water. Participants consumed chocolate malt drink once per day, 7 days per week for 8 weeks. Participants in CmdEx group consumed 300 ml of chocolate malt drink 30 minutes before performing aerobic dance exercise.

Aerobic dance exercise program: The participants of aerobic dance exercise group (Ex) and combined chocolate malt drink consumption with aerobic dance exercise group (CmdEx) were required to attend aerobic dance classes for 3 sessions per week, one hour per session (from 5.30 pm to 6.30 pm) for 8 weeks.

The aerobic dance exercise program of this study consisted of one sessions of 'floor aerobic dance exercise' and two sessions of a 'step board' aerobic dance exercises in a week. The one hour session started with 10 to 15 minutes of warming up period, 30 to 35 minutes of dance period and ended with 5 to 7 minutes of cooling down, conditioning and toning.

The aerobic dance exercise program prescribed in the present study generally involved continuous, controlled movement of the legs and trunk and intermittent movement of the arms. These include movements that extend, flex, abduct, adduct and rotate the leg and foot like side stepping, fast walking, forward and backward stepping, leg lifts, placing foot to the front, side and behind, forward and side-lunging, heel rises and also some high impact exercises like jumping. In the floor aerobic dance exercise sessions, participants were required to do upper and lower limbs movements according to the beat of the music played. In the 'step board' exercise sessions, participants were required to step up and step down the step board while dancing. Heart rate monitor (polar watch, $710, US) were worn by the participants throughout the dancing sessions to estimate the intensity of aerobic dance exercise.

Measurement of aerobic capacity: Twenty meter shuttle run test was conducted to determine participants' predicted maximal oxygen uptake (VO_{2max}). This test was conducted before and after 8 weeks of intervention period. The equipments that had been used in this test were measuring tape, pre-recorded CD, CD player and marker cones. The test procedure began with 5 min of warm-up. The participants ran on a flat, non-slippery area which was marked with cones separated by 20 meters distance. Participants' predicted VO_{2max} were calculated based on the number of completed shuttles by using an online formula (http://www.

topendsports.com/testing/beepcalc.htm).

Blood biochemical analysis: Serum total calcium was analysed calorimetrically by using an automatic analyzer (Hitachi Automatic Analyzer 912, Bohringer Mannheim, Germany) with commercially available reagent kits (Randox, UK). Serum osteocalcin is a bone formation marker, which was analysed by using a commercially available enzyme immunological test kit (N-MID® Osteocalcin ELISA). 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 1CTP is a bone resorption marker, which was analysed by an available enzyme immunoassay kit (Human C-telopeptide of type 1 collagen (1CTP), ELISA Kit). The concentration was determined by using a photometric reader (Molecular Device, Versamax tunable micro reader, USA). Reagent kits from BioAssay Systems, USA were used to determine Superoxide dismutase enzyme activity (EnzyChromTM Superoxide Dismutase Assay Kit, ESOD-100) and glutathione peroxidase enzyme activity EnzyChromTM Glutathione Peroxidase Assay Kit ,EGPX-100).

Statistical analysis: Statistical analysis was done by using Statistical Package for Social Science (SPSS) version 20.0. All values are presented as mean±standard deviations (SD). Repeated measure Analysis of variance (ANOVA) and Bonferroni post hoc test were performed to determine the significance of the differences between and within groups. The difference was considered statistically significant at $p<0.05$.

RESULTS

Anthropometric Characteristics of the Participants

A total of 44 healthy young female's participants which are recruited in the present study had completed the study. Anthropometric characteristics of the participants are illustrated in Table 1. No participant had discontinued the program during the experimental period.

