

EVALUATING THE COMBINED EFFECT OF HONEY AND WALKING ON BONE HEALTH IN POSTMENOPAUSAL WOMEN AGED 50 TO 65 YEARS

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ABSTRACT

This study examined the combined effects of honey consumption and walking exercise on the bone speed of sound (SOS), t-score and z-score, bone metabolism markers in postmenopausal women aged 50 to 65 years. Forty participants were assigned to four groups: control (C), honey supplementation alone (H), walking exercise alone (Ex) and combined honey supplementation and walking exercise (HEX) groups. The walking program was conducted for 30 minutes per session, three sessions per week for six weeks by participants in Ex and HEX groups. Honey drink was consumed by participants in H and HEX groups with 20 grams of honey diluted in 300 ml water daily for six weeks. Participants' bone SOS, T-score and Z-score, serum ALP and ICTP were determined at pre and post test. It was found that 37.8% of the participants had normal bone health status, 30.4% had osteopenia, and 29.7% had osteoporosis. There were no statistically significant differences in bone SOS, T-score, Z-score, serum ALP and ICTP between pre- and post tests in all the groups. Six weeks of combined honey supplementation and walking exercise did not significantly improve bone SOS and bone metabolism markers in postmenopausal women. Future studies with higher frequency and intensity of exercise and longer intervention duration are recommended for determining the beneficial effects of this combination on improving bone health and metabolism in postmenopausal women.

Keywords: honey, bone speed of sound, walking, postmenopausal women

INTRODUCTION

Exercise is one of the crucial ways to improve bone mineral density and bone metabolism, i.e., bone formation and resorption (Ooi et al., 2011; Sahrir et al., 2018; Ooi & Sahrir, 2018). Bone metabolism changes can be detected by blood bone metabolism markers, such as serum alkaline phosphatase as bone formation marker and serum carboxyterminal telopeptide of type 1 collagen (ICTP) as bone resorption marker (Seibel, 2005). During bone formation, collagen fibres and certain organic substances are synthesized by osteoblast cells and stored in the bones along with minerals. Meanwhile, osteoclast cells degrade minerals and collagen fibres during bone resorption. The degraded bone matrix components are transported into the blood (Ooi & Sahrir, 2018).

Weight-bearing exercises are practical in increasing bone density by stimulating osteoblastic activity, generating endogenous electrical activity, and increasing serum osteocalcin levels (Benedetti et al., 2018). Walking is a type of weight bearing exercise. It is one of the best forms of exercise for all ages and genders. Walking briskly for at least 30 minutes or more per week can provide significant health benefits (Morabia & Costanza, 2004; Ford & Swaine, 2012; Chen, 2014). According to Chen (2014), fifteen to thirty minutes of walking daily could increase an individual's physical fitness level, including bone health.

Nutrition also plays a crucial in improving and maintaining bone health (Wadiah et al., 2015). Honey is rich in carbohydrates vitamins, and minerals such as calcium, phosphorus and magnesium, which are essential for bone formation and mineralization (Kamaruzzaman et al., 2019). Studies suggest that honey can enhance calcium absorption and support bone formation, as evidenced by increased BMD in young rats fed honey over 52 weeks (Ariefdjohan et al., 2008; Chepulis & Starkey, 2008). Additionally, previous studies have demonstrated that honey has similar effects to hormone replacement therapy on bone health density in postmenopausal women (Hussain et al., 2012) and prevents uterine atrophy in the postmenopausal state in rats (Zaid et al., 2010). These effects are likely mediated through honey's mineral content and bioactive compounds, which promote osteoblastic activity and inhibit osteoclastic activity.

Menopause marks a significant transition in a woman's life, signaling the end of her reproductive years. Postmenopausal women experience a significant decline in estrogen levels, which can increase the risk of developing osteoporosis and cardiovascular disease. Appropriate treatment, including hormone therapy and lifestyle modifications, can help mitigate these risks and maintain optimal health in the postmenopausal years (Baker et al., 2018).

Bone mineral density (BMD) and speed of sound (SOS) measured by qualitative ultrasound (QUS) were reported to be correlated (Calero et al., 2000; Omar et al., 2006). Therefore, quantitative ultrasound measurement of bone SOS is an alternative to BMD measurements. Bone SOS is affected not only by BMD but also by qualitative properties such as the structure and thickness of bone (Foldes et al., 1995; Escobio-Prieto et al., 2023). In the present study, bone SOS was used to reflect BMD, and serum ALP and ICTP were measured for reflecting bone formation and resorption respectively.

