Onion decreases the ovariectomy-induced osteopenia in young adult rats

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Abstract

It has been suggested that fruit and vegetable consumption are associated with good bone health. Onion, in particular, has been verified in its efficacy in bone resorption activity. In this study, we further investigated the effects of an onion-containing diet on ovariectomy-induced bone loss using methods of serum marker assay, histomorphometric analysis and biomechanical tests. Sixty-four female Wistar rats (14-week-old) with sham operations or ovariectomy were assigned to 6 groups: CON, sham-operated control group; OVX, ovariectomized group; ALN, ovariectomized rats treated with alendronate (1 mg/kg/day, p.o.); and 3% ON, 7% ON and 14% ON, ovariectomized rats fed with diets containing 3%, 7% and 14% (wt/wt) onion powder, respectively. Animals were sacrificed after a six-week treatment course. In the serum marker assay, alendronate and all three onion-enriched diets significantly decreased serum calcium level ($p<0.05$). Both 14% ON group and the ALN group even showed similarly lower level of serum osteocalcin ($p<0.05$), suggesting a down-regulation of bone turnover. The histomorphometric analysis showed that ovariectomy markedly decrease bone trabeculae. The ALN and 14% ON rats were 80% and 46% higher, respectively, in BV/TV than the OVX rats ($p<0.05$), and the rats fed with onion-enriched food showed a lesser ovariectomy-induced bone loss in a dose-dependent manner. Additionally, both ALN and 14% ON groups had significantly more trabeculae, and fewer osteoclasts ($p<0.05$), but the protective efficacy from the 14% onion-enriched diet was slightly inferior to that of alendronate. Ovariectomy also significantly decreased tissue weight and biomechanical strength in the OVX group ($p<0.05$). The ALN and 14% ON groups equivalently showed a lesser decrease in tissue weight, though the difference was not significant. On the other hand, both the ALN and 14% ON groups represented similar biomaterial properties of femurs, and both reduced the ovariectomy-induced decrease in bending load and bending energy ($p<0.05$). The present study further verified that an onion-enriched diet could counteract ovariectomy-induced bone loss and deterioration of biomechanical properties.

Keywords: Osteoporosis; Phytotherapy; Alendronate; Osteoclast; Menopause

Introduction

The increasing incidence of osteoporosis and its related fractures are important global health issues. Osteoporosis affects about 75 million people in Europe, USA and Japan [1]. In addition, one-third of women, as well as one-fifth of men over 50, may develop osteoporotic fractures in their lifetimes [2–4]. It was
predicted that, by 2050, more than 50% of worldwide osteoporotic hip fractures would occur in Asia [5]. Among the pharmacological treatments, hormone therapy (HT), selective estrogen receptor modulators (SERMs) and bisphosphonate have been shown to be effective either in increasing bone mineral density (BMD) and/or reducing fracture rates [6]. However, the side effects of these medications, including gastrointestinal tolerance problems in bisphosphonate and the potential malignancies in HT [7,8], may preclude their long-term use. Growing evidence of the benefits of natural foods for bone health provide an alternative option for prevention and/or treatment of osteoporosis.

Cross-sectional studies have shown that people, including postmenopausal women, healthy women, and elderly men, consuming more fruits and vegetables accumulated a higher bone mineral density (BMD) [9–15]. In addition, increased fruit and vegetable consumption showed benefits for bone health and bone development in young people [15–17]. In animal studies, it had been demonstrated that short-term treatment with diverse fruits, vegetables and herbs could effectively reduce bone resorption activity [18–20]. One recent study showed the effects of 5%, 15%, and 25% prune-enriched diets in reversing bone loss in young adult ovariectomized rats [21]. The 25% prune-enriched diet even showed an equally protective effect on bone volume ratio (BV/TV, %) as 17β-estradiol E2. Similarly, a summary of animal studies strongly suggested the positive effects of onion on good bone health. A short-term treatment (10 days) with onion (0.03–1.5 g/rat/day) has been shown to decrease the ovariectomy-induced bone resorption rate in a dose-dependent manner [22]. A dose at 1 g/rat/day has even served as a positive control treatment for down-regulating bone resorption [19,20]. The aim of this study was to investigate whether an onion-enriched diet could prevent estrogen deficiency induced osteopenia. For this purpose, serum bone markers and long bone biomechanical testing were measured and tissue histomorphometry was performed to investigate the dose-dependent bone protective effects of onion on 14-week-old ovariectomized Wistar rats for a period of six weeks.

