

Does three months genistein and daidzein in the form of soy protein supplementation have any effects on bone formation markers after menopause?

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ABSTRACT

Postmenopausal osteoporosis is caused by a sharp decrease in estrogen levels leading to an increased rate of bone remodeling. Dietary supplements are preferred as alternative therapeutic options for many women instead of estrogen therapy. These alternative therapies include the use of natural substances such as soy isoflavones due to their weak estrogenic activity and affinity for estrogen receptors.

Present study was carried out as a "before and after clinical trial" on 25 postmenopausal women aged 45-64 years. Soy protein at 35g level containing 98.3mg isoflavones (containing 47.2 genistein and 37.8 daidzein) were given to subjects daily for 12 weeks. Blood and urine samplings were done in 3 stages, in the beginning and at the end of 6th and 12th week. Repeated measurement analysis was employed to analyze any possible changes in food intake and biochemical variables in 3 stages. The level of significance was set at below 5 percent ($P < 0.05$). Mean body mass index and physical activity level had no change and mean daily intake of macronutrients and important micronutrients were not different at 6 and 12 weeks compared to the start of the study. The results showed a total serum alkaline phosphatase (TALP) significant increase while the other bone formation indicators namely osteocalcin and insulin growth factor binding protein (IGFBP3) did not change significantly. These data suggest that soy protein or its isoflavones may increase bone formation by supplementation.

Keywords: Human, soy; isoflavones; genistein; daidzein; bone; formation markers; menopause

INTRODUCTION

Postmenopausal osteoporosis is caused by a sharp decrease in estrogen levels that leads to an increased rate of bone remodeling [1]. Currently estrogen therapy, especially bisphosphonates, calcitonine and raloxifene is used to treat postmenopausal osteoporosis [2]. Although hormone replacement therapy is effective in reducing postmenopausal bone loss [3, 4], it is associated with a higher risk for breast, endometrial, and ovarian cancer; cardiovascular disease; venous thromboembolism and stroke [4, 5]. Dietary supplements are preferred as alternative therapeutic options for many women [6]. These alternative therapies include the use of natural or plant-based substances such as soy isoflavones [7-10]. Phytoestrogens, including isoflavones, are being used as an alternative therapeutic agent to ERT because of their weak estrogenic activity and affinity for estrogen

receptors, specifically estrogen receptor- α (ER- α) and estrogen receptor- β (ER- β), which are present in bone, brain, bladder, and vascular epithelia [11, 12]. Isoflavones stimulate osteogenesis at low concentrations and inhibit osteogenesis at high concentrations in osteoblasts and osteoprogenitor cells [13, 14]. Isoflavones also influence the production of cytokines derived from osteoblasts and osteoprogenitor cells in a similar biphasic manner [15]. By contrast, bone resorption data show that, instead of having biphasic effects, isoflavones only inhibit osteoclast formation and activity [16]. Phytoestrogens can act by binding or phosphorylation, resulting in the commitment of osteoprogenitor cells to differentiate into either osteoblasts or adipocytes [13]. Evidence from epidemiological studies of elderly women in Asian nations suggests that high consumption of isoflavones from soy products may be protective against fractures in comparison with Western

populations [17]. Because of the differences in experimental design, results from human studies are variable and conflicting. It seems finding the critical doses of phytoestrogens that exert beneficial effects on bone is very important to preventive interventions. Thus, the aim of this study was to evaluate the formative effects of isoflavone supplementation on postmenopausal females' bone in I.R. of Iran.

MATERIALS AND METHODS

Participants

This study recruited 25 postmenopausal women aged 45-64 years, who had experienced at least 1-10 years of menopause according to their own statements. They were recruited between January 2004 and March 2004 from osteoporosis clinic of Endocrine Research Centre at Tehran Medical University (Shariati Hospital) for bone density measurement.

Study design

Present study was carried out as a "before and after type of clinical trial" to examine how bone formation markers were affected by soy isoflavone supplementation for 12 weeks.

