EFFECTS OF COMBINED AEROBIC DANCE EXERCISE AND HONEY SUPPLEMENTATION ON BONE METABOLISM, MUSCULAR STRENGTH AND IMMUNE FUNCTIONS IN WOMEN

by

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ABSTRACT

EFFECTS OF COMBINED AEROBIC DANCE EXERCISE AND HONEY SUPPLEMENTATION ON BONE METABOLISM, MUSCULAR STRENGTH AND IMMUNE FUNCTIONS IN WOMEN

INTRODUCTION: Although combination of physical activity with supplementation has being investigated on its effects in maintaining and enhancing bone health, little is known about the effectiveness of combination of aerobic dance exercise with honey supplementation on bone metabolism markers, muscular strength and immune function in women. **PURPOSE:** The aim of this study was to investigate the effects of combined aerobic dance exercise and honey supplementation on bone metabolism, muscular strength and immune function in women. **METHODS:** Forty four subjects, healthy sedentary women (25-40 year-old) were age and body mass matched, and subsequently being assigned into four groups with n=11 per group: Control group (C), honey supplementation group (H), aerobic dance exercise group (Ex) and combined aerobic dance exercise with honey supplementation group (HEx). Aerobic dance exercise was carried out for one hour per session, three times per week for eight weeks. Honey drink was consumed by H and HEx groups, in a dosage of 20g of honey diluted in 300ml of plain water, for 7 days per week for a total of 8 weeks duration. In HEx group, the subjects were required to consume honey drink 30 minutes before performing exercise on the exercise days. Before and after 8 weeks of experimental period, subjects' anthropometry, muscular strength and power were measured. Meanwhile, blood samples were taken in order 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), and immune functions determination through full blood counts and immunophenotyping measurements. **RESULTS:** The end of 8 weeks of experimental period, combination of aerobic dance exercise with honey supplementation (HEx) exhibited more discernable beneficial effects on left and right lower limb peak torque (muscular strength) and power compared to the other three Ex, H and C groups. Regarding bone metabolism, the percentage of increment in 1CTP, a bone resorption marker, and PTH concentrations in HEx group were the lowest compared to the other experimental groups. With regards to immune functions, significant increases in T cytotoxic (CD8) and total B cells (CD19) counts ($p<0.05$) were observed in HEx group after 8 weeks of experimental period. Additionally, the percentage increase in lymphocyte counts, T helper (CD4), T cytotoxic (CD8) and total B cells (CD19) counts after 8 weeks were the highest in HEx group compared to the other three experimental groups. **CONCLUSION:** The results of present study suggest that combination of aerobic dance exercise and honey supplementation elicited more beneficial effects on bone health, muscular strength and power, and immune functions generally compared to aerobic dance exercise or honey supplementation alone in sedentary women.

ABSTRAK

KESAN GABUNGAN SENAMAN TARIAN EROBIK DAN PENGAMBILAN MADU KE ATAS METABOLISMA TULANG, KEKUATAN OTOT DAN FUNGSI IMUNITI DI KALANGAN WANITA

PENGENALAN: Walaupun gabungan aktiviti fizikal dan suplimentasi telah dikaji keberkesanannya dalam mengekalkan dan meningkatkan kesihatan tulang, namun pengetahuan tentang keberkesanan gabungan senaman tarian erobik dan pengambilan madu ke atas penanda metabolisma tulang, kekuatan otot dan fungsi imuniti dikalangan wanita masih belum diketahui dengan meluas. **MATLAMAT:** Tujuan kajian ini adalah untuk menyiasat kesan gabungan senaman tarian erobik dan pengambilan madu ke atas metabolisma tulang, kekuatan otot dan fungsi imuniti di kalangan wanita. **KAEDAH:** Empat puluh empat peserta wanita yang sihat dan sedentari (berumur 25-40 tahun), selepas disuaipadankan umur dan berat badan, mereka diagihkan kepada empat kumpulan dengan n=11 bagi setiap kumpulan: Kumpulan kawalan (C), kumpulan pengambilan madu (H), kumpulan senaman tarian erobik (Ex) dan kumpulan gabungan senaman tarian erobik dan pengambilan madu (HEx). Senaman tarian erobik telah dijalankan selama sejam untuk setiap sesi, tiga kali seminggu selama lapan minggu. Minuman madu telah diambil oleh kumpulan H dan HEx dalam dos 20g madu yang dilarutkan dalam 300ml air selama 7 hari seminggu dalam tempoh 8 minggu. Dalam kumpulan HEx, subjek diminta untuk minum minuman madu 30 minit sebelum memulakan senaman pada hari senaman. Sebelum dan selepas 8 minggu tempoh penyelidikan, antropometri subjek, kekuatan dan kuasa otot diukur. Selain itu, sampel darah diambil untuk menentukan kepekatan serum kalsium keseluruhan, serum osteocalcin (penanda pembentukan tulang), serum C-terminal telopeptide of type 1 collagen