Bone Metabolism Markers

Bone formation markers: Serum osteocalcin (OC) and serum alkaline phosphatase (ALP): The bone formation markers of serum osteocalcin (OC) and serum alkaline phosphatase (ALP) concentrations in all the groups at pre-and post tests are presented in Table 2 respectively. After 8 weeks of intervention period, there was no significant simple effect of intervention on serum osteocalcin (OC) concentrations ($F=0.34$, $p>0.05$) between all the experimental groups. Furthermore, no significant main effect of time on serum osteocalcin (OC) concentrations ($F=0.599$, $p>0.05$) between pre- and post tests for all experimental groups. The present results showed that percentage increment of serum osteocalcin (OC) concentrations was the highest in CmdEx group (+7.90%) compared to Ex (+2.25%), C (+1.27%) and Cmd (-1.21%) groups. After 8 weeks of intervention period, no significant simple effect of intervention on serum ALP concentrations ($F=0.117$, $p>0.05$) was observed between all the groups. Statistically significant main effect of time on this serum bone formation marker was observed ($F=37.78$, $p<0.01$) between pre- and post tests in Cmd, Ex and CmdEx groups. In post test, there were significant greater values of serum ALP as compared to the pre test values in Cmd, Ex and CmdEx groups.

Bone resorption marker: Serum C-terminal of type 1 collagen (1CTP): Mean serum C-terminal of type 1 collagen (1CTP) concentrations of all groups is presented in Table 2. After 8 weeks of intervention period, no significant simple effect of intervention on serum 1CTP concentrations ($F=0.087$, $p>0.05$) was observed between all the groups. Statistically significant main effect of time on serum 1CTP concentrations was observed ($F=3.136$, $p<0.05$) between pre- and post tests in Ex group, in which there was significant lower post test serum 1CTP value as compared to the pre test value in Ex group. The percentage reduction of mean serum 1CTP concentrations was the highest in Ex group (-25.02%).

Serum total calcium: Mean serum total calcium concentrations for all groups are presented in Table 3. There were no significant simple effect of intervention on serum total calcium concentrations ($F=0.547$, $p>0.05$) between all the experimental groups after 8 weeks of intervention period. Furthermore, no significant main effect of time on serum total calcium concentrations ($F=4.029$, $p>0.05$) between pre- and post tests was observed among all the experimental groups.

Antioxidant Enzyme Activities: Glutathione Peroxidase (Gpx) and Superoxide Dismutase (SOD) Activity

Mean of glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity of all groups are presented in Table 4. There was no significant simple effect of intervention on GPx

Groups (N=44)	Height (cm)		Weight (kg)		Body fat (%)		Body mass index (kg/m²)	
	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Control (C)	155.21±5.20	154.96±5.19	56.66±7.98	56.19±7.57	29.96±7.57	30.92±7.45	23.16±3.69	23.47±3.63
Chocolate malt drink (Cmd)	152.79±5.63	152.87±5.70	51.47±9.88	52.36±9.56	28.14±6.46	29.36±6.11	22.02±3.44	22.26±3.21
Exercise (Ex)	152.60±3.63	152.73±3.70	55.21±14.79	55.21±13.84	32.33±13.39	32.16±12.38	23.70±6.11	23.57±5.80
Combined (CmdEx)	154.86±6.09	155.03±5.99	56.13±10.79	57.03±10.37	31.18±8.14	31.27±7.85	23.56±4.71	23.69±4.14

Table 1: Means age, body height, body weight and percentage of body fat of the participants.