To date, information is lacking on the additional beneficial effects of combined honey supplementation and exercise compared to honey supplementation alone and exercise alone on bone parameters and bone metabolism in postmenopausal women. It was hypothesised that the combination of Tualang honey supplementation and walking exercise would produce greater improvements in bone speed of sound, reflecting bone mineral density, and more favourable changes in bone metabolism markers (serum alkaline phosphatase and serum ICTP) compared to either honey supplementation alone or walking exercise alone in postmenopausal women aged 50 to 65 years. Therefore, the present study was carried out to investigate the combined effects of Tualang honey supplementation and walking exercise on bone speed of sound, which can reflect bone mineral density and bone metabolism markers in 50 to 65 years old postmenopausal women.

METHODS

Participants

Forty postmenopausal women were involved in this study. Participants were screened in order to determine the inclusion criteria. The inclusion criteria were postmenopausal women aged 50-65 years old, naturally menopause for more than one year, free from any chronic health problems, non-smoker, and previously and currently do not engage with any other training program. While the exclusion criteria were exercised more than once per week, consuming honey as a daily supplementation, using hormonal replacement therapy (HRT) or any drugs that can affect bone formation and resorption. The study was approved by the human ethic committee of Universiti Sains Malaysia (JEPeM Code: USM/JEPeM/18020139).

Participants Grouping and Randomisation

The participants were age-matched and randomly assigned into four groups using a computer-generated randomisation sequence. Group allocation was concealed to minimise selection bias. The group consisted of sedentary control without honey consumption and walking exercise (C), honey consumption alone (H), walking exercise alone (Ex), and combined walking exercise with honey consumption (HEX).

Tualang Honey Supplementation

Honey drinks were consumed by the participants in H group and HEX group with a dose of 20 g of honey diluted in 300 ml of water for seven days per week for a total of 6 weeks. Tualang honey (Agromas, Malaysia), which is a local Malaysian honey provided by the Federal Agriculture Marketing Authority, Malaysia, was used in this study. In the HEX group, the participants were required to consume a honey drink one hour before walking exercise on the exercised days. Participants adherence to honey supplementation was monitored by asking participants to maintain a daily honey intake log, and participants received reminder to consume honey through WhatsApp every morning.

Walking Exercise Programme

The participants in both Ex and HEX groups were required to attend a walking exercise program, with 30 minutes per session (from 5.45 p.m. to 6.15 p.m.) three times per week for six weeks. The walking exercise intensity should be maintained within 50%-65% of the participants' estimated heart rate maximum (HR_{max}), i.e. moderate exercise intensity. To ensure the training intensity was maintained within the targeted range, the researcher calculated the participant's individual estimated maximal heart rate (HR_{max} = 220 – age), i.e. targeted exercise heart rate before the commencement of exercise sessions, and recorded the participant's post-exercise heart rate after a walking session. If the walking pace did not elicit a heart rate that should fall within the targeted exercise heart rate mentioned above, the participants were requested to change their pace during the subsequent walking session. The participants wore heart rate monitors (Polar watch) for recording their post-exercise heart rate accurately. Adherence to the walking program was monitored using exercise logs book and participants were asked to record the duration and intensity of each walking session. All logs were reviewed weekly by the study team to ensure accuracy.

Anthropometric parameters, bone speed of sound, T-score and Z-score measurements

Anthropometric parameters such as body height and body weight were measured. Body height was measured by a stadiometer (Seca 220, Germany) and body weight was measured by a body composition analyzer (Tanita model TBF-410, Japan).

The participants' bone speed of sound (SOS), T-score, and Z-score were determined using a bone sonometer (Sunlight Mini Omni™, Petah Tikva, Israel). The participants' middle shaft tibia of the leg and distal radius of their arms for both dominant and non-dominant legs and arms were measured. Bone SOS reflects bone mineral density. Besides providing bone SOS, T-score and Z-score readings, the measurement from the bone sonometer also provides information on categories of the participants' bone health status, i.e. 'normal,' 'osteopenia,' or 'osteoporosis' based on the T score value. The T-score is the relevant measure particularly

when screening for osteoporosis. It provides valuable insights into an individual's bone mineral density (BMD) compared to a reference population. It compares a patient's BMD to that of a healthy 30-year-old person. A T-score of -1.0 or higher shows that an individual has normal bone health status, osteopenia has a T-score between -1.0 and -2.5, and osteoporosis has a T-score of -2.5 or lower. The Z-score is the comparison to the BMD of a healthy age-matched person. In this present study, the participants' categories of 'normal,' 'osteopenia,' or 'osteoporosis' was based on the measurement of radius of non-dominant arm (Yue et al., 2022; Baim et al., 2008; Frisoli et al., 2021).