Materials and methods

Animals

Female Wistar rats (n=64) were housed under controlled conditions including a room temperature of 22±1 °C with a 12:12 h light–dark cycle. All animals were fed with standard Purina Rodent Chow 5001 (Labdiet®, Richmond, IN, U.S.A.) containing 0.95% calcium and 1.07% phosphate (wt/wt of dry food) and tap water ad libitum. The procedures of the animal study, including the raising, feeding, and the whole surgical process, followed the APS’s “Guiding Principles in the Care and Use of Animals” and were approved by the Committee of Animal Study at National Cheng Kung University (Document Serial No. 940204).

Experimental design and treatments

The use of rats younger than 3 months is not suitable for ovariectomy due to the concurrence of bone remodeling and bone growth [23]. Thus, we used adult virgin female Wistar rats with an age of 14 weeks. One week before the ovariectomy or sham operation, all animals (13 weeks old) were given demineralized water and a diet of “semi-purified” diet powder (AIN-76A, TestDiet®, Richmond, IN, USA) with animal protein (20% casein) containing 0.52% calcium and 1.12% phosphate. Three days after surgery, sham-operated animals (n=8) were set as the control group and ovariectomized rats (n=56) were randomly assigned to five groups (Fig. 1): the OVX group (n=11), fed with the AIN-76A diet without any addition; the ALN group (n=12), fed with the AIN-76A diet + alendronate (Calbiochem Co., Merck Taiwan Ltd, Taiwan) at a dose of 1 mg/kg b w/day (p.o.), which has been used by others in either growing or aged ovariectomized rats [24,25]; and three onion treatment groups (n=11 for each), the 3% ON, 7% ON and 14% ON groups, fed with an AIN-76A diet containing 3%, 7% and 14% (wt/wt) onion powder, respectively. The onion powder (Chiseng®, Taipei, Taiwan), which was made from fresh onion without any additives, is commercially available. The treatment course for all groups was six weeks (Fig. 1).

The female Wistar rats ate, on average, 15–20 g/day of AIN-76A powder according to the evaluation in the week before ovariectomy surgery. Since three or four rats were housed per cage, we used ad libitum feeding. Thus, the diet for each group was freshly prepared everyday and was given at a mean amount of 25 g per rat.

Serum bone marker assay

After the treatments were completed, the animals were sacrificed under deep anesthesia with a high dose of sodium pentobarbital (intraperitoneally, 65 mg/kg) and bloodletting via carotid arteries. Blood samples were collected and centrifuged (4 °C) at 1500 × g for 20 min. Serum was separated immediately and was stored at −75 °C for future bone metabolic marker assay. Serum calcium, and phosphorus were determined with an automatic chemistry analyzer.
(Beckman Synchron LX20, Diamond Diagnostics, Holliston, MA, USA). ELISA kits, including RatTRAP™, Rat-MID™ and Rat/Mouse IGF-I (Nordic Bioscience Diagnostics, Herlev, Denmark), were used to analyze serum tartrate-resistant acid phosphatase (TRAP) activity, osteocalcin, and insulin-like growth factor-I (IGF-I), respectively. Assay procedures followed the instructions included in package of each kit. A microplate photometer (Multiskan Ascent, Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to measure the absorbance of each well after a series of sample preparations and reactions.