(1CTP) (penanda resorpsi tulang), hormon paratiroid (PTH) dan penentuan fungsi imuniti melalui pengukuran ‘full blood counts’ dan ‘immunophenotyping’. **KEPUTUSAN:** Selepas 8 minggu tempoh penyelidikan, gabungan senaman tarian erobik dan pengambilan madu (HEx) memberikan kesan baik yang lebih nyata ke atas kekuatan dan kuasa otot kaki kanan dan kiri berbanding ketiga-tiga kumpulan lain iaitu kumpulan Ex, H dan C. Berkenaan metabolisma tulang, peratusan peningkatan 1CTP, penanda resopsi tulang dan kepekatan PTH bagi kumpulan HEx adalah paling rendah berbanding dengan kumpulan penyelidikan yang lain. Merujuk kepada fungsi imuniti, peningkatan yang signifikan bagi bilangan sel T cytotoxic (CD8) dan jumlah sel B (CD19) ($p<0.05$) telah diperhatikan dalam kumpulan HEx selepas 8 minggu tempoh kajian. Tambahan pula, peratusan peningkatan dalam bilangan sel limfosit, T helper (CD4), T cytotoxic (CD8) dan jumlah sel B (CD19) selepas 8 minggu adalah paling tinggi dalam kumpulan HEx berbanding dengan kumpulan kajian yang lain. **KESIMPULAN:** Keputusan kajian ini mencadangkan bahawa gabungan senaman tarian erobik dan pengambilan madu mendatangkan kesan baik yang lebih nyata ke atas kesihatan tulang, kekuatan dan kuasa otot, dan fungsi imuniti keseluruhannya jika dibandingkan dengan senaman tarian erobik atau pengambilan madu sahaja di kalangan wanita yang sedentari.

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CHAPTER I

INTRODUCTION

Osteoporosis is a disease characterised by a loss of bone mass and the structure deterioration of bone tissue, resulting in bone fragility and an increase in susceptibility to break (fractures) (Mitchel *et al*., 1998; Weiss *et al.*, 2000; Gannotti *et al*., 2007). This disease imposes major burden on the health economy (Weiss *et al*., 2000) and being recognised as one of the major public health problems. Many treatments have been developed with the aim of preventing bone loss and increasing bone mass, these include involvement in physical activity programmes (Craciun *et al*., 1998; Kehoe, 2006) and through adequate nutritional intake (Morris *et al*., 1997).

Regular physical activities are claimed to be able to elicit changes in structures and biomechanical properties of bones and consequently increase bone strength. Osteogenic effect is believed to be associated with exercise (Morris *et al*., 1997). Normal bone development is influenced by nutritional and physical factors (Wallace *et al*., 2000). Thus, enhancement of bone mineral acquisition and adequate nutritional intake especially during growth may be useful as a preventive strategy against osteoporosis (Morris *et al*., 1997).

Many studies have investigated the influence of exercise and mechanical load on bone tissue (Peng *et al*., 1994; Barengolts *et al*., 1996). According to Rittweger (2006), the mechanical strain generated by exercise constitutes one of the most important stimuli to bone formation. Bone tissue responds better to dynamic exercises (Barengolts *et al*., 1993) such as those consisting of jumps, rather than static loading (Robling *et al*., 2002, Turner and Robling, 2003).

Evidence to date suggests that bone response to exercise is governed by site specificity (Turner and Robling, 2003; Matthews *et al*., 2006; Magkos *et al*., 2007) and load dependency (Morris *et al*. 1997). Bones need to be subjected to additional mechanical or gravitational forces for increasing their mass and structural density. Matthews *et al*. (2006) provides the evidence that mechanical loading gives the maturity-specific effects on bone. Morris *et al*. (1997) found that total body bone mineral content (BMC) increased at a significantly greater rate in the exercise group compared with the controls.

Maximising peak bone mass may reduce the risk of osteoporotic fracture in later life, and weight bearing exercise during growth can enhance peak bone mass (Matthews *et al*., 2006). Continuation of weight-bearing exercise after growth is mandatory to prevent excessive bone loss in adults. Dancing may provide an ideal osteogenic stimulus, particularly at the hip region, because the various jumps and landings that typify dancing impart unusual and ranging from low to high-impact loads on the skeleton. These loads are experienced primarily in the lower limbs (Khan *et al*., 1998), and the upper limbs may serve as a quasi-control site. Significant increases in bone mineral density were found by supervised 18 month high impact training, and were effectively maintained with subsequent unsupervised regular aerobic and step exercises (twice per week), in prevention of osteoporosis among healthy premenopausal women (Heinonen *et al*., 1999).