Blood Sample Collection and Analysis of Bone Formation and Bone Resorption Markers

Blood samples were taken at 8.30 a.m. immediately before and after the six-week experimental period. Four ml of resting venous blood samples were drawn from the participants' antecubital veins after an overnight fast of 8 hours. Post-exercise blood samples were taken from the participants in the Ex and HEX groups between 8.30 a.m. to 10.30 a.m., i.e. 14 – 16 hours after completing the exercise in the evening. Serum samples were analysed for two bone metabolism markers, i.e. bone formation marker of serum alkaline phosphatase (ALP) and bone resorption markers of serum carboxyterminal telopeptide of type 1 collagen (ICTP). Serum ALP analysis was performed in an accredited pathology laboratory (BP Clinical Lab, Malaysia). Serum ICTP concentration was analysed using a commercial kit human ICTP ELISA kit (Wuhan EIAab Science Co, China) and measured on VersaMax ELISA microplate reader (Molecular Devise, USA).

Statistical Analysis

Data were analyzed using the statistical software in the Statistical Package for Social Science (SPSS) Version 22.0. Descriptive analysis was performed. Repeated measures of mixed ANOVA, followed by a post-hoc Bonferroni test was also performed. Data are expressed as frequency and percentage, as well as means and standard deviation (S.D.). Statistical significance was accepted at $p < 0.05$.

Sample Size Calculation

The sample size used in this study was calculated using G Power Software. The study's power was set at 80% with a 95% confidence interval and 30% effect size. The calculated sample size was 9 per group. 10% was estimated as the dropout rate, equal to one participant per group. There were 4 study groups in this study, so the total number of participants recruited was 40, with 10 participants per group.

RESULTS

Anthropometric parameters and post exercise heart rate of the participants

Thirty-seven participants with a mean age of 56.8 ± 2.9 years, mean body weight of 65.0 ± 9.7 kg, and mean body height of 154.2 ± 4.8 cm completed the study. One participant from each of the control, exercise alone, and combined honey and exercise groups did not complete the study due to personal reasons.

The estimated target range of the participants' exercise heart rate ranged from 79 bpm to 111 bpm. The average post-exercise heart rate measured was 109 bpm. Meanwhile, the estimated walking speed was approximately 4 km per hour. In addition, the walking distance covered was approximately 2 km in one walking exercise session.

Bone Speed of Sound, T-Score, Z-Score, and categories of bone health status of the participants

The mean bone speed of sound, T-score, and Z-score of participants' radius of all the groups are presented in Table 1. The categories of bone health status of the participants of dominant and non-dominant arm are illustrated in Figure 1. It was found that 37.8% of the participants had normal bone health status, 30.4% had osteopenia, and 29.7% had osteoporosis.

The mean bone speed of sound, T-score, and Z-score of participants' tibia of all the groups are presented in Table 2. There was no significant interaction effect between time and intervention on the bone speed of sound ($df=3$, $F=0.644$, $p=0.592$), T-score ($df=3$, $F=0.578$, $p=0.634$), and Z-score ($df=3$, $F=0.723$, $p=0.546$) of the dominant leg among the experimental groups. There were no significant main effects of time or intervention ($p>0.05$) on the bone speed of sound, T-score, and Z-score of the dominant leg.

Regarding non-dominant leg, there was no significant interaction effect between time and intervention on the bone speed of sound ($df=3$, $F=1.117$, $p=0.356$), T-score ($df=3$, $F=1.204$, $p=0.324$), and Z-score ($df=3$, $F=1.110$, $p=0.359$) among the experimental groups. Similarly, there were no significant main effects of time or intervention ($p>0.05$) on the bone speed of sound, T-score, and Z-score after six weeks of intervention of the non-dominant leg.