**Bone samples preparation**

The left tibiae were removed, fixed with a 4% neutral paraformaldehyde in PBS solution (pH 7.4) for 48 h and then decalcified with a 10% ethylenediamine tetraacetic acid (EDTA) solution (pH 7.4) at 4 °C for 4 weeks. After decalcification, each bone sample was cut along the coronal plane and embedded with paraffin for further tissue section and histological staining. For geometric and biomechanical measurement, the left femora were removed and the soft tissues were cleaned. Then, the bone tissues were wrapped in 0.9% sodium chloride soaked gauze and aluminum foil, and stored at −20 °C for future biomechanical testing.

**Bone histomorphometric analysis**

Histological staining, including hematoxylin and eosin (H & E) staining and tartrate-resistant acid phosphates (TRAP) staining, was performed on two nonconsecutive sections (5 μm) for each sample, which were the 1st and the 11th sections in sequence. H & E staining was performed according to our previous studies [26]. The histomorphometric study of the metaphysis of the proximal tibiae was performed as previously described with image analysis software (Image Pro Plus 6.1 for Windows; Media Cybernetics, Silver Spring, MD, USA) [27]. Parameters measured included the bone volume ratio (BV/TV, %); mean trabecular tissue area (N. Oc/T. A, 1/mm²) was measured as an index of osteoclasts. The TRAP-positive multinucleated osteoclast along the metaphysis of the proximal tibiae was performed as previously described with image analysis software (Image Pro Plus 6.1 for Windows; Media Cybernetics, Silver Spring, MD, USA). The cross-sectional moment of inertia (CMSI) from the photographs using the software Image Pro Plus 6.1 for Windows (Media Cybernetics, Silver Spring, MD, USA).

**Statistical analysis**

The data are presented as means±SEM. For the data of serum markers, histomorphometry and body weight gain, one-way analysis of variance (ANOVA) was used to evaluate the differences among groups. For geometric and biomechanical data, one-way analysis of covariance (ANCOVA) was used to evaluate the differences among groups, and body weight data was set as a covariate. When significant levels (p<0.05) were revealed, pair-wise comparisons between groups were made using the Fisher’s least significant difference (LSD) method. Power values regarding the size of groups were calculated using α=0.05. Statistical analysis software, SPSS (11.5 version, SPSS, Chicago, IL), was adopted for processing the data of the present study.

**Results**

**Body weight gain (one-way ANOVA)**

After surgery, body weight gain was typically and consistently higher in the ovariectomized rats in each group when compared with the sham-operated control rats (p<0.05) (Table 1). Except for the slightly lower body weight gain of the ALN and 14% ON group in the first week, the interventions we used, including alendronate and three onion-enriched diets, did not significantly affect body weight gain throughout the experimental period.

**Serum bone markers (one-way ANOVA)**

After the six-week treatment, the ALN and three onion-enriched groups similarly showed significantly lower serum calcium levels as compared to the CON and OVX groups (p<0.05) (Table 2). In osteocalcin assay, the ALN group showed

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Table 1

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>OVX</th>
<th>ALN</th>
<th>3% ON</th>
<th>7% ON</th>
<th>14% ON</th>
<th>Power</th>
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<tr>
<td>1st week</td>
<td>13.9±</td>
<td>35.0±</td>
<td>28.8±</td>
<td>32.1±</td>
<td>31.5±</td>
<td>28.0±</td>
<td>1.00</td>
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<td>1.3±</td>
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<td>2.3±</td>
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<td>1.5±</td>
<td>2.5±</td>
<td></td>
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<tr>
<td>2nd week</td>
<td>27.1±</td>
<td>50.7±</td>
<td>46.9±</td>
<td>53.4±</td>
<td>48.6±</td>
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<td></td>
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<td>3.6±</td>
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<td>3rd week</td>
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<td>60.5±</td>
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<td>36.8±</td>
<td>67.9±</td>
<td>63.8±</td>
<td>71.2±</td>
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<td>3.4±</td>
<td>4.5±</td>
<td>5.1±</td>
<td></td>
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<tr>
<td>5th week</td>
<td>36.1±</td>
<td>76.8±</td>
<td>73.2±</td>
<td>79.5±</td>
<td>67.5±</td>
<td>66.9±</td>
<td>1.00</td>
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<tr>
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<td>5.7±</td>
<td>3.2±</td>
<td>4.1±</td>
<td>5.4±</td>
<td>4.8±</td>
<td></td>
</tr>
<tr>
<td>6th week</td>
<td>44.9±</td>
<td>81.5±</td>
<td>76.3±</td>
<td>79.5±</td>
<td>68.2±</td>
<td>75.1±</td>
<td>0.984</td>
</tr>
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<td></td>
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<td>5.4±</td>
<td>5.0±</td>
<td>3.9±</td>
<td>5.9±</td>
<td>5.8±</td>
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</tr>
</tbody>
</table>

