Improvement of Bone Strength and Dermal Thickness Due to Dietary Edible Bird’s Nest Extract in Ovariectomized Rats

Noriko MATSUOKAw1, Megumi MATSUMOTOW2,†, Wakoto BUKAWAW,†, Hideyuki CHII4, Keizo NAKAYAMA,1 Hiroshi HARAW,2 and Takamitsu TSUKAHARAW1,†

1Kyoto Institute of Nutrition and Pathology, Inc., 7-2 Furuiketani Tachikawa Ujitawara, Kyoto 610-0231, Japan
2Laboratory of Nutritional Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kitaku, Sapporo, Hokkaido 060-8589, Japan
3CombiCorporation, 5-2-39 Nishihori, Sakura-ku, Saitama, Saitama 338-0832, Japan
4Department of Food Science and Human Nutrition, Faculty of Human Life Science, Fuji Women’s University, Ishikari, Hokkaido 061-3204, Japan

Received October 4, 2010; Accepted December 6, 2010; Online Publication, March 7, 2011

[doi:10.1271/bbb.100705]

Note

Improvement of Bone Strength and Dermal Thickness Due to Dietary Edible Bird’s Nest Extract in Ovariectomized Rats

Keizo NAKAYAMA,1 Noriko M ATSUOKAWA,1 Hiroshi HARAW,2 and Takamitsu TSUKAHARAW1,†

1Kyoto Institute of Nutrition and Pathology, Inc., 7-2 Furuiketani Tachikawa Ujitawara, Kyoto 610-0231, Japan
2Laboratory of Nutritional Biochemistry, Division of Applied Bioscience, Graduate School of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kitaku, Sapporo, Hokkaido 060-8589, Japan

Oral administration of edible bird’s nest extract (EBNE) improved bone strength and calcium concentration in the femur of ovariectomized rats. Dermal thickness was also increased by EBNE supplementation, whereas EBNE administration did not affect the serum estradiol concentration. These results suggest that EBNE is effective for the improvement of bone loss and skin aging in postmenopause all women.

Key words: edible bird’s nest extract; bone strength; ovariectomized rat

It is well known that ovarian hormone deficiency induces osteoporosis. A decrease in ovarian estrogen production is the predominant cause of rapid bone loss during the first decade after menopause.1,2) Osteoporosis is a serious public health problem worldwide, and is a major contributor to the high frequency of bone fracture in elderly women. The most effective approach to preventing postmenopausal osteoporosis is hormone replacement therapy such as estradiol,3) but this therapy increases the risk of breast and uterine cancers.4,5)

Edible bird’s nest (EBN) is a famous Chinese traditional food that is made from the nest of Collocalia swiftlets. EBN is made from regurgitated saliva of male swiftlets, and therefore contains mucinous glycoproteins such as condroitin glycosaminoglycans (GAG), and sialylglycoconjugates.6,7) Studies have been carried out on the tonic effects of EBN.8,9) EBN administration stimulates mitosis hormones and the epidermal growth factor in the repair of cells and stimulation of the immune system. On the other hand, EBN extract (EBNE) was developed recently, and is obtained by Pancreatin F degradation of EBN.10) EBNE consists of water-soluble compounds including condroitin GAG. Nakagawa et al. have reported that EBN is rich in proteoglycans containing nonsulfated chondroitin GAG.7) Condroitin GAG is one of the main components of the bone, and the aim of the present study as to evaluate the preventive effect of dietary EBNE against osteoporosis using an ovariectomized rat model. We further analyzed the dermal thickness of the ovariectomized rats, because the condroitin GAG is an important component of the dermis.11)