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Groups (N=44)	Serum osteocalcin concentration (µg/ml) (Mean±SD)			Mean percent difference (%)	Serum ALP concentration (µg/ml) (Mean±SD)			Mean percent difference (%)
	Pre-test	Post-test	Mean difference between pre- and post		Pre-test	Post-test	Mean difference between pre- and post	
Control (C)	25.12±9.19	25.44±7.34	0.32± 3.53	1.27	52.55±5.73	58.36±9.09	5.82±7.73	11.06
Chocolate malt drink (Cmd)	23.19±5.04	22.91±5.59	- 0.28±5.21	-1.21	56.82±13.86	63.91±17.82**	7.09±5.17	12.48
Exercise (Ex)	21.79±4.80	22.28±6.73	0.49±4.70	2.25	59.55±17.1	67.18±21.83**	7.64±7.27	12.81
Combined (CmdEx)	23.30±6.00	25.14±3.47	1.84±1.53	7.90	57.36±9.72	64.36±14.21**	7.00±9.02	12.20
Groups (N=44)	Serum 1CTP concentrations (µg/ml) (Mean±SD)			Mean percent difference (%)	Serum total calcium concentration (µg/ml) (Mean±SD)			Mean percent difference (%)
	Pre-test	Post-test	Mean difference between pre- and post		Pre-test	Post-test	Mean difference between pre- and post	
Control (C)	150.66±172.64	136.26±159.99	-14.4±84.78	-9.56	2.22±0.18	2.31±0.15	0.09±0.20	4.05
Chocolate malt drink (Cmd)	166.31±120.38	161.56±113.63	-4.75±68.37	-2.86	2.30±0.13	2.33±0.13	0.03±0.15	1.30
Exercise (Ex)	203.02±161.74	152.22±109.10*	-50.8±96.54	-25.02	2.37±0.09	2.40±0.15	0.03±0.11	1.27
Combined (CmdEx)	76.97±51.41	65.39±34.79	-11.58±46.27	-15.04	2.30±0.13	2.33±0.12	0.03±0.09	1.30

*significantly different from pre-test (p<0.05)
**significantly different from pre-test (p<0.01)

Table 2: Mean serum osteocalcin, alkaline phosphatase (ALP), C-terminal telopeptide of type 1 collagen (1CTP), total calcium concentrations at pre- and post tests (Mean±SD).

Groups (N=44)	Glutathione peroxidase activity (U/g) (Mean±SD)			Mean percent difference (%)	Superoxide dismutase activity (U/g) (Mean±SD)			Mean percent difference (%)
	Pre-test	Post-test	Mean difference between pre- and post		Pre-test	Post-test	Mean difference between pre- and post	
Control (C)	265.57±46.18	279.64±28.21	14.07±52.28	5.30	1.76±0.59	1.64±0.62	-0.12±0.32	-6.82
Chocolate malt drink (Cmd)	271.82±42.26	306.02±25.61	34.21±52.31	12.58	1.65±0.48	1.55±0.51	-0.1±0.23	-6.06
Exercise (Ex)	242.16±33.20	293.98±33.86*	51.82±55.23	21.40	1.55±0.35	1.49±0.34	-0.06±0.34	-3.87
Combined (CmdEx)	231.02±78.50	291.14±47.03**	60.11±111.37	26.02	1.43±0.22	1.64±0.64	0.20±0.62	14.69

*significantly different from pre-test (p<0.05)
**significantly different from pre-test (p<0.01)

Table 3: Mean of glutathione peroxidase (GPx) activity and superoxide dismutase (SOD) activity at pre- and post tests.

activity (F=0.876, p>0.05) for all the experimental groups after 8 weeks of intervention period. There were statistically significant main effect of time on GPx activity between pre- and post tests for Ex (F=13.493, p<0.05) and CmdEx (F=13.493, p<0.01) groups. In post test, there were significant greater values of GPx activity as compared to the pre test values in Ex and CmdEx groups. The present results showed that the percentage increment of mean of GPx activity was the highest in CmdEx group (+26.02%) compared to Ex (+21.40%), Cmd (+12.58%) and C (+5.30%) groups. There was no significant simple effect of intervention on SOD activity (F=1.509, p>0.05) for all the experimental groups after 8 weeks of intervention period. Furthermore,

Groups (N=44)	Aerobic capacity (mL/kg/min) (Mean±SD)			Mean percent difference (%)
	Pre-test	Post-test	Mean difference between pre- and post	
Control (C)	23.41±2.23	24.16±3.27	0.75±1.79	3.20
Chocolate malt drink (Cmd)	25.47±3.81	26.12±4.13	0.65±2.57	2.55
Exercise (Ex)	23.49±0.002	25.9±2.10***	2.41±1.38	10.26
Combined (CmdEx)	25.46±3.60	27.86±4.35***, +	2.4±1.63	9.43