Data were presented as mean±SEM. Power, power values calculated using α=0.05; *Mean±SEM values within a group not sharing a common superscript are significantly different (p<0.05).
Table 2
Serum markers of bone metabolism

<table>
<thead>
<tr>
<th></th>
<th>CON</th>
<th>O VX</th>
<th>ALN</th>
<th>3% ON</th>
<th>7% ON</th>
<th>14% ON</th>
<th>Power</th>
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</thead>
<tbody>
<tr>
<td>Calcium (mg/dl)</td>
<td>9.68±</td>
<td>9.43±</td>
<td>8.96±</td>
<td>8.90±</td>
<td>8.83±</td>
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</tr>
<tr>
<td>Phosphorus (mg/dl)</td>
<td>0.07a</td>
<td>0.09a</td>
<td>0.11b</td>
<td>0.15b</td>
<td>0.10b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRAP (U/L)</td>
<td>1.84±</td>
<td>1.47±</td>
<td>1.55±</td>
<td>1.95±</td>
<td>1.68±</td>
<td>1.75±</td>
<td>0.314</td>
</tr>
<tr>
<td>Osteocalcin (ng/ml)</td>
<td>123.4±</td>
<td>166.7±</td>
<td>109.2±</td>
<td>199.3±</td>
<td>150.6±</td>
<td>129.1±</td>
<td>0.29</td>
</tr>
<tr>
<td>IGF-I (ng/ml)</td>
<td>931.9±</td>
<td>1020.2±</td>
<td>913.9±</td>
<td>1003.2±</td>
<td>976.2±</td>
<td>899.0±</td>
<td>0.221</td>
</tr>
</tbody>
</table>

Data were presented as mean±SEM. Power, power values calculated using $\text{IGF-I}$

the lowest value and was significantly lower than the OVX and 3% ON groups ($p<0.05$). The 14% ON groups also showed significantly lower serum osteocalcin level when compared to the 3% ON group ($p<0.05$). The TRAP and IGF-I assays in both ALN group and 14% ON group also showed a numeric lower level as compared with CON group or OVX group, but not reaching statistical significance.

**Histomorphometry study (one-way ANOVA)**

As shown in Figs. 2 and 3, the ovariectomy procedure caused significantly less bone volume ratio (BV/TV, %), less trabecular number (Tb. N., 1/mm), and increased trabecular separation (Tb. Sp., mm) in the OVX group as compared with the CON group ($p<0.05$). In addition, the CON rats had a significantly higher BV/TV than the rats in the ALN and three onion-enriched diet groups ($p<0.05$) (Figs. 2A and 3). Alendronate-treated rats showed a significantly higher BV/TV as compared to the rats in the OVX, 3% ON and 7% ON groups ($p<0.05$), but not the rats in the 14% ON group. The 14% ON group was significantly higher in BV/TV than the OVX and 3% ON groups ($p<0.05$). Linear regression analysis showed that the BV/TV data were significantly related to the ratio of onion-contained in the diet ($r=0.489, p<0.05$) (Fig. 2B).