Muscle contraction forces act directly or indirectly on bone and are responsible for overloading bone tissue to produce an osteogenic stimulus. 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 (Iwamoto *et al*., 1999). Forces generated by the muscles on the bone tissue during the performance of the exercise increased bone metabolism and promoted osteogenesis (Mullender *et al*., 1996). Muscle contractions had an important role in stimulating osteogenesis, probably due to the overload produced during the exercise.

Physical activity is also believed to have close relationship with immune function. Exercise induces physiological changes in the immune system. The benefits of exercise may result from the direct effect on immune response modulations or from the mechanism of psychological effects of exercise (Nieman, 1992). Exercise may be considered a type of stress that affects immune response differently dependent on the duration, intensity and frequency of the stress (Hoffman-Goetz and Pederson, 1994). Generally, many researchers found that low intensity (Lim and Hong, 2009) and moderate intensity exercise improves immunity (Nieman *et al*., 1993), conversely strenuous exercise and overtraining decrease immunity and raise infection risk (Nieman, 1998; Woods *et al*., 1999; Kohut *et al*., 2005).

Besides regular weight-bearing exercise, nutrition also plays an important role in enhancing and maintaining bone health. It was reported that a group of researchers from Purdue University, U.S.A. has shown in their preliminary study that taking honey along with supplemental calcium appeared to enhance calcium absorption in rats, and could therefore play a role in boosting bone health (Philip, 2005). It has been reported that honey contains moisture, sugars such as glucose and fructose, enzymes, trace essential elements such as iron, copper, zinc and calcium and vitamins such as vitamin

A, C and E (Al-Waili, 2003). These elements are believed to be beneficial for maintaining and enhancing bone health. Besides, it also have properties of antioxidant (Al-Waili, 2003), antimicrobial (Lusby *et al*., 2005; Klein *et al*., 2000), anti-inflammatory (Zaharil *et al*., 2011), and immunomodulatory (Klein *et al*., 2000) as well as for the promoting wound healing (Zaharil *et al*., 2011) and enhancement of fertility effects (Mahaneem *et al*., 2011).

Understanding the relationship between exercise load and immune system has potential implication for public health. Aerobic dance exercise has been shown to be effective in producing great osteogenic effects (Khan *et al*., 1998; Matthews *et al*., 2006). It is one of the weight bearing exercises which requires ones to work against gravity and involves low, moderate and high impact or load activities. It is believed that aerobic dance exercises can help to develop and maintain strong bone. Since the combined effects of an aerobic dance exercise regimen with honey supplementation on bone, muscular strength and immune functions have not been investigated in women with age ranging from 25 to 40 years-old, the present study was proposed for determining the effectiveness of combination of aerobic dance exercise and honey supplementation on bone health, muscular strength and immune function in women.

1.1 OBJECTIVES OF THE STUDY

1. To investigate the effects of combined aerobic dance exercise and honey supplementation on bone metabolism in women.
2. To investigate the effects of combined aerobic dance exercise and honey supplementation on muscular strength in women.
3. To investigate the effects of combined aerobic dance exercise and honey supplementation on immune function in women.

1.2 SIGNIFICANCE OF THE STUDY

The present study is an extension of a pilot study by using animal model, which has been carried out to investigate the combination effects of jumping exercise and honey supplementation on bone properties and bone metabolism in female rats (Ooi *et al*., 2010; Somayeh, 2011). In this pilot study, it was found that jumping exercise could elicit signifcant beneficial effects on bone properties and bone metabolism in female rats. Thus, the present study was proposed for transferring the pilot study results from animal to human. If the present study can find that combined aerobic dance exercise and honey supplementation could elicit beneficial effects on bone metabolism, muscular strength and immune function of women, thus this combination can then be proposed for developing age-specific exercise and nutritional promotion programmes, by formulating guidelines for the enhancement and maintenance of bone health, muscular strength and immune function in women.

1.3 HYPOTHESIS

H_o: There are no significant differences in bone parameters, muscular strength and immune functions in combined aerobic dance exercise and honey supplementation group compared to sedentary without supplementation control, honey supplementation group or aerobic dance group.

H_A: There are significant differences in bone metabolism, muscular strength and immune functions in combined aerobic dance exercise and honey supplementation group compared to sedentary without supplementation control, honey supplementation group or aerobic dance group.

1.4 OPERATIONAL DEFINITIONS

Aerobic dance exercise programme: The exercise sessions was carried out for one hour per session, 3 times per week for a duration of 8 weeks. The subjects were required to attend 2 sessions of ‘high and low impact exercise’ and 1 session of ‘step board’ aerobic dance exercise in a week (Appendix A), (please refer to section 3.2.6 of ‘Methodology’ for the details of aerobic dance exercise programme).

