Effect of a Combination of Jumping Exercise and Honey Supplementation on the Mass, Strength and Physical Dimensions of Bones in Young Female Rats

Somayeh Sadat Tavafzadeh¹, Foong-Kiew Ooi¹, Oleksandr Krasilshchikov¹ and Siti Amrah Sulaiman²

¹ Sport Science Unit, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia.
² Department of Pharmacology, School of Medical Sciences, Universiti Sains Malaysia, Kelantan, Malaysia

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*Corresponding author: Email: somatavaf@yahoo.com

Summary

The study investigated the effects of combined jumping exercise and honey supplementation on bone properties in young female rats. Forty eight 12-week old female rats were divided into four groups: control group (C), honey supplementation group (H), jumping group (J), and combined jumping and honey supplementation group (JH). Jumping exercise consisted of 40 jumps per day for 5 days per week at a height of 40 cm. Honey was supplemented to the rats at a dosage of 1g / kg body mass / rat / day via force feeding for 7 days per week. After 8 weeks of experimental period, right hind leg tibial and femoral wet and fat free day weights (bone mass), maximal load (bone strength), mid shaft maximum and minimum diameters were measured. Tibial wet and fat free dry weights (583.33 ± 29.82, P<0.01; 412.50 ± 24.89 P<0.05), tibial and femoral maximal load (101.55 ± 9.88; 133.97 ± 23.78, P<0.05), tibial mid-shaft minimum diameter (2.35 ± 0.13, P<0.05) and femoral mid-shaft maximum diameter (4.00 ± 0.16, P<0.05) were significantly increased in the combined jumping exercise and honey supplementation group. However, these discernable improvements in bone were not observed in the group with jumping exercise alone or the honey supplementation only group. The results of the present study suggest that a combination of jumping exercise and honey supplementation elicited more discernable beneficial effects on tibia and femur bone generally when compared to either jumping exercise or honey supplementation alone in young female rats.

Keywords: jumping exercise, honey supplementation, bone health

Introduction

Osteoporosis is a systemic skeletal disease characterised by low bone density and micro-architectural deterioration of bone tissue, with a consequent increase in bone weakness, fragility and susceptibility to fracture. It is the result of gradual bone loss with ageing and it poses a serious public health threat that affects millions of women and men all over the world (Swaim et al., 2008). It is believed that mechanical strain generated by exercise is one of the most important stimuli for bone formation (Renno et al., 2007). Thus exercise plays an important role in preventing and reducing bone loss.

High impact exercise such as jumping is considered to be beneficial for eliciting bone health in animals (Umemura et al., 1997). Previous studies have shown that 8 weeks of jumping exercise had beneficial effects on bone mass, strength, mineral density, and bone formation in the lower limbs of rats (Honda et al., 2001; Honda et al., 2003; Ooi et al., 2009). Similarly, results of a study carried out by Notomi et al., (2000) also showed that jumping exercise, which required the animals to be loaded with extra weights while exercising, could increase the mass and strength of the lumbar vertebrae and mid femur of rats, by stimulating periosteal bone formation, accelerating cortical drift, and reducing the endocortical mineral apposition rate.

Regarding the effects of supplementation on bone health, several previous studies indicated that supplementation such as soy or soy isoflavones elicited positive effects on bone (Omi et al., 1994; Arjmandi et al., 1996; Arjmandi et al., 1998; Arjmandi et al., 2002). Arjmandi & Smith (2002) reported that soy isoflavones exerted beneficial effects on bone by stimulating bone formation and...
supplementation, one researcher reported that a combination of exercise in combination with calcium supplementation (Prince et al., 1995) showed that bone loss could be reduced or prevented by performing a two years study in postmenopausal women with low bone density in growing male rats (Choi, 2004). In humans, a two years study in postmenopausal women with low bone density showed that bone loss could be reduced or prevented by performing exercise in combination with calcium supplementation (Prince et al., 1995). It has been reported that the acute increase in osteoclastic activity after endurance cycling exercise can be suppressed by prior intake of a calcium load through drinking high-calcium mineral water (Guillemant et al., 2004).

Honey has long been used within various traditional medical systems. It is also well known for its beneficial actions within the wound environment (Lusby et al., 2005). It has been found that honey increased calcium absorption after acute feeding in growing rats (Ariefdjohan et al., 2008). Chapels & Starkey (2007) reported that bone mineral density was significantly higher in honey-fed rats compared to those fed with a sugar-free diet after 52 weeks. These findings indicate that honey may enhance bone health. To date, the effects of combined honey supplementation and jumping exercise on bone have not been determined. Therefore, the present study was designed to determine the effectiveness of 8 weeks of combined jumping exercise and honey supplementation on bone in young female rats.