Female Sprague-Dawley rats (5 weeks old, Clea Japan, Tokyo) were housed in individual stainless steel cages with wire-mesh bottoms. The cages were placed in a room under controlled temperature (23–25°C), relative humidity (40–60%), and lighting (lights on 8:00–20:00). The rats had free access to water and a semi-purified diet based on the AIN93G formulation for an acclimation period of 7. They were handled in accordance with the guidelines for animal studies of the Kyoto Institute of Nutrition and Pathology. The acclimated rats were divided into four groups. Three groups underwent bilateral ovariectomy (OVX) and the other underwent bilateral laparotomy (sham). One group of OVX rats were fed an AIN93G-based normal diet (C group, n = 12), and the rats in the other groups were fed an AIN93G-based diet supplemented with EBNE (10 or 100 mg/kg, 2 mg or 20 mg/kg in conversion of EBN; EL and EH groups at n = 8 and n = 10 respectively) for 10 weeks. The sham group was fed an AIN93G-based normal diet (S group, n = 9). The food intake of the OVX rats in each group was adjusted to the average intake of the sham group each day (pair feeding). All the rats were given free access to water. Body weight was measured at every week. On the last day, the rats were anesthetized (sodium pentobarbital of 50 mg/kg body weight; Somnopentyl, Kyoritsu, Tokyo), and were sacrificed after collection of aortic blood. Blood was centrifuged (1,300 g for 10 min) to obtain the serum. Both femurs were removed, and carefully cleaned of adherent tissue, and the left femurs were used to measure the bone strength. The right femurs were freeze-dried to measure mineral and hydroxyproline concentrations. A portion of dorsal skin 3 × 3 cm in size was also collected from each rat and soaked in 10% formalin for histological evaluation. The uterus was removed and weighed to confirm the success of the ovariectomy in each rat.

1 To whom correspondence should be addressed. Tel: +81-774-99-7331; Fax: +81-774-99-7332; E-mail: tsukahara@kyoto-inp.cc
2 Present address: Meiji Dairies Research Chair, Creative Research Initiative Sousei (CRIS), Hokkaido University, Kita-21, Nishi-10, Kita-ku, Sapporo, Hokkaido 001-0021, Japan
Concentrations of Sham and Ovariectomized Rats Fed the Control or the EBNE Diet for 10 Weeks

Table 1. Final Body Weight, Femoral Weight (mg), Calcium (mmol/g, femur), Phosphorus (mmol/g, femur), and Hydroxyproline (nmol/g, femur) Concentrations of Sham and Ovariectomized Rats Fed the Control or the EBNE Diet for 10 Weeks

<table>
<thead>
<tr>
<th>Final body weight</th>
<th>Dry weight</th>
<th>Calcium</th>
<th>Phosphorus</th>
<th>Hydroxyproline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>321 ± 14.6</td>
<td>0.512 ± 0.013</td>
<td>8.10 ± 0.364&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.73 ± 0.863&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>354 ± 6.2</td>
<td>0.501 ± 0.015</td>
<td>6.77 ± 0.301&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.11 ± 0.692&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low-EBNE</td>
<td>370 ± 4.6</td>
<td>0.528 ± 0.011</td>
<td>7.59 ± 0.405&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>7.90 ± 0.459&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>High-EBNE</td>
<td>363 ± 3.4</td>
<td>0.533 ± 0.010</td>
<td>8.32 ± 0.568&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.32 ± 0.605&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values represent means ± SEM; n = 9 (Sham), 12 (control), 8 (low EBNE), 10 (high EBNE). Means not sharing a common letter differ significantly, p < 0.05.