***; significantly different from pre-test ($p<0.001$),
+; significantly different from respective control (C) group ($p<0.05$)

Table 4: Mean of aerobic capacity at pre- and post tests.

no significant main effect of time on SOD activity ($F=0.082$, $p>0.05$) between pre- and post tests for all the groups. The study results showed that the percentage increment of mean SOD activity was the highest in CmdEx group (+14.69%) compared to Ex: -3.87%, C: -6.82% and Cmd: -6.06%.

Aerobic Capacity

The mean of aerobic capacity in all groups at pre- and post tests are presented in Table 4. There was a significant simple effect of intervention on aerobic capacity between CmdEx ($F=2.973$, $p<0.05$) and C group respectively. Furthermore, there were statistically significant main effect of time on aerobic capacity between pre- and post tests for Ex and CmdEx ($F=29.527$, $p<0.001$) groups. In post test, there were significant greater values of aerobic capacity as compared to the pre test values in Ex and CmdEx groups. The present results showed that the percentage increment of mean aerobic capacity was the highest in Ex group (+10.26%) compared to CmdEx (+9.43%), C (+3.20%) and Cmd (+2.55%) groups.

DISCUSSION

One of the notable finding in the present study is that there were significant increases ($p<0.01$) in serum alkaline phospahtase (ALP) in Cmd, Ex and CmdEx groups (Table 3), implying Cmd alone, Ex alone and combined Cmd with Ex may give beneficial effect on bone health by stimulating the bone formation. The finding of increased in serum ALP as result of exercise in the present study was in an agreement with the another study conducted by Lau, et al.,[14] which found that serum ALP was significantly increased in Ex group and the percentage increment was the highest (+12.81%) in the Ex group compared to the other groups.

Another notable findings of the present study is that the percentage of serum osteocalcin concentrations was the highest in CmdEx group (+7.90%) compared to Ex (+2.25%), C (+1.27%) and Cmd (-1.21%) groups (Table 3). This finding indicates that CmdEx may have greater potential in increasing bone formation compared to other groups.

Regarding combined effects of exercise and nutritional supplementation on bone formation marker in humans, the finding of the present study with increased serum ALP in Ex and CmdEx was in consistent with Ooi, et al.[15] which reported that bone formation marker which is serum ALP was significantly increased in young female participants after 6 weeks of consuming honey combined with 6 weeks of aerobic dance exercise. The present finding is also consistent with the finding of an animal study conducted by Gala, et al.[21] Gala, et al.[21] reported that bone formation marker of ALP in ovariectomized rats which performed exercise alone and ovariectomized rats with combined exercise program and diet supplemented with calcium showed increased in ALP compared to ovariectomized control rats group. In another previous study conducted by Wagner, et al.[22] it was reported that serum bone alkaline phosphatase was significantly increased after taking calcium during weight loss training program. In that study, the participants consumed 800 mg of calcium per day and exercise 3 times per week for 12 weeks. A previous study conducted by Tartibian, et al.[23] also reported that serum ALP and serum procollagen type 1 C-terminal (PICP) (bone formation markers) was significantly increased in exercise alone and combined exercise, calcium and vitamin D supplementation groups. In that study, the participants exercised 30 to 45 minutes with 70%-80% maximum intensity for 9 weeks. Additionally, participants in the combined exercise and supplements group consumed 1000 mg of calcium and 200 IU of vitamin D per day. The findings of these previous studies were similar with the present finding which showed that whether exercise alone or exercise combined with nutritional supplementation may elicit beneficial effects on bone formation.