Materials and methods

Animals

Forty eight eleven-week old Sprague-Dawley female rats were placed in the experimental room to acclimatise to the environment for a period of seven days. Throughout the study, the environment was maintained under constant temperature of 26-29°C and relative humidity of 70-75%. The rats were exposed to a constant 12:12 light/dark cycle, with the light period starting from 7.00 p.m to 7.00 a.m for the entire experimental period.

The reversed light/dark cycle, was implemented to allow jump training during the day. After one week of acclimatisation, the rats were weighed for their initial body mass. Then the rats were block-randomised into four initial body-mass-matched groups with 12 rats per group (6 rats per cage). At the end of the experimental period, the rats were weighed once again to obtain the final body mass. Then they were anaesthetised, one at the time, by placing them for 2-3 minutes in a desiccated jar containing a chloroform-soaked gauzed pad before being decapitated using a small guillotine (Scientific Research Instrument, U.K.). The right hind leg was dissected for the measurement of various bone parameters. The research protocol was approved by animal research committee, USM, No.: USM/Animal Ethics Approval/2008/(39)(121).

Experimental design

This study consisted of 8 weeks of jumping exercise and honey supplementation. Rats with initial body mass ranged 190-220 grams were randomly assigned to four groups, with twelve rats in each (n=12): the control group was sedentary without supplementation (C), honey group; the sedentary group had honey supplementation (H); the jumping group had jumping exercise without honey supplementation (J), and the combined jumping and honey group had jumping exercise and honey supplementation (JH). All the rats were sacrificed at the age of twenty week-old.

Training programme

Rats in the jumping group and in combined jumping and honey supplementation group were trained to jump using a previously described protocol (Umemura et al., 1995; Umemura et al., 1997; Ooi et al., 2009). Jumping exercises were carried out from 8:30 a.m. to 11:30 a.m. Each rat was subjected to the exercise for 5 minutes duration per day for 5 days per week. Each rat was placed in a specially designed wooden box, measuring 30.5 x 30.5 x 40 cm in length, width and height respectively, and with a copper strip base that formed an electrical grid. The jumping exercise was initiated by applying electrical stimulation to the base of the box through a stimulator (Grass S48, U.S.A.). The stimulator was set to automatically deliver a stimulus of 50-80 V for 1 second and at 3-second intervals. To begin the exercise session, the rats were placed on the electrical grip with the stimulator turned off. When the stimulator was turned on, the rats jumped from the floor of the box to catch the top edge of the box with their forepaws. Upon reaching the top, they were then immediately repositioned by hand to the floor of the box to repeat the procedure. The requirement for electrical stimulus decreased over time when the rats became accustomed to the jumping exercise.

The jump training began with an initial jumping height of 20 cm, after which the height was increased gradually to 40 cm by the third day. After a few days of training the rats began to jump without electrical stimulation. The rats that refused to jump were stimulated by the low voltage of electrical stimulation. The sedentary rats in the control group (free cages activity) were not given any electrical stimulus. In order to mimic the stress induced by handling before and after jumping exercise, the sedentary rats were handled 5 days per week for 8 weeks.
**Honey supplementation**
Honey was orally supplemented to the rats at the dosage of 1 g / kg body mass / rat / day for 8 weeks via force feeding (gavages), 30 min. prior to the jumping exercise. Body mass of the rats was measured biweekly, and the dosage of honey was calculated based on the most recent body mass.

**Bone harvesting and measurements**
Immediately after sacrificing, the right hind leg of the rat was dissected. After removal of the flesh from the tibia and femur, they were then soaked in saline to prevent dehydration for subsequent measurement of bone wet weight, length, mid shaft maximum and minimum diameters (physical dimensions). Tibia and femur wet weight, to the nearest 0.01 mg, were determined on an electrical balance (AA-160, Denver Instrument company, U.S.A.) immediately after the bone harvesting. The tibia and femur length, to the nearest 0.01 mm, were then measured with a digital sliding caliper (A.S.M., Germany), from the top of the tibia head to the distal point of tibia, and from the top of the femur head to the distal point of the femur respectively. The caliper was then set at half the length of the measured tibia and femur, and the mid-point was then marked with a marker pen on the tibia and femur. At the point, the bone mid shaft maximum diameter was measured from the anterior to posterior region of the bone, whereas the bone minimum diameter was measured from the medial to lateral region of the bone. The bones were then wrapped in saline-soaked gauze pads, to prevent dehydration and put into labelled plastic bags and stored at -80ºC (Heto Ultra Freezer 3410, Denmark) for the measurement of mechanical property and bone fat free dry weight at a later date (Ooi et al., 2009).