Bone formation marker: Serum alkaline phosphatase (ALP)

Figure 2 (A) illustrates the results of serum ALP. There was no significant interaction between time and intervention ($df=3$, $F=0.048$, $p=0.986$). There was a significant main effect of time ($df=1$, $F=6.824$, $p=0.013$), but no significant main effect of intervention ($df=3$, $F=0.559$, $p=0.646$). Further analysis revealed no significant differences ($p>0.05$) between pre- and post-tests in serum ALP across all groups.

Bone resorption markers: Serum C-terminal telopeptide of type I collagen (ICTP)

Figure 2(B) shows the means serum ICTP for all groups at pre-and post-tests and percentage of change across time. The study results indicate no significant interaction between time and intervention ($df=3$, $F=0.603$, $p=0.618$) on serum ICTP among all the experimental groups. Additionally, no significant main effect of time ($df=1$, $F=0.107$, $p=0.745$) or intervention ($df=3$, $F=0.115$, $p=0.951$) on serum ICTP. The percentage change of serum ICTP concentration in the C, H, and Ex groups was +2.34%, +6.99%, and +6.92%, respectively. The HEx group, on the other hand, had a slight decrease (-0.87%) in serum ICTP levels.

Table 1: Means bone speed of sound, T-score, and Z-score of participants' radius of dominant and non-dominant arms

Variables	Groups	Bone speed of	T-score	Z-score
		sound (SOS) (Mean±SD)	(Mean±SD)	(Mean±SD)
Radius of dominant arm	C (n=9)	4155.0±139.3	-0.3±1.2	1.1±1.6
	H (n=10)	3996.4±271.1	-0.8±2.5	0.7±2.5
	Ex (n=9)	4031.2±181.0	-1.4±1.6	-0.0±1.5
	HEx (n=9)	4040.9±98.6	-1.3±0.9	0.5±0.9
Radius of non-dominant arm	C (n=9)	4161.2±126.4	-0.2±1.1	1.1±1.2
	H (n=10)	3999.4±275.0	-0.8±2.5	0.5±2.7
	Ex (n=9)	3935.0±123.1	-2.2±1.1	-0.8±1.0
	HEx (n=9)	3957.9±83.2	-2.0±0.7	-0.7±0.9

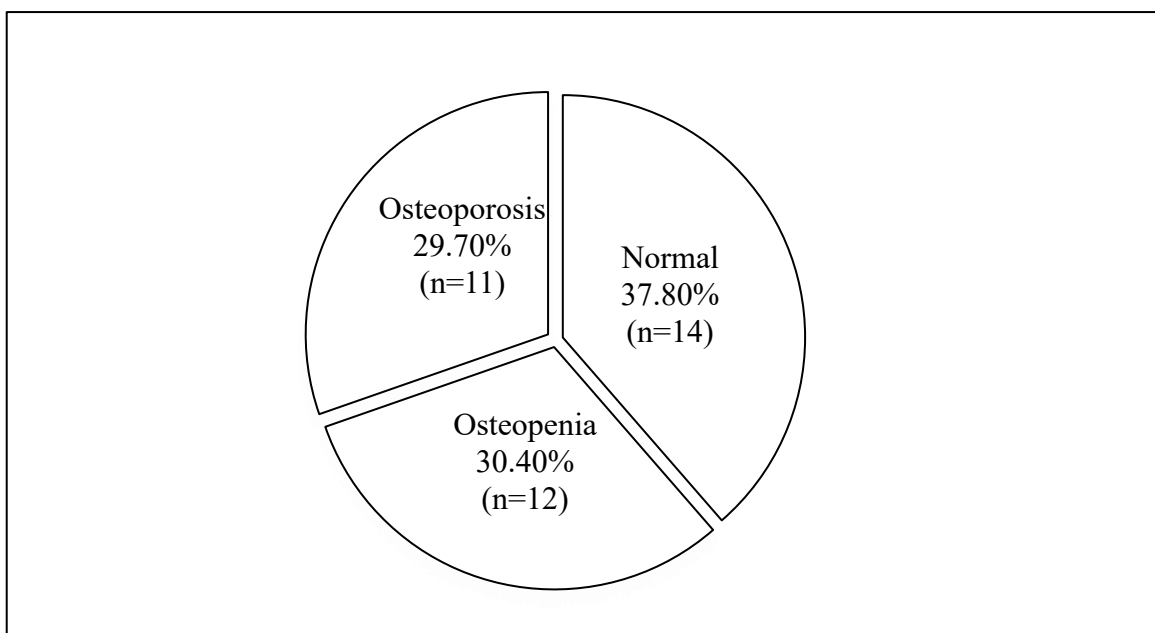


Figure 1: Categories of bone health status of the participants

Table 2: Means bone speed of sound, T-score, and Z-score of participants' tibia of dominant and non-dominant legs at pre test and post test

