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Role of Soy Protein on Bone Turnover

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Abstract
Bone mass loss is one of the commonest menopause symptoms, resulting from cessation of estrogen production. Compounds which have estrogen – like biological activity similar to "Isoflavones" present in plants especially soy, may reduce bone loss in postmenopausal women, because as they are similar in structure to estrogens. This study, therefore, was undertaken to assess the effect of soy protein on bone metabolism biomarkers in postmenopausal women with osteopenia. This "before and after" clinical trial was carried out, on 15 postmenopausal women with osteopenia, between 45 to 64 years of age. The subjects were asked to consume 35 gram/day of soy protein for 12 weeks. Blood and urine samples, were taken at 0, 6 and 12 weeks of the study. Anthropometric measurements and a 2-day dietary recall were done at the beginning of the study, and at the 6 and 12 weeks. The food consumption data were analyzed by "Food Proccessor" software. Repeated measurement analysis was utilized to determine the changes in biochemical indices, anthropometric and dietary data. P-values less than 0.05 were considered as significant. Comparison of weight, BMI, physical activity and dietary intake of subjects during the study did not show any significant differences. Soy protein consumption, showed significant reductions in deoxypyridinoline (biochemical marker of bone resorption) and significant increase in total alkaline phosphatase (biochemical marker of bone formation).There were no significant differences in serum osteocalcin, C- telopeptide, insulin- like growth factor binding protein 3 (IGFBP3), and type-I- collagen telopeptides. Considering the beneficial effects of soy protein consumption on bone metabolism biomarkers, inclusion of this inexpensive and available food item in postmenopausal women diet, may reduce bone loss and could be recommended for the prevention of osteoporosis.

Keywords: *Soy protein, Isoflavones, Postmenopaus, Bone turnover*

Introduction
Osteoporosis is a serious problem for postmenopausal women which increases the risk of bone fracture and worsens with age, increasing from 4% in 50-to 59- y-old bracket to 50% in 80 y-old women. Bone fractures are also prevalent in these women (1). Today estrogen therapy,especially bisphosphonates, calcitonine and raloxifene is employed to prevent and treat osteoporosis (2). However, side effects such as breast cancer and endometrial adenocarcinoma (3) have limited the acceptance of these medications among women (4) and only 3-8% of menopausal women give consent to this treatment (5).
Epidemiologic studies have shown osteoporotic fractures, cardiovascular disease, postmenopausal symptoms and some cancers to be less prevalent in Asians comparing their western counterparts. Hip fracture, for example, is 50-60% less frequent among Asian compared to western women (6). This advantage is gradually anihilated as Asian adapt western lifestyle (7). These observa-

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tions, prompted researchers to scrutinize Asian dietary habits. Soy is a part of Asian traditional diet, showing some relationship with the above mentioned diseases (8).

Isoflavones are phytoestrogens similar to women's estrogens which are bound to cellular estrogen receptors in various organs, thus phytoestrogens affinity is weak compared to human's estrogens. Recent studies have shown that cells have two types of estrogen receptors α and β. Human estrogens have more affinity to α-receptors, wherease, isoflavones have high affinity to β-receptors. β-receptors exist in brain, bone, bladder and vascular epithelium, being important in the function of non-steroid estrogens (9).

Estrogen-like compounds such as isoflavones existing in plant foods specially soy, (10) can curb reduced bone density in menopausal women, due to their structural similarity (11). Some studies have not, however, supported clearly the role of soy isoflavones in preventing osteoporosis (12).

The aim of this study was assessing relation between soy protein intake as a major source of isoflavones and bone turnover in postmenopausal women.

Materials and Methods

This clinical trial of before and after type, was carried out in 2003. Women referring to the osteoporosis clinic of Endocrine Research Centre at Tehran Medical University for bone density measurement were screened and 15 postmenopausal 45 to 64 years old women were selected. Those women from 1 to 10 y postmenopause who were non-smokers and free from, diseases entered this study.