Honey supplementation: Malaysian local honey (Gelam Honey supplied by Federal Agriculture Marketing Authority, FAMA) (Appendix B) was consumed by subjects of honey (H) and combined aerobic dance exercise and honey supplementation (HEx) groups in a dose of 20g of honey diluted in 300ml of plain water, for 7 days for a total of 8 weeks duration. The subjects of HEx group were required to consume honey water 30 minutes before performing aerobic dance exercise on the exercised days.

Bone metabolism: Measurements of blood parameters such as serum total calcium, serum osteocalcin (bone formation marker), serum C-terminal telopeptide of type 1 collagen (1CTP) (bone resorption marker) and parathyroid hormone.

Muscular strength and power: Measurement of left and right lower limb peak torque (strength) and power of the subjects (Appendix C).

Immune functions: Measurements of blood parameters of full blood counts: white blood cells (WBC), neutrophil, lymphocyte counts, and immunophenotyping: serum T lymphocytes (CD3, CD4 and CD8), B lymphocytes and natural killer (NK) cells.

CHAPTER 2

LITERATURE REVIEW

2.1 BONE

2.1.1 Bone tissue

According to the Dorland's Pocket Medical Dictionary (2001), bone can be define as a hard, rigid form of connective tissue constituting most of the skeleton of vertebrates and composed mainly of calcium salts. A bone is made up of several different tissues working together such as osseous tissue (bone), cartilage, dense connective tissues, epithelium, adipose tissue and nervous tissue. Bone tissue is a complex and dynamic living tissue because it involves continuously growing process, remodeling i.e. building new bone tissue, breaking down old bone tissue and repairing themselves (Tortora and Grabowski, 2003). It is surrounded by an abundant matrix of intercellular materials which contained 50% of crystallised mineral salts, 25% of water, and 25% of collagen fibers. Bone consists largely of a mineralized extracellular matrix which mass and architecture result from a balance between production and resorption. The major components of the organic matrix are such as type I collagen, osteopontin and osteocalcin (Scott *et al*., 2008).

Osteocalcin is used as a bone formation marker. It is the major non-collagenous protein of bone matrix which consists of 49 amino acids. Osteocalcin is synthesised in bone by osteoblasts. After production, it is partly incorporated into the bone matrix and partly delivered to the circulatory system. Circulating level of osteocalcin reflects the rate of bone formation. Determination of serum osteocalcin has proved to be valuable for monitoring bone metabolism. Osteocalcin is an indicator of osteoblastic activity in human serum and plasma. It values may vary depending upon the person's age (years after menopause), circadian rhythm, rate of glomerular filtration and duration of treatment. All of these factors are controlled in this study except the glomerular filtration rate.

Type 1 collagen is the most abundant collagen type in the body, and it is the only collagen type found in mineralised bone, where it accounts for more than 90% of the organic matrix. The carboxyterminal telopeptide region of type 1 collagen is known as 1CTP. This peptide is found in an immunochemically intact form in blood, where it seems to be derived from bone resorption and degradation of loose connective tissues. Increase serum concentrations of 1CTP are hence seen in conditions associated with increased lysis of bone. Thus, 1CTP is used as a bone resorption marker.

2.1.2 Bone functions

Besides providing attachment for most of the skeletal muscle, serves as the structural framework for the body by supporting soft tissues and assist in the body part movements, bones also protect the internal organs from injury. For example, brain is surrounded by several cranial bones, spinal cord in the vertebrae and other vital organs such as heart and lungs are well protected in the rib cage. They also store several minerals mainly calcium and phosphorus, and play an important role in minerals homeostasis in the human system. Some part of certain bones in the body which is known as red bone marrow, involving in hemopoiesis which produces red blood cells, white blood cells and platelet (Tortora and Grabowski, 2003).

2.1.3 Bone cells

They are three types of bone cell in the bone: osteoblasts, osteocytes and osteoclasts. Osteoblasts are bone-forming cells which synthesise and secrete collagen fibers and other organic components needed to build the matrix of bone tissue. Matured osteoblasts form osteocytes. Alkaline phosphatase, a serum index of bone formation, is abundant on the plasma membrane of osteoblasts.

Osteocytes is the mature bone cells, and exist as the main cells in bone tissue. These cells are originally osteoblasts that differentiated and found in bone lacunae. The main role of osteocytes is involvement in activation of bone turnover, regulation of extracellular calcium to provide means of communication required in mechanotransduction (Khan *et al*., 2001).