On the day of mechanical testing the stored tibiae and femurs were thawed for approximately 1 hour at room temperature, and then soaked in 0.9% saline. The tibiae and femurs were then wiped dry and loaded, one at a time, onto an electromechanical testing system (Instron machine 8874, U.K.), and subjected to the three-point bending until fracture for the determination of bone mechanical property, i.e. maximal load. The distance between the two bottom supports of the tester was set 16 mm apart, and the cross-head speed was set at 10 mm / min (Ooi et al., 2009). After positioning each bone on the support, a force was applied to the tibial and femoral mid shaft from the lateral to the medial surface. The maximum force required to break the bone was referred to as bone maximal load, which was an indicator of bone strength.

After the measurement of the mechanical property, the broken tibiae and femurs were immersed in chloroform-methanol solvent (2:1 by volume respectively) for one week to remove the fat from the bones (Ooi et al., 2009): Following this, the bones were removed from the solvent and were then oven dried and sterilized at 80ºC for 24 hours (hot air oven, Sanyo Gallenkamp, U.K.). After drying, the fat free dry weight to the nearest 0.01 mg was determined on an electronic balance (Sartorius, Germany).

**Statistical Analysis**
Statistical Package for Social Sciences (SPSS) version 12.0 was used for the statistical analysis. All the data had been reported as mean ± standard deviation (SD). One way analysis of variance (ANOVA) was performed to determine the significant differences between groups. When the one way ANOVA revealed a significant difference, post hoc (least significant differences test) was used to determine the differences between specific means. 'P' of <0.05 was considered as statistically significant and used for all the comparisons.

**Results**
There were no differences in the initial mean body mass of the rats. After 8 weeks of experimentation, no significant differences were observed in final body mass among all the groups either (Table 1).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tibial Wet Weight (mg)</th>
<th>Tibial Fat Free Dry Weight (mg)</th>
<th>Femoral Wet Weight (mg)</th>
<th>Femoral Fat Free Dry Weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>545.17 ± 26.84</td>
<td>380.50 ± 25.79</td>
<td>738.42 ± 40.97</td>
<td>500.17 ± 36.11</td>
</tr>
<tr>
<td>H</td>
<td>544.17 ± 37.11</td>
<td>379.50 ± 31.18</td>
<td>753.50 ± 67.59</td>
<td>500.25 ± 46.93</td>
</tr>
<tr>
<td>J</td>
<td>571.25 ± 41.19</td>
<td>392.58 ± 26.41</td>
<td>748.08 ± 56.57</td>
<td>494.42 ± 37.57</td>
</tr>
<tr>
<td>JH</td>
<td>585.33 ± 29.82** **</td>
<td>412.50 ± 24.89* *</td>
<td>771.00 ± 50.94</td>
<td>519.92 ± 35.36</td>
</tr>
</tbody>
</table>

* = p<0.05; ** = p<0.01 compared to control group (C);  = p<0.05; ++ = p<0.01 compared to honey supplementation group (H).
C = control group; H = honey group; J = jumping group; JH = combined jumping and honey group.

Table 1. Tibial and femoral wet weight and fat free dry weight. (Means ± SD)
Jumping exercise and honey on bones in rats

Table 2. Tibial and femoral length, maximum diameter and minimum diameter (Means ± SD).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Tibial Length (mm)</th>
<th>Tibial Maximum Diameter (mm)</th>
<th>Tibial Minimum Diameter (mm)</th>
<th>Femoral Length (mm)</th>
<th>Femoral Maximum Diameter (mm)</th>
<th>Femoral Minimum Diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>38.05 ± 0.85</td>
<td>3.15 ± 0.13</td>
<td>2.24 ± 0.15</td>
<td>34.57 ± 0.56</td>
<td>3.28 ± 0.27</td>
<td>2.89 ± 0.13</td>
</tr>
<tr>
<td>H</td>
<td>38.50 ± 0.95</td>
<td>3.27 ± 0.24</td>
<td>2.21 ± 0.81</td>
<td>34.95 ± 0.88</td>
<td>3.83 ± 0.14</td>
<td>3.00 ± 0.13</td>
</tr>
<tr>
<td>J</td>
<td>37.98 ± 0.74</td>
<td>3.22 ± 0.16</td>
<td>2.26 ± 0.63</td>
<td>34.43 ± 0.81</td>
<td>3.88 ± 0.09</td>
<td>2.93 ± 0.17</td>
</tr>
<tr>
<td>JH</td>
<td>38.17 ± 0.59</td>
<td>3.29 ± 0.18</td>
<td>2.35 ± 0.13*</td>
<td>34.57 ± 0.56</td>
<td>4.00 ± 0.16*</td>
<td>2.94 ± 0.17</td>
</tr>
</tbody>
</table>

* = p<0.05 compared to control group (C). + = p<0.05 compared to honey supplementation group (H).