The finding of the present study showed that serum ALP was significantly increased in Cmd alone group. The present finding is inconsistent with Lau, et al.,[18] findings. They found that there were no statistically significant different on serum ALP in Cmd alone group. The discrepancy between the finding

of Lau, et al.,[18] and the present study may be due to the variation of gender. In the present study, the participants are young females while in the previous study, the participants are young males. These study findings have confirmed our speculation that gender may play a role in affecting bone metabolism based on the fact that there is difference in bone mineral density between males and females. In the present study, it was also observed that serum ALP increased non-statistically to similar extent to other experimental groups, nevertheless there were no statistically significant differences among the groups.

Regarding effects of exercise on bone resorption in the present study, it was found that serum C-terminal telopeptide of type 1 collagen (1CTP), a bone resorption marker was significantly decreased in exercise alone (Ex) group (Table 3), this study finding showed that by performing one hour of aerobic dance exercise three times per week, not only increase in bone formation marker of serum ALP as mentioned earlier could be observed, decrease in bone resorption marker of serum 1CTP could also be observed. Collectively, the present findings imply that physical activity or exercises may elicit beneficial effects to bone health by stimulating bone formation and decrease bone resorption. Nevertheless, the absence of statistically significant changes in serum 1CTP in combined exercise and supplementation group needs further investigation, and this observation may imply that effects of exercise alone on bone reportion marker can be different from exercise combined with supplementation.

Several previous studies have showed similar finding with reduction in bone resorption marker as results of exercise as in the present study. For instance, Eliakim, et al.,[24] reported a significant decreased in urinary N-terminal telopeptide cross-links, a bone resorption marker after 5 weeks of endurance-type training consist of running, aerobic dance, competitive sports and occasional weight lifting in the participants. It was reported in another study by Brahm, et al.,[25] that there was a significant decreased in 1CTP, a bone resorption marker in marathon runners compared to the sedentary control group. In an animal study conducted by Ooi, et al.,[13] it was found that serum 1CTP levels were significantly lower in rats, which received exercise loads of 40 jumps per week for 24 weeks compared to the sedentary control rats.

In the present study, no statistically significant differences were found in serum total calcium among all the experimental groups (Table 3). Recently, Marhasiyah, et al.,[17] found that there was significant greater serum total calcium in post test compared to the pre test in honey supplementation alone (H) group. Their finding is inconsistent with the present finding, in which chocolate malt consumption alone did not show any significant increase of serum total calcium in Cmd group. Comparison between Marhasiyah, et al.,[17] and the present study showed that honey drink may have greater potential in increasing serum total calcium than chocolate malt drink. The other factor which may cause differences in the finding of Marhasiyah, et al.[17] and the present study can be variation of the age of the participants recruited, where the present study recruited young females with age range between 19-25 years old while previous study by Marhasiyah, et al.[17] recruited adult women with age range between 25-40 years old. These findings have confirmed our speculation that age can play a role in affecting bone metabolism, based on the fact that there is difference in bone mineral density between young and older populations.

Miyazaki, et al.,[26] found that GPx was significantly increased among all the participants who performed running at 80% of maximal heart rate. Their finding is in agreement with the findings of the present study. During exercise, aerobic metabolic rate can increase up to 10 folds. As a result, it stimulates the enhancement leakage of oxygen from the mitochondria to the cytosol.[27] This reaction gives rise to reactive oxygen species (ROS) which can induce damage to the cells in our body. An increase in ROS during exercise has been considered to be an oxidative stress. That is the reason why antioxidant enzymes such as GPx rise during exercise. They act as scavenger to prevent ROS from damaging the body cells.[28] The mechanism stated above is parallel with the present finding in which GPx activities were significantly increased after 8 weeks of exercise.