Osteoclasts are giant cells and it is derived from the fusion of as many as 50 monocytes and concentrated in the endosteum. It is usually found in contact with a calcified bone surface and within the resorption cavity it created. The cell able to synthesise powerful lysosomal enzymes and acids, and release them via it ruffled border to digest the protein and mineral components of the underlying bone matrix. This bone resorption process i.e. breakdown of the bone matrix, is part of the normal development, growth, maintenance and repair of bone (Khan *et al*., 2001).

2.1.4 Bone remodeling

Bones continues to renew themselves even after reaching their adult shape and size. Bone remodeling is an ongoing process, whereby osteoclasts first carve out small tunnels in old bone tissue and then osteoblasts rebuild it.

The purposes of remodeling is, to renew bone tissue before deterioration sets in, and redistributes bone matrix along lines of mechanical stress which allows the bone to adjust it strength through the strategically placing.

The breakdown of matrix by osteoclasts is called bone resorption. In this process, an osteoclast attaches tightly to the bone surface at the endosteum or periosteum. Then, it releases protein digesting lysosomal enzymes to digest collagen fibers, and other organic substances and acids to dissolve the bone minerals. Several osteoclasts carve out a small tunnel in the old bone. The degraded bone proteins and matrix minerals mainly calcium and phosphorus was excreted by the osteoclasts to the insterstitial fluid. These bone resorption product was then diffused into the nearby blood capillaries. Once a small area of bone has been resorbed, osteoclasts depart and osteoblasts move in to rebuild the bone in that area. In order to achieve homeostasis, bone resorbing actions of osteoclasts must balance the bone making actions of osteoblasts. A loss of too much calcium or inadequate formation of new tissue weakens bone tissue (Tortora and Grabowski, 2003).

2.1.5 Osteoporosis

Osteoporosis is a disease characterised by a loss of bone mass and the structure deterioration of bone tissue, resulting in bone fragility and an increase in susceptibility to break or fractures (Mitchel *et al*., 1998; Weiss *et al*., 2000; Gannotti *et al*., 2007). This disease imposes major burden on the health economy (Weiss *et al*., 2000) and being recognised as one of the major public health problems.

Osteoporosis is defined by WHO as 2.5 standard deviations or more below the mean (Gannotti *et al*., 2007). Progressive loss of bone tissue implies a long lasting imbalance between bone formation and resorption (Brown *et al*., 1984). Many treatments have been developed with the aim of preventing bone loss and increasing bone mass, including physical activity programmes (Kehoe, 2006 and Craciun *et al*., 1998). Bone turnover markers are referred to the bone formation and bone resorption markers.

2.2 EXERCISE, BONE HEALTH AND MUSCULAR STRENGTH

Maximising peak bone mass may reduce the risk of osteoporotic fracture in later life and weight bearing exercise during growth can enhance peak bone mass (Matthews *et al*., 2006). Continuation of weight-bearing exercise is mandatory to prevent excessive bone loss in adults.

Osteogenic effect is associated with exercise (Morris *et al*., 1997). Exercise is a subcategory of physical activity that is planned, structured, repetitive and purposive in the sense that improvement or maintenance of one or more components of physical fitness is the objective (Reid *et al*., 1999). Many studies have investigated the influence of exercise and mechanical load on bone tissue (Peng *at al*., 1994; Barengolts *et al*., 1996). According to Rittweger, (2006), the mechanical strain generated by exercise constitutes one of the most important stimuli to bone formation. During growth, bone tissue responds better to dynamic exercises (Barengolts *et al*., 1993) such as those consisting of jumps, rather than static loading (Robling *et al*., 2002, Turner and Robling, 2003). It is proven that appendicular bones have been shown to respond best to high strain rates (Bourrin, 1994; Bassey, 1998) with a rest between loads

(Cheng, 2002) and a period of several hours between loading sessions (Chien, 2000).

Evidence to date suggests that bone response to exercise is governed by site specificity (Turner and Robling, 2003; Matthews *et al*., 2006; Magkos *et al*., 2007) and load dependency (Morris *et al*., 1997). Bones need to be subjected to additional mechanical or gravitational forces for increasing their mass and structural density. Matthews *et al*., (2006) provide the evidence that mechanical loading gives the maturity-specific effects on bone. Morris *et al*., (1997) found that total body bone mineral content (BMC) increased at a significantly greater rate in the exercise group compared with the controls. Jumping provides bone loading of the lower limb, but not the upper limb. This is an example of a target bone loading activity which refers to force generating activity that provides stimulus to a specific bone. Uniquely, bone accommodates to static exercises, reaching mechanosensory saturation, after which bone mass and strength increase no more, unless the externally applied loads magnitude is increased exceed the normal loading patterns (Robling *et al*., 2002).

Turner and Robling (2003) stated that mechanical loads which act on bones creates a gradient within the bone's fluid filled lacunar-canalicular network and promotes a cascade of cellular events, including elevation of intracellular calcium, expression of growth factors and increase of bone matrix production. This mechanism increases the osteogenesis process in the specific site of loading bones.