C = control group; H= honey group; J = jumping group; JH = combined jumping and honey group.

Tibial wet weight was significantly higher in JH when compared to C and H respectively (P<0.01). Similarly, fat free dry weight was significantly higher in JH when compared to C and H respectively (P<0.05). However, femoral wet weight and fat free dry weight were not significantly different among the groups (Table 1).

Tibial maximal load was significantly higher in J compared to H and C respectively. Tibial maximal load was also significantly higher in JH compared to H and C respectively (Figure 1). Maximal load of right femur in JH was significantly higher (P<0.05) compared to C (Figure 2).

Results of tibial and femoral physical dimensions are illustrated in Table 2. There were no significant differences in tibial length and femoral length among the experimental groups (Table 2). Tibia maximum diameter was not significantly different among all of the groups. However, tibial minimum diameter was higher (P<0.05) in JH as compared to C and H respectively (Table 2). The present study results reveal that femoral maximum diameter was significantly higher in JH as compared to H and C respectively (P<0.05). Nevertheless, there were no significant differences in femoral minimum diameter among the groups (Table 2).

Discussion

The most notable finding in the present study is that a combination of jumping exercise and honey supplementation elicited more discernable beneficial effects in tibia and femur bone properties generally when compared to jumping exercise or honey supplementation alone in young female rats.

In the present study, the absence of significant changes in the final body mass between exercise and control groups might be due to the jumping exercise not being sufficiently demanding metabolically or calorie consuming to affect body mass. The lack of significant effects of honey supplementation on body mass in the honey supplementation group may be due to the prescribed dosage of honey not being high enough to affect body mass of the rats. In a previous study, Carmody et al. (2007) reported that rats fed by
ooney were 13.3% lower in body mass compared to those fed by sucrose. However, in two other studies, soy protein supplementation has also been reported to have no effect on body weight in the rats (Choi, 2004; Figard et al., 2006).

The present findings of no changes in tibial wet weight and fat free dry weight in the jumping exercise alone group are in contrast with a few previous studies where 8 weeks of jumping exercise as a high-impact exercise has been reported to increase tibial wet and fat free dry weights in the rats (Umemura et al., 1995; Honda et al., 2001; Umemura et al., 2002; Umemura et al., 2008). Differences in these studies could be due to differences in strain of the animals, or the experimental environment, e.g. food given, room temperature and humidity. The strain of the rats used by Umemura et al. (2002) were Fischer 344, and Wistar rats were used in studies done by Umemura et al. (2008) and Honda et al. (2001), however, Sprague Dawley rats were used in the present study. According to the experimental environment, the temperature was set at 23°C in Umemura et al. (2002, 2008) and Honda et al. (2001), however, the temperature was at the range of 26-29°C in the present study. The chow (CE-2, CLEA, Japan) consumed by the rats in Umemura et al. (1995), differed from the chow (Golden Coin, Malaysia) that was used in the present study. The effects of exercise on bone tissue may also vary according to the type of exercise, intensity, duration and frequency. It is interesting to note that greater values of tibial mass were only observed when jumping exercise was combined with honey supplementation in the present study and indicates that enhancement in both the mineral phase and the organic matrix of the bone occurred only when jumping exercise was combined with honey supplementation. Another notable finding in the present study was that 8 weeks of combined jumping exercise and honey supplementation elicited improvement in tibial mass but not in femoral mass, which reflects that bones may respond differently to mechanical loading and nutritional supplementation according to their bone site.