In the present study, we found that GPx activity was significantly increased in the combined exercise with chocolate malt drink consumption group. In a previous study conducted by Kan, et al.,[29] it was found that the GPx activity was significantly increased with the supplementation of flavonoids after performing an exhaustive swimming exercise in mice. Tauler, et al.,[30] also found that GPx activities were significantly increased after taking antioxidant diet supplementation combined with exercise training in amateur trained male athletes. In another previous study conducted by Tessier et al.,[31] it was found that the erythrocyte GPx activity level were significantly increased with 10 weeks of endurance training program combined with supplementation of selenium, which act as antioxidant. The present and previous study findings indicate that exercise combined with antioxidant diet supplementation may enhance the level of antioxidant enzyme activities.

The present finding showed that there was no statistically significant difference in SOD activity level among all the experimental groups but the highest increment was observed in the combined group (+14.94%). Several previous studies showed different finding with the present finding. For instance, Kan, et al.,[29] found that SOD activity level was significantly increased with supplementation of polyphenols and flavonoids rich which is Ixora parviflora extract after an exhaustive swimming in mice. Miyazaki, et al.,[26] also found that SOD activity was significantly increased after 12 weeks of intense training in young males. The inconsistent finding between previous and present studies may be due to the variation in the duration of intervention period and types of antioxidant diet consumption among the studies.

The absence of statistically significant changes in SOD activities in the present finding was consistent with the several

other previous studies. For instance, Fadillioglu, et al.,[27] found that there was no significant difference before and after exercise program in plasma and erythrocyte SOD levels among all the groups. Similarly, SOD activity level was not statistically increased after running over 5 kilometers in sedentary men.[32] In another previous study conducted by Tauler, et al.,[30] it was found that there was no statistically significant difference of erythrocyte and lymphocyte SOD activity level in combined of supplemented selenium and exercise training group. The present and previous studies findings may indicate that either exercise alone or exercise combined with antioxidant diet supplement may not give beneficial effect to elevate SOD activity level. Nevertheless, the present finding of the percentage changes of SOD (+14.69%) were the highest in CmdEx group compared to other groups may imply that CmdEx may have greater potential in enhancing antioxidant enzyme activity compared to other groups.

The exercise intensity in the present study was considered moderate to high based on the observation that the exercise heart rate of the participants ranged from 140 bpm to 170 bpm throughout the exercise sessions, which represent 70% to 85% heart rate maximum. The present study showed that participants' aerobic capacity was significantly increased after 8 weeks of study period in exercise and combined groups (Table 4). Other than that, it was also found that there was significant difference between the combined group compared to the control group respectively after 8 weeks of study period. Similar finding between the previous study and the present study have been reported. Donnelly, et al.,[33] found that there was a significant increased in maximal oxygen consumption in the participants across 18 months of exercise. Besides, Rognmo, et al.,[34] also found that the peak oxygen uptake (VO_{2peak}) was increased significantly before and after training in high and moderate intensity throughout 10 weeks of training. Women with Systemic Lupus Erythematosus (SLE) who underwent 12 weeks of cardiovascular training program showed significant increased in maximum oxygen consumption compared to the sedentary control group. These findings imply that exercise may improve aerobic capacity in human.[35]

To date, studies of combined exercise and nutritional supplementation on aerobic capacity are lacking, this made the comparison between the present findings with previous related study difficult. More future studies regarding combination of exercise and nutritional supplementation on aerobic capacity are warranted.

CONCLUSION

As a conclusion, the present study found that either aerobic dance exercise alone or combination of aerobic dance exercise with chocolate malt drink consumption give more beneficial effects on bone health, antioxidant enzyme activities and aerobic capacity compared to the consumption of chocolate malt drink alone and sedentary without chocolate malt drink consumption in young females. Therefore, both consumption of chocolate malt drink with 45 g of MILO® diluted in 300 ml of plain water combined with 3 days per week aerobic dance exercise, and aerobic dance exercise performed at 3 times per week have potential to be proposed for formulating guidelines in planning exercise and nutrition promotion program for the maintenance of bone health, enhancing antioxidant enzyme activities and increasing cardiorespiratory endurance in young females.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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