According to Magkos *et al*., (2007), effects of exercise on each skeletal site is expected to respond vary and uniquely according to different exercise types, intensity, duration and frequency. From low-level gravitational forces to high-level impacts, bone has a remarkable ability to modify its mass and architecture in response to a wide range of loads. A variety of types and magnitudes of mechanical stimuli have been shown to influence human bone cell metabolism in vitro, including fluid shear, tensile and compressive strain, altered gravity and vibration (Scott *et al*., 2008).

Experiment using laboratory animals have shown that mechanical loading of the skeleton is necessary for maintaining bone mass. In an animal study, high impact exercise strengthened bone in osteopenic ovariectomised rats (Honda *et al*., 2003). This finding is in agreement with a study which demonstrated that jump resistance training in rats increased bone formation (Notomi *et al*., 2000). Notomi *et al*., (2000). hypothesised the increased in bone formation is due to an increase in the number of osteoblasts and a decrease in bone resorption due to decrease in the number of osteoclasts cells.

Studies involving athletes in high-impact competitive sports such as gymnastics demonstrate that high levels of participation in childhood and adoslescence confer additional bone gain when compared with controls (Bass *et al.,* 1998; Nickols-Richardson *et al*., 1999; Lehtonen-Veromaa *et al*., 2000). Impact-producing exercise is more osteogenic than aerobic endurance type of exercise. Efficacy impact loading is proportional to the ground reaction force (GRF) exerted by the impacts but additional improvements are negligible with increased repetitions (Welch et al., 2008). Many researchers have suggested that dynamic high-impact exercises and resistance training are more beneficial to bone tissue, and producing greater strain than walking (Notomi *et al*., 2000; Honda *et al*., 2003).

Dancing may provide an ideal osteogenic stimulus, particularly at the hip region, because the various jumps and landings that typify dancing impart unusual and high-impact loads on the skeleton (Michel *et al*., 1991). These loads are experienced primarily in the lower limbs (Khan *et al*., 1998), and the upper limbs may serve as a quasi-control site. Matthews *et al*., (2006) demonstrate the positive effects of dance training on the skeleton in peripubertal (age 11-14 years) girls as well as the maintenance of an already existing bone mineral advantage. In their study, ballet dancers had 0.6-1.3% significantly greater BMC (bone mineral content) than controls. At the Femoral neck, dancers had 4% greater BMC than controls in prepuberty and the exercise-induced bone gains can be maintained. They also reported that ballet dancing provides a model of mechanical loading patterns required to site-specifically modulate bone.

Aerobic dance consists of sequences of choreographed movements performed rhythmically to music. Since this is a weight bearing activity, the lower body does most of the work; however, there is also a simultaneous and varying contribution from the upper body musculature. Since its inception for community classes in the 1970s, two main styles of dance, high impact and low impact, have emerged. High impact dance is composed predominantly of running, hopping and jumping movements in which both feet momentarily leave the floor (Bell *et al*., 1994). The impact of this exercise on vulnerable areas such as the shins, knees and vertebral column has led to a high incidence of injuries (Richie *et al*., 1985). Classes have therefore been modified to include more low impact dance, which is characterised by at least one foot remaining in contact with the floor at all times and lower ground forces are produced (Reeves and

Moss 1992). The incidence of overuse injury is reduced and exercise duration prolonged because the workload is spread more evenly between lower and upper musculature (Mostardi and Senechal 1981), which benefits for those embarking on a training programme for the first time. Aerobic dance has been shown to raise heart rates to levels above 55% of maximum and so to constitute a viable cardiovascular training mode (ACSM, 2006). Muscle contraction forces act directly or indirectly on bone and are responsible for overloading bone tissue to produce an osteogenic stimulus. 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 (Iwamoto *et al*., 1999). Forces generated by the muscles on the bone tissue during the performance of the exercise increased bone metabolism and promoted osteogenesis (Mullender *et al*., 1996). Muscle contractions had an important role in stimulating osteogenesis, probably due to the overload produced during the exercise. On the other hand, Daly *et al*., (2004) mentioned that although it has been documented that young and old athletes have both greater muscle and bone masses, there are no conclusive data to prove that the stimuli created by greater muscle contraction leads to a proportional bone increment. Nevertheless, it is generally believed that strong muscle is associated with high force production which consequently producing great osteogenic effects.