Baseline assessment and sample collection

Information on weight, height, body mass index, two 24-hr food consumption recall and physical activity were collected at the start, 6 and 12 weeks of the study. Blood and urine samplings were done in 3 stages, in the beginning and at the end of the 6th and 12th 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.

Intervention

Soy protein at 35g level containing 98.3mg isoflavones were given to subjects daily. Isoflavones profiles of utilized soy protein are shown in table 1. Subjects were provided with a special cup for measuring soy and cooking instructions were also given to the subjects. A few reductions were done in some groups' serving of food like meat and bread to avoid exceeded intake of protein. They were emphasized to not change their food habits and physical activity level.

Markers of bone formation

Total alkaline phosphatase was assayed calorimetrically with Hitachi 902 autoanalyzer, osteocalcin by IRMA method using Biosource kit and Wizard gamacounter, IGFBP3 by ELISA using Biosources diagnostics. Urinary creatinine was determined by calorimetric method [18].

Table 1 – Isoflavones profile of the consumed soy protein.

| Isoflavones profile | Milligram per gram of soy protein |
|---------------------|-----------------------------------|
| Genistin | 1.35 |
| Daidzin | 1.08 |
| Glycitin | 0.265 |
| Genistein | 0.063 |
| Daidzein | 0.041 |
| Total | 2.81 |

Statistical analysis

We used Food Processor software and SPSS program for food consumption survey and statistical analysis of the data, respectively. All values are presented as the mean \pm S.D. Quantitative variables were then examined by Kolmogorof-Smirnof (KS) to ensure normality of distribution. Repeated measurement analysis was employed to analyze any possible changes in food intake, intervening and biochemical variables in 3 stages. The purpose of this analysis was to ensure lack of significant changes of the variables. The level of significance was set at below 5 percent ($P < 0.05$).

RESULTS

From among 25 participants, 10 subjects were excluded for reasons such as irregular soy intake, gastrointestinal disorders, traveling, stroke and HRT. 15 women, therefore, remained in this intervention till the end of study. Table 2 shows the characteristics of subjects who participated in our assessment. Mean body mass index and physical activity level had no change and mean daily intake of macronutrients as well as important micronutrients did not differ at 6 and 12 weeks compared to the start of the study (Table 3). To analyze changes in 3 stages repeated measurement analysis was utilized. Three months soy protein supplementation resulted in a significant increase for total serum alkaline phosphatase (TALP) while the other bone formation indicators namely osteocalcin and insulin growth factor binding protein (IGFBP3) remained unchanged.

Table 2. Subjects' characteristic data at the beginning of the study (n=15).

| Characteristic data | Mean \pm SD (n = 15) |
|--------------------------|------------------------|
| Age (year) | 52.9 \pm 4.3 |
| Duration of Menopause | 5.47 \pm 3.4 |
| Weight (kg) | 68 \pm 7.5 |
| Height (m) | 157.2 \pm 7.2 |
| BMI (kg/m ²) | 27.4 \pm 3 |

Table 3. Subjects mean food intake in 3 stages of the study (n=15).

| Macro/Micronutrients intakes | at the start | 6 weeks | 12 weeks | P value** |
|------------------------------|----------------|----------------|----------------|-----------|
| Energy (kcal) | 1933.4 ± 302.5 | 2033.2 ± 420.3 | 1902.8 ± 308.6 | NS |
| Protein (g) | 74.6 ± 12.3 | 71.9 ± 17.4 | 74.8 ± 10.2 | NS |
| Fat (g) | 62 ± 13.5 | 65.45 ± 17.25 | 63.68 ± 13.08 | NS |
| Carbohydrate (g) | 269.14 ± 53.31 | 282.62 ± 66.5 | 257.48 ± 59.65 | NS |
| Calcium (mg) | 999.3 ± 460.2 | 966.8 ± 443.7 | 1014 ± 436 | NS |
| Phosphorus(mg) | 873.2 ± 228.9 | 853.1 ± 273.9 | 866.4 ± 200.6 | NS |
| Fiber (g) | 14.72 ± 2.79 | 13.91 ± 2.91 | 14.4 ± 2.69 | NS |

* Mean ± SD

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

Table 4. Change of bone formation markers in three stage of study.