Groups		Bone speed of sound (SOS)		T-score		Z-score	
		Mean±SD		Mean±SD		Mean±SD	
		Pre test	Post test	Pre test	Post test	Pre test	Post test
Tibia of dominant leg	C (n=9)	3760.6±145.2	3773.3±130.4	-2.0±1.4	-1.8±1.3	-0.9±1.8	-0.7±1.5
	H (n=10)	3763.6±185.5	3766.2±212.8	-1.9±1.8	-2.0±1.9	-0.7±1.8	-0.7±2.1
	Ex (n=9)	3831.7±168.3	3779.0±159.2	-1.3±1.7	-1.8±1.6	-0.1±1.7	-0.6±1.4
	HEx (n=9)	3773.6±108.1	3772.6±97.5	-1.8±1.1	-1.8±1.0	-0.8±0.9	-0.6±1.1
Tibia of non-dominant leg	C (n=9)	3709.6±161.8	3677.2±255.6	-2.5±1.6	-2.8±2.5	-1.4±1.9	-1.7±2.8
	H (n=10)	3735.5±120.4	3800.2±143.7	-2.2±1.2	-1.5±1.4	-1.0±1.2	-0.4±1.5
	Ex (n=9)	3842.2±197.8	3816.1±176.8	-1.2±2.0	-1.4±1.7	-0.0±1.9	-0.3±1.6
	HEx (n=9)	3726.6±96.3	3724.1±116.5	-2.3±0.9	-2.3±1.1	-1.2±1.1	-1.2±1.2