Information on weight, height, body mass index, two 24-h food recalls and physical activity were collected at the 0, 6 and 12 weeks of the study. Thirty five gr soy proteins were given to subjects daily. Subjects were provided with a special cup for measuring soy. Cooking instructions were also given to the subjects.

Blood and urine samplings were done in 3 stages, at the beginning and at the end of 6^{th} and 12^{th} week. Blood and urine samples were kept frozen until the end of the twelfth week at-80°C. Serum biochemical indicators were measured on the same day for all samples. Total alkaline phosphatase was assayed calorimetrically by Hitachi 902 autoanalyzer osteocalcin by IRMA method using Biosource kit and Wizard gamacounter, IGFBP3 and c-telopeptide by ELISA using Biosources and Bioscience diagnostics respectively.Type I collagen telopeptide was determined by RIA method using Orion- Diagnostica on Wizard gama counter and urinary creatinine by calorimetric method (13). Food Processor software was used for food consumption survey and SPSS (version 11.5) for statistical analysis of data. All quantitative variables were then examined by Kolmogrof-Smirnof (KS) to ensure normality of distribution.To analyze any possible changes in food intake, intervening and biochemical variables in 3 stages, repeated measurement analysis was utilized. The purpose of this analysis was to ensure lack of significant changes of the variables. Significant level was set at below 5 percent (P<0.05).

Results

Mean of subjects mean age was 52.9 ± 4.3 years, duration of menopause 5.47 ± 3.4 years and height 157.4 ± 7.2 centimeters. Mean of weight, body mass index and physical activity level remained unchanged. Mean of food consumption patterns were not different at 12 weeks compared to the beginning of the study (Table 1). After 12^{th} week of soy consumption, urinary deoxypyridinoline (DPD) decreased and seral total alkaline phosphatase (TALP) significantly increased (P<0.05). Changes of these

two markers are shown in Figs. 1 and 2. Osteocalcin as a formation marker did not change significantly (F3). Other indicators namely insulin growth factor binding protein (IGFBP3), c-telopeptide and type-I collagen telopeptides have no significant change.

Table 1: Mean of food intake at 0, 6 and 12 weeks of the study (n=15)

Variables	Energy (kcal)	Protein (g)	Calcium (mg)	Phosphore(mg)
at the start	1933.4 ± 302.5*	74.6 ± 12.3	999.3 ± 460.2	873.2 ± 228.9
6 weeks	2033.2 ± 420.3	71.9 ± 17.4	966.8 ± 443.7	853.1 ± 273.9
12 weeks	1902.8 ± 308.6	74.8 ± 10.2	1014 ± 436	866.4 ± 200.6
P value**	**NS****	**NS**	**NS**	**NS**

* Mean ± SD

** significant level was set at below 5 percent ($P<0.05$).

*** To analyze changes in 3 stages, repeated measurement analysis was utilized.

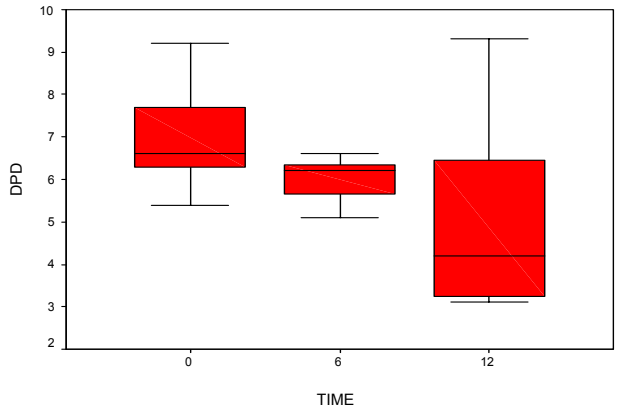

Fig. 1: Changes of DPD in 3 stages of study in subjects (n=15).