Bone maximal load, which is an indicator of bone strength, reflects the ability of bone to resist fracture. It was remarkable to observe in the present study that honey supplementation alone did not enhance bone strength, unless it was combined with jumping exercise. The lack of significant changes in bone strength with nutritional supplementation alone has been reported earlier. In a study by Deyhim et al. (2003) it was reported that isoflavones supplementation did not increase bone strength. In another study it was reported that 8 weeks of isoflavones combined with calcium supplementation did not enhance peak load and peak displacement or toughness in the bone of the rats (Breitman et al., 2003). The reason why a combination of jumping exercise and honey supplementation may elicit additional beneficial effects compared to jumping exercise or honey alone is uncertain. Nevertheless, one possible reason might be that jumping exercise is a type of high impact dynamic exercise, hence higher volume of blood would be delivered to the working muscles during jumping exercise due to the rhythmic nature of dynamic loading as compared to other lower impact and static exercises (Borer, 2005; Ooi et al., 2009). Thus, the enhancement in blood supply to the muscle and subsequently to the bone might increase the supply of nutrients derived from honey to the bone during jumping exercise. The vital nutrients contained in the honey which could enhance bone health are vitamin D, vitamin K and minerals such as calcium, phosphorous and magnesium. It is speculated that the improvement in bone mass and strength which could be seen in the JH group may be due to the contribution of the enhancement of these vital vitamins and minerals in the bone, which consequently increased mineralization and facilitated deposition of bone matrix.

Bone length, maximum and minimum diameters are indicators of bone physical dimensions. In the present study, tibial and femoral length were similar among all the groups implying that honey supplementation, jumping exercise and combination of both did not affect bone length. It was also found that there were significantly greater tibial minimum diameter and femoral maximum diameter in JH group compared to H and C groups respectively. However, significant differences were not evident among J, H, and C groups. These observations highlighted that combination of jumping exercise and honey elicited greater beneficial effects on bone diameters than jumping exercise or honey supplementation alone.

The reason for the lack of exercise effect on bone length in the present study is unclear. It is possible that the loading stimulus generated by the jumping exercise was not high enough to affect longitudinal growth of bone in the rats. The other probable reason for the lack of significant changes in bone length following exercise could be that the growth plate was closed at the bone distal site as the rats were exercising at age 12 to 20 weeks-old. It was previously reported by Iwamoto et al. (1999) that the growth plate at the distal tibia site was closed when rats were 12 week-old. These authors suggested that exercise in an early age might be more effective in stimulating bone longitudinal growth.

Even though jumping exercise did not affect bone in the present study, muscle contractions during exercise could possibly act on the surface of the bone shaft, and stimulate bone adaptation at the bone shaft. Thus bone enlargement may occur more obviously at the diameter of the bone shaft rather than length. Vainionpaa et al. (2007) mentioned that bone may respond differently to loading according to the bone region, which is due to different bone regions experiencing diverse loading environments with similar movement. This was confirmed in our study by the evidence of greater mid-shaft minimum diameter observed in the tibia, and greater mid shaft maximum diameter exhibited in the femur of the JH group when compared to C and H groups respectively.

Comparable findings reported by Umemura et al. (1995)
showed that the diameter of the tibia and femur were significantly greater after 8 weeks of both run training and jump training compared to sedentary rats. Similarly, 12 weeks of treadmill exercise has been shown to have significant effects on tibial diameter of rats (Baumbach et al., 2003). However, no significant changes in femur diameter have been reported after 12 weeks of either swimming or running mode of exercise in rats (Figard et al., 2006). The reason for the lack of effects in the jumping exercise only group on bone diameters in the present study is uncertain. However, the possible reason could be due to differences with previous studies in terms of strain and age of the animals, or experimental environment compare to previous studies. In terms of differences in the strain of animals used, Sprague Dawley rats were used in the present study, however, Fischer 344 rats were used in Umemura et al. (1995) and Baumbach et al. (2003). In terms of age differences between the present study and previous studies, in the study done by Umemura et al. (1995), rats started exercise at the age of 5 weeks old, however, 12 week-old rats were used in the present study. Regarding differences in experimental environment, room temperature was higher in our study (26-29°C) compare to 23°C used in Umemura et al. (1995). Consistent results in femur diameter between the present study and the study reported by Figard et al. (2006) can be due to the similarities in terms of age, sex and strain of the animals used in these both studies.

It was found in the present study that honey supplementation alone did not affect bone length and diameters. However, significant changes in bone physical dimensions were observed when supplementation was combined with jumping exercise. The probable reason might be that jumping exercise may increase absorption of vitamins and mineral contained in the honey into the bone through the enhancement of blood flow to the muscle, and subsequently to the bone due to the rhythmic nature of dynamic loading elicited by jumping exercise as mentioned earlier. The vital vitamins and minerals contained in honey may have enhanced bone growth in diameters with accumulation of more mineral and collagen matrix in the bone.

In conclusion, the present study showed that a 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. Nevertheless, further study using human subjects is warranted to confirm the present study results. If the beneficial combination effects of jumping exercise and honey supplementation can be seen in human beings, guidelines for exercise and nutritional supplementation programmed for enhancing bone health can be proposed.

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References