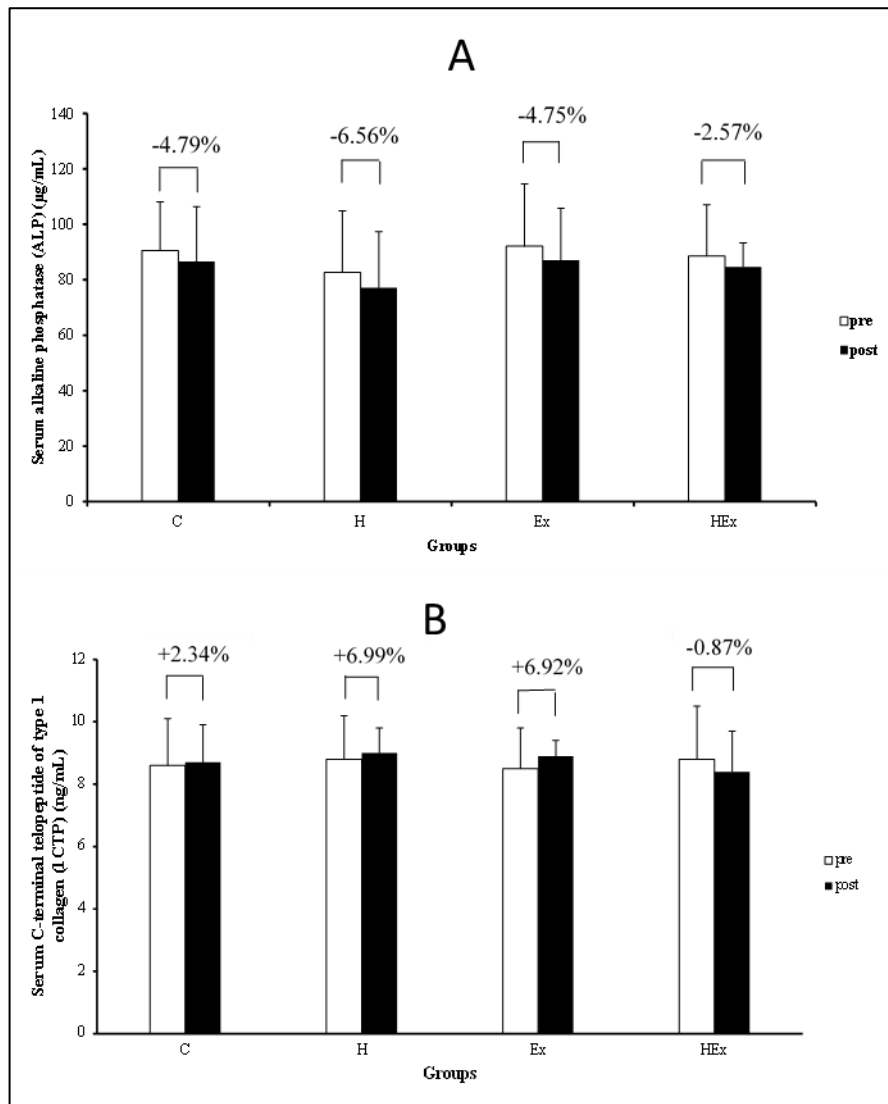


Figure 2: (A) Means serum alkaline phosphatase (ALP) concentration, and (B) means serum C-terminal telopeptide of type 1 collagen (1CTP) concentration at pre test and post test

DISCUSSION

The present study evidenced that the occurrence of osteoporosis was 29.7% among 37 Malaysian postmenopausal women with a mean age of 57 years old. It was also evidenced that 30.4% of the participants were in osteopenia state, and 37.8% of the participants had normal bone health status. These results provide new evidences on the prevalence of osteoporosis and osteopenia among Malaysian postmenopausal women.

In the present study, there were no significant differences in bone speed of sound, T-score, and Z-score of both dominant and non-dominant legs between pre- and post tests in all the groups. The results reflected that there were no changes in bone mineral density in all the groups after six weeks of the study period. It was speculated that the study period was too short to elicit changes in bone mineral density. This findings suggest that the six week intervention period may have been too short to elicit significant changes in BMD or bone metabolism in postmenopausal women. Bone remodeling is a slow physiological process, particularly in older adults, and significant changes in BMD generally require at least 6 to 12 months of consistent weight-bearing exercise or nutritional supplementation (Watson et al., 2015; Gianoudis et al., 2014). The duration of six weeks was based on previous studies in youngers populations that reported short term changes in bone metabolism markers (Ooi et al., 2011; Ooi & Azlina, 2017), but with different age gaps, explaining the lack of effect observed in the present participants.

The present study's findings was not consistent with previous studies (Ooi et al., 2011; Ooi and Azlina, 2017) which showed the beneficial effect of consuming honey and exercise on bone formation marker after intervention in young population. For instance, Ooi et al. (2011) found that six weeks of aerobic dance exercise performed three times per week, one hour per session combined with daily consumption of 20g of honey diluted in 300 ml of plain water elicited more beneficial effects on blood ALP, i.e. blood bone formation marker in young females aged 19 to 29, compared to supplementation with honey alone or exercise alone. A statistically significant increase in blood ALP was also observed in a previous study with honey combined with circuit training carried out by Ooi and Azlina (2017). They found that six weeks of combined circuit training and daily honey consumption of 20g of honey diluted in 300 ml of plain water elicited beneficial effects on increasing bone formation markers of blood ALP in young males. It is generally known that bone response varies with age. Thus, bone metabolism may differ between older populations and young females with the combination of exercise and honey supplementation. Therefore, an extension of the previous study of Ooi et al. (2011) which involved young females has been carried out by Rahim et al. (2016) to assess the impact of combining aerobic dance exercise and honey supplementation on bone metabolism markers in adult women aged 25 to 40 years old. The authors reported that after six weeks of study, the honey combined with aerobic dance exercise group exhibited the highest increase in serum ALP level, with a percentage increment of +31.79%.

Similar, in animal study, reported that administration of Tualang honey to ovariectomized rats for two weeks significantly restored the tibia bones compared to control non-honey treated rats (Zaid et al., 2010). Previous investigators claimed that carbohydrate constituents and gluconic acid, the major organic acid in honey could enhance calcium absorption in rats' bones (Ariefdjohan et al., 2008). Nevertheless, the present study did not show the beneficial effect of honey on enhancing bone speed of sound, an indicator of bone mineral density in postmenopausal women. These contrasts suggest that age, baseline bone health and intervention intensity are critical determinants of bone responsiveness.