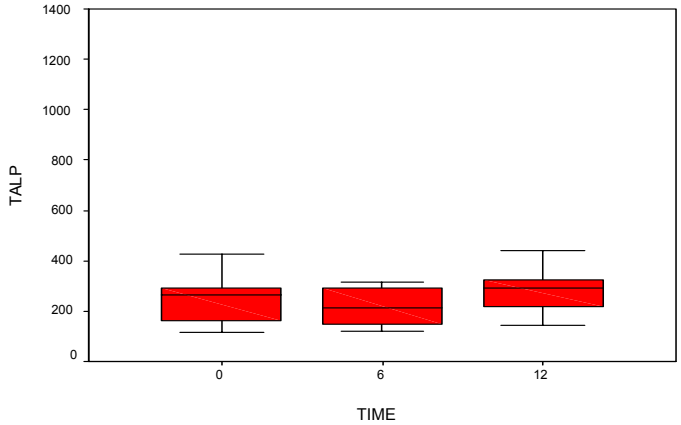

Fig. 2: Changes of TALP in 3 stages of study in subjects (n=15).

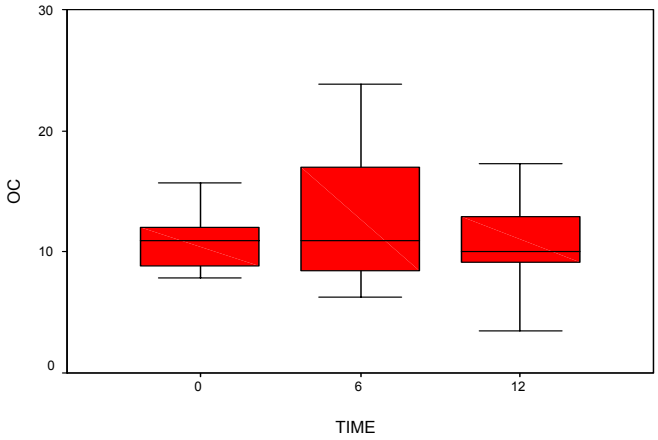

Fig. 3: Changes of Oc in 3 stages of study in subjects (n=15).

Discussion

Some studies have shown the effects of isoflavones on bone turnover. In these researchs, bone resorption markers such as DPD (14-16) and formation markers like Oc and TALP (14-17) have been assessed. In most studies which DPD changes were determined, finding have shown reduction in this marker (18-20) and our results are similar to finding of other investigators. The effect of soy isoflavones on DPD is so strong that Uesagi et al (23) observed consuming 61.8 mg of isoflavone for 4 weeks resulted in a significant reduction in urinary DPD. It can be said that DPD acts as a bridge between collagen fibrils which enter urine with collagen breakdown. Because of specificity of DPD as a bone resorption marker, its significant reduction in our study suggests soy intake may prevent degradation of collagen, the major protein in bone matrix (13).

TALP is a bone formation marker which soy protein consumption in our study caused increase in its concentration among postmenopausal women. Arjmandi et al observed a slight but insignificant increase in TALP on rats (14-16) while Register et al showed a significant fall in TALP in monkeys after 12 weeks (17). Cause of this reduction is less efficacy of isoflavone in monkeys. This animal can convert only 30 to 50 % of daidzein to equol and accordig to some investigations equol showes more estrgen- like properties than daidzein (21, 22).

Other seral indicators of bone turnover showed no significant changes in our study. The changes observed in this study are not contradictory to other studies and slight differences observed may be attributed to sample size, isoflavone dosing, and period of intervention and dissimilarity of the subjects. Different studies have reported intake of 70-90 milligrams of isoflavones per day to be effective. In our study, the intake was 35gr of soy protein, containing 98 milligram of isoflavones which is in accordance with other studies (24, 25). Some studies have also shown, less amounts of isoflavones can be effective in longer period of time (26).

Results of this study indicated that soy protein have a role in prevention of bone loss especially in high risk groups like postmenopausal women.

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