The amounts of calcium, phosphorus, and hydroxyproline in the right femurs were measured after hydrolysis at 110°C for 24 h with 6 mol/L, HCl. The calcium concentration in the solution was measured by atomic absorption spectrophotometry after appropriate dilution with 0.1 mol/L, HCl. The phosphorus concentration was determined by the molybdovanadate method. The hydroxyproline concentration was determined by a colorimetric method, in which the hydrolyzed solution was oxidized with chloramine-T and applied to colorimetry by Ehrlich reaction at 558 nm. The maximum breaking force of the left femoral diaphysis (the center of the femur) was measured as the bone strength. A three-point bending test was performed with a rheometer (RE-3305 Rheonert; Yamaden, Tokyo) under the following conditions: 1.0 cm of sample space; 30 mm/min of prager speed; 20 kg of load range. Skin samples collected from the rats were immersed into paraffin wax and sliced 4 μm thick. A slice section was stained with Masson’s trichrome staining to evaluate a thickness of the collagen fibrils (the dermis). Thickness of the collagen fibrils (the dermis) was measured by light microscopy (BX51, Olympus, Tokyo) equipped with a digital camera (DP25, Olympus) and analysis software (DP2-BSW, Olympus). For each section, a cross section of collagen fibrils was evenly divided to 10 segments, and the center point of each segment was measured as the thickness. The calcium and phosphorus concentrations in the serum were analyzed by Japan Clinical Laboratories (Kyoto) by standard analytical methods. The estradiol concentration in the serum was analyzed by BML (Tokyo) by ELISA techniques.

Either a complete randomized design 1-way ANOVA or the Kruskal-Wallace test, depending on the results of the Bartlett test, was used to analyze the differences in a given parameter among the groups. Scheffe’s F test (parametric and non-parametric) was used for multiple comparisons when needed. Differences among the treatment groups were considered significant at p < 0.05. All data were analyzed by StatLight 2000 (Yukms, Tokyo).

Food intake and body weight gain through the experiment were not affected among the groups (data not shown). The uterine weights of all the O VX rats were significantly lower than those of the sham rats (0.03–0.04 vs. 0.27 g/100 g, B.W.). The maximum breaking force of the femur was higher in the EH group than in the C group (Fig. 1A). The calcium and phosphorus concentrations in the femur were also higher in the EH group than in the C group (Table 1). Although the mean hydroxyproline concentration was highest in the EH group, followed by the S group, the EL group, and the C group, this was not statistically significant among the groups. EBN contains condroitin GAG, but the value of condroitin GAG in EBNE was only 0.6% as analyzed by Japan Food Research Laboratories (Tokyo). Also, in our previous study, oral administration of EBN (23.18 mg/kg) to ovariectomized rats in the same experimental design did not affect bone strength. Condroitin GAG in the EBNE used in the present study was solubilized by an extraction procedure including protease hydrolysis, and the beneficial effects of EBNE on bone might depend on solubilized condroitin GAG. However, some other products of EBN hydrolysis are effective components, such as glycoprotein including...
sialylglycoconjugates,

since EBNE contains this at approximately 10%. Some other components such as sialylglycoconjugates may be effective for improvement of bone strength in ovariectomized rats. The phosphorus concentrations in the femur and serum were higher in the EH group than in the C group in this study (Tables 1 and 2). Phosphorous is associated with improvement of bone strength in ovariectomized rats, but we do not have any explanation for this association. Further study is needed to elucidate the association between phosphorus concentration and bone strength.

Many investigators have suggested that functional foods, such as soy bean, dry plum, and olive, prevent osteoporosis. Isoflavones, a major component of foods, such as soy bean, dry plum, and olive, prevent osteoporosis. However, hyperdoses of isoflavones have been suggested to induce breast cancer. It has been reported that circulating estrogen levels are positively associated with breast cancer risk. In the present study, EBNE ingestion did not affect the serum estradiol concentration (Table 2). This anti-osteoporosis product may not increase breast cancer risk, at least under dietary 100 mg/kg administration.

The mean thickness of collagen fibrils was dose-dependently increased by EBNE supplementation (Fig. 1B). The values for the EH group were significantly higher than for the C group. It is noteworthy that oral administration of EBNE increased dermal thickness in ovariectomized rats, because thinning of the dermal layer is associated with skin aging in human subjects.

In conclusion, oral administration of EBNE increased bone strength and dermal thickness in ovariectomized rats. We did not find any anti-osteoporosis mechanism of EBNE in this study, and this needs further elucidation.

ACKNOWLEDGMENT

We thank Mr. H. Nishikawa and Ms. Y. Nakamoto of the Kyoto Institute of Nutrition and Pathology for technical assistance.

REFERENCES