Previous studies have shown that exercise alone could reduce bone resorption markers (Yamazaki et al., 2004; Woitge et al., 1998; Marini et al., 2020) and increase bone formation markers (Fujimura et al., 1997). Serum ALP is one of the markers of liver function tests, and only 50% of the measured serum ALP activity is contributed by the bone. The normal range of serum ALP is between 41 and 133 U L⁻¹ (Gowda et al., 2009). Generally, serum ALP changes can be attributed to liver disease or bone growth (Limdi & Hyde, 2003). As we did not measure the isoenzyme of bone-specific ALP (BALP), which originates mainly from

the bone, we could not precisely conclude the effects of walking exercise on bone formation in the present study.

In the present study, it was observed that there were no statistically significant differences between pre- and post tests in serum 1CTP in all the groups. Similarly, Rahim et al. (2016) observed that after eight weeks, there was no significant change in serum 1CTP levels in adult women aged 25 to 40 who combined honey consumption with aerobic dance. A previous study by Maimoun et al. (2005) also showed that serum 1CTP did not change after exercise in active elderly subjects. The authors mentioned that bone tissue of the elderly population may have become less responsive to exercise.

The present study observed that the combined honey and walking exercise (HEx) group showed a non-statistically significant decrease (-0.87%) in 1CTP in the post-test compared to the pre-test. In addition, no statistically significant increases in 1CTP were observed in the control group (C) (+2.34%), honey group (H) (+6.99%), and exercise group (Ex) (+6.92%), respectively. This finding reflects that honey combined with exercise may have potential to benefit bone health by decreasing bone resorption.

In a previous animal study done by Tavafzadeh et al. (2015), a lower level of serum 1CTP was observed in the combined jumping exercise with the honey group compared to the control and other experimental groups, i.e., honey alone and jumping exercise alone in young female rats. The result of this animal study was similar to the present study, which found that combined honey and exercise may have potential to reduce serum 1CTP.

Martin et al. (1991) mentioned that normal muscle function and load bearing are necessary to prevent or retard bone loss in the elderly. Bevier et al. (1989) also mentioned that in postmenopausal women, if muscular strength is a determinant of bone density, the force of muscle contraction must impact an anatomically related skeletal site. It is generally known that walking exercise involves muscle contraction of the lower limbs and subsequently impose forces on bone for enhancing bone health. The prescribed six weeks of walking exercise with 30 minutes per session and three sessions per week could not elicit beneficial effects on enhancing bone speed of sound, which reflects bone mineral density of the legs in postmenopausal women in the present study. Hatori et al. (1993) reported that lumbar BMD increased with fast walking (7.2 km/h) performed at intensities exceeding the anaerobic threshold in postmenopausal women. Mohebbi et al. (2023) evidenced that slower walking (6.2km/h) at intensities below the threshold was insufficient to increase lumbar BMD. Martin & Notelovitz (1993) observed that walking speeds of less than 6.4 km/h did not increase lumbar BMD. Similarly, the present study found no increase in bone speed of sound after six weeks of walking exercise in postmenopausal women. It is speculated that the participants in the present study walked with a walking speed of approximately 4.0 km/h, and this walking speed may not be high enough to elicit positive effects on BMD which was reflected by bone speed of sound.

Previous studies reported that bone mineral density could be increased by merely 1% after one year of exercise intervention in postmenopausal women (Watson et al., 2015; Gianoudis et al, 2014). Thus, it was unsurprising that the present study did not observe a significant increase in bone speed of sound with six weeks of walking exercise intervention. Similarly, Bassey & Ramsdale (1995) also reported no significant increase in bone mineral density of postmenopausal women who performed 12 months of weight-bearing exercise.

CONCLUSION

In conclusion, the present study found that the combination of walking exercise and honey supplementation did not produce significant changes in bone speed of sound or bone metabolism markers in postmenopausal women aged 50 to 65 years over the study period. These findings suggest that, under the frequency, intensity, and duration applied in this study, the combined intervention may be insufficient to elicit measurable improvements in bone health parameters in this population.

Nevertheless, this study provides important baseline evidence on the effects of a low-intensity, lifestyle-based intervention that is safe and easily implemented. The absence of significant effects highlights the need for future studies with higher exercise intensity or frequency, longer intervention duration, and possibly larger sample sizes to determine whether more robust protocols are required to induce meaningful changes in bone health among postmenopausal women.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

CNN conducted the study, FKO designed, conducted the study and edited the manuscript, NSA designed and conducted the study, CKC designed the study and edited the manuscript, NHHH designed the study, MM designed the study, NZZ conducted the study, MAA conducted the study and NAFA edited the study.

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