| Bone metabolism biomarkers* | Stage of study | Mean ± SD (n = 15) | P value† |
|-----------------------------|----------------|--------------------|----------|
| TALP | At the start | 237.5 ± 85.4 | <0.05 |
| | 6 weeks | 300.4 ± 294.1 | |
| | 12 weeks | 281.3 ± 80.5 | |
| OC | At the start | 11.4 ± 4.8 | NS |
| | 6 weeks | 12.7 ± 5.7 | |
| | 12 weeks | 11.5 ± 4.8 | |
| IGFBP3 | At the start | 3304.6 ± 728.6 | NS |
| | 6 weeks | 3221.6 ± 534.4 | |
| | 12 weeks | 3103.9 ± 639.8 | |

* TALP: Total alkaline phosphatase (gr/dl); OC: Osteocalcin (ng/ml); IGFBP3: Insulin like growth factor binding protein 3 (ng/ml)

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

DISCUSSION

In the present study, the daily consumption of 35 g soy protein containing 47.2 genistein and 37.8 daidzein for three months resulted in significant increase in TALP in menopausal women with osteopenia, while other parameters were not significantly difference. In Arjmandi et al studies on rats a slight insignificant increase in TALP was seen [19-21] and their study on postmenopausal women showed, biomarkers of bone formation, i.e. osteocalcein, IGF-I and BSAP were all significantly elevated [22]. Totally, comprehensive human studies on the effect of soy on biomarkers of bone formation have been reported to either increase [23, 24] or no change [25,26] as a result of soy supplementation. In monkeys soy protein consumption induced a significant fall in TALP after 12 weeks [27]. In animal models such as monkey only 30 to 50% of daidzein was converted to equol and it has been shown that equol possesses more estrogen-like properties than daidzein. This is why isoflavone efficacy has been less pronounced in monkeys and the

results have been reported as reduced TALP levels [28, 29].

Albertazzi observed genistein as a component of soy isoflavone reduced osteocalcin, a marker of bone formation, by 3.6% compared to baseline and 0.31% compared to placebo (30). In another study, at 24 months, genistein significantly increased the levels of bone-specific alkaline phosphatase and insulin-like growth factor I [31].

Vitolins et al. reported that daily consumption of 25 g soy protein with 5, 42 or 58 mg isoflavones had no bone preserving effects in peri- and post-menopausal women in a two-year study [32]. Findings from two studies have shown similar effects of soy protein and its isoflavones on bone [33, 34]. In contrast, other studies have suggested that isoflavone-rich soy milk containing 80 to 90 mg isoflavones [35] or soy products with 40 to 60 mg isoflavones on a daily basis [36] can exert bone protective effects.

Atkinson et al showed that loss of lumbar spine bone mineral content and bone mineral density was significantly lower in the women taking the

isoflavone supplement than in those taking the placebo. There were no effects on markers of bone resorption, but bone formation markers like bone-specific alkaline phosphatase were significantly increased ($P=0.04$) in the intervention group compared with placebo in postmenopausal women [37].

Isoflavone intervention stimulated bone formation and significantly increased serum BAP. These effects occur even with <90 mg/day of isoflavones consumption for less than 12 weeks [38]. Based on Devareddy et al study results, soy protein and its isoflavones have a modest beneficial effect in established osteoporosis as was evident by improvements in certain structural parameters and higher doses of isoflavones may be required to reverse the loss of tibial microstructural properties [39]. Additionally, the other study indicate that soy protein isoflavones increase mRNA levels of bone-specific alkaline phosphatase, an indicator of osteoblastic activity, and several of bone

matrix proteins including type I collagen and osteocalcin in ovariectomized rats [40] which can explain the alkaline phosphatase increases observed in our study.

CONCLUSION

According to several studies, soy isoflavones induce bone formation based on at least three mechanisms: stimulation of activity, proliferation, and differentiation of osteoblasts; protection of osteoblasts from apoptosis and enhancement of bone formation rate as assessed by bone histomorphometry [41-44]. Based on these data it can be concluded that soy protein or its isoflavones may increase bone formation by supplementation.

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