Further histomorphometric analysis of architecture parameters showed that trabecular sections were 14–17% thicker in rats fed 7% and 14% onion-enriched diets than those in the ALN group, but not at a significant level (Fig. 2C). Though the 14% ON group was significantly less in trabecular number than the CON and ALN groups, the rats in those three groups had significantly higher trabecular number and less trabecular separation as compared to the OVX, 3% ON and 7% ON groups ($p<0.05$) (Fig. 2D and E). In osteocalcin number per trabecular area, the OVX group was significantly higher than the other five groups ($p<0.05$) (Fig. 2F).

**Femur weight, geometry, and biomechanical testing (one-way ANCOVA)**

In order to eliminate the effects of body weight on size-related parameters and extrinsic biomechanical properties, an analysis of covariance was used to adjust and analyze the mean values using body weight as a covariate [29]. The CON group was significantly higher in femoral wet weight than the OVX, 3% ON and 7% ON groups ($p<0.05$) (Table 3). The ALN and the 14% ON groups were numerically higher in wet weight than the three other ovariectomized groups, but did not show a significant difference. The CON group was also significantly higher in femoral fat-free dry weight (FFDW) than all ovariectomized groups ($p<0.05$). However, the ALN group showed a smaller decrement of FFDW and was higher than the OVX ($p=0.057$) and 3% ON groups ($p=0.007$). As compared to the CON group, the five ovariectomized groups had lower values for three cross-sectional geometric measurements, ranging from −2.0% to −13.6% lower (Table 3), but showed no statistical significance. Also, the ALN and 14% ON groups showed less decrease in CSA, cortical thickness and CSMI as compared with OVX group.

Regarding the femoral three-point bending test, the CON rats showed the highest maximal load value and were significantly different from rats in the OVX, 3% ON and 7% ON group (Fig. 4A). In fracture load value, the CON group was also the highest, but only significantly higher than the 3% ON group. Treatments with alendronate and 14% onion-enriched diets could prevent the deterioration of bending strength; the ALN and 14% ON groups were significant higher in values of maximal load and fracture load than the 3% ON group ($p<0.05$) (Fig. 4A and B). Paralleling loading values, the CON group showed the highest energy to maximal load and fracture load with significantly higher data than those of the OVX group ($p<0.05$) (Fig. 4C and D). Again, the alendronate and 14% onion-enriched diets respectively preserved the properties of bone bending energy. The ALN group showed significantly higher energy to maximal load than the OVX and 3% ON groups, and significantly higher energy to fracture load than the OVX group. On the other hand, the 14% ON group also had a higher bending energy than the OVX group in energy to maximal load ($p=0.032$) and fracture load ($p=0.059$), though a statistically significant difference was not revealed in the later measurement (Fig. 4D). In bending stiffness, the CON group showed significant higher than all ovariectomized groups ($p<0.05$) except for the 14% ON group. The rats treated with 14% ON showed a slightly higher stiffness and were significantly higher than the 3% ON group (Fig. 4E).

**Discussion**

In this study we showed that onion-enriched diets could counteract the ovariectomy-induced osteopenia in young adult rats in a dose-dependent manner. The efficacies of the highest dose of onion (14%) in preventing bone loss were similar to those of alendronate treatment in many measurements.

**Body weight gain**

Previous work showed that onion is effective in intact male rats and does not affect specific body weight [19,20]. A previous study using rutin, an extract from onion, as a treatment showed no effect on ovariectomy-induced weight gain [31]. Therefore, there is no reason to assume that onion displays an estrogen-like
Fig. 2. Histomorphometric analysis of proximal tibiae among different groups. A. bone volume ratio (BV/TV, %); B. linear regression between BV/TV and dietary onion consumption ratio; C. mean thickness of the trabeculae (Tb.Th, μm); D. trabecular number (Tb. N., 1/mm); E. trabecular separation (Tb. Sp., mm); F. number of osteoclasts per unit tissue area (N. Oc/T. A, 1/mm²). Ovariectomy caused much decrease of BV/TV (%), and trabecular number, increased trabecular separation and number of osteoclasts. However, the ALN and 14% ON groups could counteract the ovariectomy-induced changes. Power, power values calculated using α=0.05; "a"Mean±SEM values within a group not sharing a common superscript are significantly different (p<0.05).
effect. In order to minimize the interference from body weight-induced mechanical loading on bone, alendronate was chosen as positive control treatment due to its nominal effect on ovariectomy-induced body weight gain [32]. During the six-week treatment period, all five ovariectomized groups showed similar trends of body weight gain. Neither the alendronate, nor the three different onion-enriched diets showed any obvious modification in the increment of body weight gain induced by ovariectomy and, thus, reduced the interference from body weight on bone metabolism among different treated groups.