Physical exercises programmes have been widely used as part of osteoporosis prevention and treatment. However, some unclear points exist with respect to the most adequate exercise modality and intensity, capable of eliciting the most efficient osteogenic response from the bone tissue

(Bayramoglu *et al*., 2005; Scott *et al*., 2008). Physical activity is known to enhance the mechanical competence of bone. However, information about the optimal type of exercise is limited. Normal bone development is influenced by nutritional and physical factors (Wallace *et al*., 2000). Thus, enhancement of bone mineral acquisition and adequate nutritional intake may be useful as a preventive strategy against osteoporosis (Morris *et al*., 1997).

One of the aims of this study was to evaluate the contribution of aerobic dance exercise combined with honey supplementation to change in bone metabolism markers and muscular strength. Since changes in bone mineral density is expected cannot be seen in a short period of 8 weeks time, thus changes in blood bone metabolism markers were observed instead of bone mineral density in the present study.

2.3 HONEY SUPPLEMENTATION AND BONE HEALTH

Honey is a natural product of bees formed from nectar collected from flowering vegetation. It has been reported that honey contains moisture, sugars such as glucose and fructose, enzymes, trace essential elements such as iron, copper, zinc and calcium, and vitamins such as vitamin A, C and E (Al-Waili, 2003). Besides, it also have properties of antioxidant (Al-Waili, 2003), antimicrobial (Klein *et al*., 2000; Lusby *et al*., 2005), anti-inflammatory (Zaharil *et al*.,2011), and immunomodulatory (Klein *et al*., 2000) as well as for promoting wound healing (Zaharil *et al*., 2011) and enhancement of fertility effects (Mahaneem *et al*., 2011). According to Klein *et al*., (2000), honey is among many naturally occurring nutraceuticals ingested for thousands of years for their medicinal purposes.

More than 95% of the solids of floral and honeydew honeys are carbohydrate in nature, largely simple sugars or monosaccharides, being fructose and glucose the major constituents (White, 1979). In nearly all honey types, fructose predominates and only a few honeys appear to contain more glucose than fructose. Fructose and glucose together account for 85-95% of honey carbohydrates (White, 1979). However, fructose and glucose contained in honey are able to decrease with time (Cavia *et al*., 2002).

Honey used in this study was kindly supplied by Federal Agricultural Marketing Authority (FAMA), Malaysia. It is a wild multifloral honey collected form beehives built on a tall tree, Malaleuca, locally named as Gelam tree, that grows in Terengganu, Malaysia. This honey which also locally known as Gelam honey is one of the most widely available honeys in our local setting, it has been traditionally used for medicinal purposes. Other than that, honey is traditionally consumed by the local Malaysian population as a nutrient supplementation (Mahaneem *et al*., 2011).

Exercise is believed to be beneficial for bone health when adequate nutrition is provided. In a recent study carried out by our research team in USM, Malaysia, it was found that combination of jumping exercise and honey supplementation could elicit more discernable beneficial effects on bone health in young rats compared to jumping exercise or honey supplementation alone (Ooi *et al*., 2010; Somayeh *et al*., 2011). Since the combination effects of supplementation honey on bone health in human being is unclear, further investigations is needed to prove the effectiveness of combination of honey supplementation with exercise intervention on bone health in human. Thus, the present study was proposed.

2.4 IMMUNE FUNCTIONS

Immune system is responsible to ensure the survival of an individual through prevention and recovery from infectious diseases (Burtis and Ashwood, 1999). Immunity refers to all mechanism used by the body as a protection against environmental agents that are foreign to the body. The body defence against invasion by external agents can be divided into two levels. There are: innate immunity and adaptive immunity.

Innate immunity is the body's initial defence mechanisms against invading microorganisms. It always presents and available at very short notice to protect an individual from foreign invaders. Innate immunity can be divided into two elements; body surfaces and internal components (Benjamini *et al*., 1996). The two subgroups of internal components are humoral and cellular parts. The humoral component includes lysozymes and complements, while the cellular component involves polymorphonuclear (PMN) cells, natural killer (NK) cells, macrophages and microgial cells (Parslow, 1997).

Acquired immunity is referred to a specific immunity because it relies upon the ability to recognise antigens. The response in acquired immunity is greater and faster as compared to innate immunity (Benjamini *et al*., 1996). An acquired immunity can be subdivided into two cellular components, involving two types of cells i.e. T lymphocyte and B lymphocyte cells, as well as two humoral components, immunoglobulins (Ig) and interleukins (IL) (Weir and Stewart, 1997).

Lymphocytes are the major cells in our immune system. It can be divided into 3 populations; B cells (10-15%), T cells (70-80%) and Natural Killer (NK) cells (10-15%). Activation of B cells (CD19) requires the help from the T cells

(CD4 helper T cells). During the humoral response, when B cells formed in the bone marrow encounter foreign antigens, they differentiate into plasma cells and secreting antibody immunoglobulins. The secreted antibodies bind to the antigen and preventing their binding to host cell receptors, thereby facilitating their removal from the body. The cell mediated response, which involves recognition of foreign antigens after their attachment to the host's cells occurs mainly via T cells (T helper). T cells subsets are consist of CD4, CD8 and γδ T cells. The main function of CD4 (T helper cell) is to interact with B cells and involved in B cell activation. CD8 also known as T cytotoxic cell, it kills infected host cell and abnormal cells. γδ T cells, it is the predominant T cell type in epithelial tissue which is involved during early infection. NK cell, a major lymphocyte of the innate immune response which has express receptors that can selectively recognise and kill transformed or cancer, or infected cells.

Neutrophil is one of the polymorphonuclear (PMN) cells. Its functions are to ingest and destroy invading foreign particles. When the invading particles are detected by the phagocytic cells, it engulfs the foreign invader and destroys it with enzymes which also known as lysozymes (Parslow, 1997).

2.5 EXERCISE AND IMMUNE FUNCTIONS

Exercise induces physiological changes in the immune system (Nieman, 1992). The benefits of exercise may result from the direct effect on immune response modulations or from the mechanism of psychological effects of exercise (Nieman, 1992). Exercise may be considered a type of stress that affects immune response differently dependent on the duration, intensity and frequency of the stress (Hoffman-Goetz and Pederson, 1994). Generally, many researchers found that low intensity (Lim and Hong, 2009) and moderate exercise improves immunity (Nieman *et al*., 1993), conversely strenuous exercise and overtraining decrease immunity and raise infection risk (Nieman, 1998; Woods *et al*., 1999; Kohut *et al*., 2005).

Besides, types of exercise (Natale *et al*., 2003), such as acute (Rowbottom and Green, 2000; Santos *et al*., 2006) and chronic (Woods *et al*., 1999) physical training are able to alter the immune status of an individual. Previous study reported that the level of immune response are varies during exercise, immediately after exercise and during recovery period (Woods *et al*., 1999).

2.6 HONEY AND IMMUNE FUNCTIONS

It was mentioned in Nieman (1998) that ingestion of fluids that contain carbohydrate can reduce perturbations in the immune system with less disturbance in blood immune cell counts, lower granulocyte and monocyte phagocytosis and oxidative burst activity, diminished pro- and anti-inflammatory cytokine response. It is believed that by maintaining higher plasma glucose levels and attenuating the cortisol and growth responses during heavy exercise, carbohydrate beverage ingestion may reduce stress to the immune system of athletes (Nieman, 1998). Thus, honey which contains carbohydrate may elicit beneficial effects on reducing stress to the immune system in active adults.

Besides green tea, ginseng and vitamin, honey is one of the nutraceuticals which are becoming more widely accepted as an adjunct to conventional therapies for enhancing general well being (Klein *et al*., 2000). Honey is reported to be able to stimulate the immune system of the body (Tonks *et al*., 2003). Klein *et al*., (2000) in their study found that honey has been proven to have antimicrobial activity.

Since the combined effects of an aerobic dance exercise regimen with honey supplementation on bone, muscular strength and immune function have not been determined, the present study was proposed for determining the effectiveness of combination of aerobic dance exercise and honey supplementation on bone health, muscular strength and immune function in women.

CHAPTER 3

MATERIALS AND METHODS

3.1 SUBJECTS

In this study, forty four physically healthy women, age ranges from 25 to 40 years old were recruited. Subjects recruited in this study were sedentary, free from health problems and did not have a habit of consuming honey supplementation daily before the recruitment. Subjects were matched in age, body mass, height and percent of body fat before they were assigned into the intervention groups and one control group with 11 subjects per group (n=11).

All subjects 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 were than distributed to all the subjects before giving their formal consent. Subjects' information sheets and consent forms (Appendix D). The present study was approved by the Research Ethics Committee (Human) of Universiti Sains Malaysia (Appendix E).

Participation of a subject in this study was on a voluntary basis and they were allowed to withdraw from this study at any time during the course of this study.

3.2 EXPERIMENTAL DESIGN

3.2.1 Subjects grouping

The subjects were randomly divided into four groups with 11 subjects 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. Subjects in the control group did not perform neither exercises nor taking honey supplementation. Meanwhile, aerobic dance exercise group performed one hour aerobic dance exercise per session, 3 times per week for 8 weeks. Subjects of honey group consumed 20g of Gelam honey which was diluted in 300ml of plain water (Appendix B), for 7 days per week for a total of 8 weeks duration. Subjects in combined aerobic dance exercise with honey supplementation group performed aerobic dance exercises one hour per session, 3 times per week for 8 weeks and consumed Gelam honey drink 7 days/week for 8 week with dosage same as honey group. The subjects of H and HEx were required to consume honey drink 30 minutes before performing aerobic dance exercise on the exercised days.