**Serum bone markers**

Except for serum TRACP 5b activity, the ALN group showed lower levels in serum mineral level and markers of bone formation. It has been previously verified that alendronate prevents bone loss through down-regulating bone turnover [33–35]. Since the high turnover may lead to loss of bone, such a down-regulation of bone turnover may be beneficial to the bone metabolism. The 14% ON group’s equivalent results suggested that a high onion-enriched diet could counteract bone loss through a similar pathway. In contrast with previous studies investigating the effects of dried plums [21,36], our serum markers seemed not suggest enhanced bone formation activity from onion-enriched diets. This might be due to the different status of subjects. Since Arjmandi et al. and Deyhim et al. treated their subjects after a period of estrogen deficiency [21,36], their studies demonstrated a major effect of dried plums in bone restoration. However, our subjects were treated immediately after ovariectomy. Moreover, onion and dried plums might act on bone metabolism through different pathways, which need to be further clarified.

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**Table 3**

<table>
<thead>
<tr>
<th>Tissue weight, geometry measurements</th>
<th>CON</th>
<th>O VX</th>
<th>ALN</th>
<th>3% ON</th>
<th>7% ON</th>
<th>14% ON</th>
<th>Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet weight, mg</td>
<td>1165.2±40.2(^a)</td>
<td>1047.5±33.5(^b)</td>
<td>1109.0±32.6(^b)</td>
<td>1020.1±36.3(^b)</td>
<td>1045.8±35.6(^b)</td>
<td>1079.1±33.8(^b)</td>
<td>0.556</td>
</tr>
<tr>
<td>FFDW, mg</td>
<td>704.5±19.2(^a)</td>
<td>594.3±16.0(^bc)</td>
<td>638.6±15.6(^b)</td>
<td>571.9±17.3(^b)</td>
<td>593.5±17.0(^bc)</td>
<td>603.7±16.1(^bc)</td>
<td>0.992</td>
</tr>
<tr>
<td>CSA, mm(^2)</td>
<td>6.40±0.28</td>
<td>5.81±0.23</td>
<td>6.09±0.22</td>
<td>5.77±0.25</td>
<td>5.86±0.25</td>
<td>6.20±0.23</td>
<td>0.290</td>
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<tr>
<td>Cortical bone thickness of the CS, mm</td>
<td>0.70±0.02</td>
<td>0.66±0.02</td>
<td>0.68±0.02</td>
<td>0.66±0.02</td>
<td>0.65±0.02</td>
<td>0.69±0.02</td>
<td>0.344</td>
</tr>
<tr>
<td>CSMI mm(^4)</td>
<td>5.55±0.44</td>
<td>4.83±0.37</td>
<td>5.21±0.36</td>
<td>4.80±0.40</td>
<td>5.12±0.39</td>
<td>5.28±0.37</td>
<td>0.156</td>
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</table>

Data were presented as means±SEM. FFDW, fat-free dry weight; CSA, cross-sectional area of cortical bone in fracture site; CSMI, cross-sectional moment of inertia. Data in parentheses were percentage differences relative to the CON group. Power, power values calculated using \( \alpha = 0.05\); \(^*\)Mean±SEM values within a group not sharing a common superscript are significantly different \((p<0.05)\).
two weeks after ovariectomy or orchidectomy [37]. Since TRACP 5b is released from osteoclasts, the serum activity of TRACP 5b could be partially affected by the absolute number of osteoclasts. Therefore, it was speculated that the serum TRACP 5b activity turn back to sham level due to the decrease in trabecular area and absolute osteoclast number as well [37]. This might be able to explain the inconsistency between the serum TRACP activity and histological osteoclast number per trabeculae area (N. Oc/T. A, Fig. 2F).

**Histomorphometry analysis**

Previous studies showed that onion at a dose of 1 g/rat/day could inhibit bone resorption shown as about a 20% decrease of

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Fig. 4. Evaluations of the midshaft femur biomechanical properties by using a three-point bending test. A. loading force to maximal load; B. loading force to tissue fracture; C. energy to maximal load; D. energy to tissue fracture; E. bending stiffness of tissue. Ovariectomy significantly decreased the bending load, bending energy, and stiffness as compared with controls. However, the ALN and 14% ON groups showed effects to counteract the deteriorations of these parameters. Power, power values calculated using $\alpha=0.05$; *Mean±SEM values within a group not sharing a common superscript are significantly different ($p<0.05$).
Higher dose of onion (1.5 g/rat/day) could blunt \[H^3\]-tetracycline urinary excretion [18–20]. Furthermore, a higher dose of onion (1.5 g/rat/day) could blunt \[H^3\]-tetracycline urinary excretion up to 26% in ovariectomized rats [22]. In the present study, we further investigated the effects of onion on ovariectomy-induced loss of trabecular bone tissue. As a positive control treatment, alendronate significantly prevented trabecular bone loss in bone volume ratio (BV/TV) and kept better architecture as compared to the OVX group (Figs. 2 and 3). However, the dose of alendronate (1 mg/kg/day) used in the present study could not fully counteract the effects of ovariectomy when compared to control rats (Fig. 2).

On the other hand, the rats treated with onion-enriched diets also showed a good preservation of BV/TV. A positive linear relationship between BV/TV and onion-enrichment ratio suggested the impact of onion is dose-dependent (Fig. 2A and B). The highest onion-treated (14%) group showed similar effects to the alendronate-treated group. Only the highest onion dose prevented a decrease in the number of trabeculae (Fig. 2D). Interestingly, the 7% ON and 14% ON groups both showed numerically higher average trabecular thickness (Fig. 2C). Since the trabeculae near the endo-cortical surface were thicker on average than those located in the center of the medullar canal, the preservation of more trabeculae in the central medullary canal in the ALN rats made their thickness of trabeculae (Tb.Th) on average slightly lower (Fig. 2C). A decreased number of TRAP-positive osteoclasts in onion-treated groups and the ALN group indicated that onion-enriched diets could act similarly to alendronate through a pathway of bone resorption inhibition (Fig. 2F). This decreased number of osteoclasts confirmed the onion’s anti-resorptive effects on bone, which has previously been suggested [18–20]. The 3% ON group failed to show preservation of BV/TV, which might be because the lower dose of onion treatment was only able to inhibit osteoclastic activity during the later phase of estrogen deficiency, when the bone resorption rate was slower.

Tissue weight, geometry and strength

Typically, ovariectomy induced a body weight gain in the present study. According to Frost’s mechanostat theory [38], this increment of body weight causes a higher loading on bone, thus preserving bone strength to a certain extent. In addition, it has been suggested that the ovariectomy-induced higher body weight might partially compensate for the deterioration of bone quality [39]. Several studies failed to reveal the ovariectomy-induced loss of bone strength in the midshaft of the femur, which might be due to the interference of this increased body weight [21,40,41]. Therefore, we compared the cross-sectional parameters and long bone three-point bending data among groups using a one-way analysis of covariance (ANCOVA) with body weight data as a covariate. Again, alendronate, as well as the 14% onion-enriched diet, better maintained bone quality in biomaterial properties (Fig. 4A–D), and the 14% ON group even showed a slightly higher stiffness than other ovariectomized groups (Fig. 4E).

On the other hand, the effect of ovariectomy on tissue weight was relatively modest, and moreover, only numeric differences were shown between the CON group and the OVX group in cross-sectional cortex measurements (Table 3). Nevertheless, the ALN and 14% ON groups still showed tendencies toward minor decreases in tissue weight or cross-sectional cortex measurements. Cross-sectional parameters, such as cortical thickness, cortical area or moment of inertia, would contribute to long bone mechanical properties [30]. However, the significant differences shown in three-point bending test were not revealed in cross-sectional measurements. This inconsistent data might be explained by the following: (1) Geometric parameters in midshaft femur were less affected by ovariectomy, which might be due to the lack of a Haversian system and vessels in cortical bone of young adult rats [42–44]; (2) According to the formula of three-point bending, a certain amount of change in CSMI and cross-sectional diameter would cause a fourfold change in loading values of three-point bending when the tested sample was an ideal cylinder with an equilibrium material property [30]. Therefore, a slight change in cross-sectional parameters would cause significant variation in loading values; (3) Other than geometric factors, it has been suggested that the strength of bone tissue could also be related to compositional measures, such as tissue density, collagen content or ash fraction, as well as the microstructure parameters, including collagen cross-linking or fiber orientation [45,46]. Further studies could be useful in determining the preventive effects of an onion-enriched diet on composition and microstructure of osteoporotic bone.

Possible mechanisms

Limited evidence was available to explain the actions of onions and other vegetables and fruits. One possible mechanism of bone restoration by those natural foods might be related to their base excess [10,14], which is believed to retain calcium in bones. However, Mühlbauer et al. showed that onion could enhance bone mineral through a pharmacological inhibition on bone resorption rather than its base excess [19]. The pharmacological inhibition effects might be from various extracts of onion, including rutin, and several phytoestrogens (coumestrol, isoflavones). These extracts have also been proven to have individual benefits for the bone of ovariectomy animals [31,47–49]. Recently, another extract of onion, gamma glutamyl peptide, has been proven to inhibit osteoclastic activity in vitro, and further animal study is awaited [50]. Aside from the effects of a specific extract, the synergistic effects of extracts from natural foods could be also important. As noted by Mühlbauer [51], the working dose of rutin used by Horcajada-Molteni et al. [31] is six times higher than its content in 1 g of onion and 700–800 times higher than its content in 1 g of leeks or wild garlic, which implies that those extracts might be more effective when taken together, rather than separately.

Limitations and applicability in human diet habits

Animals in this study were kept in a controlled environment and fed with a standard semi-purified diet, which is far from the practice of real human life. Natural inhibitors contained in our daily diets and various lifestyle factors (ex. alcohol consumption, smoking etc.) would counteract the benefits from onion and vegetables as well.
Though we verified onion as another whole food that may prevent bone loss in animal osteoporotic model, it would not be applicable to suggest that people consume large amounts of a specific fruit or vegetable as a strategy to prevent bone loss. Notwithstanding those limitations mentioned above, there are still ways and clues that encourage us to apply a potentially anti-resorptive diet in our lives. Indirect evidence from human studies has suggested that eating more fruits and vegetables would benefit bone health. [9–17]. Besides, plenty of vegetables and herbs commonly consumed by humans have been mentioned about their similarly inhibitive effects on bone resorption in animal studies [18–20,22]. Therefore, inclusion of an appropriate amount of these vegetables and herbs would keep variety in the daily diet and could be an effective and more practical way to decrease the incidence of osteoporosis in humans.

Conclusion

In summary, our study showed that high onion-enriched diets are able to decrease ovariectomy-induced osteopenia and deterioration of biomechanical strength. Further investigations would be valuable in investigating the cellular mechanisms of onion on bone homeostasis. Although it remains unclear how those natural foods benefit bone health, increasing the intake of fruits and vegetables might offer a potentially alternative way to improve bone health.

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