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The Effect of Isoflavones on Bone Mass and Bone Remodelling Markers in Postmenopausal Women

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Aim: Recently, isoflavones have attracted attention for their potential roles in the prevention and treatment of estrogen-related osteoporosis. However, the optimal dosage and the components responsible for the favorable effects of isoflavones on bone in humans are still unclear. This study aims to investigate the effect of low-dose isoflavones on bone mineral density (BMD) and on biochemical markers of bone turnover in early postmenopausal women.

Materials and Methods: Ninety participants between 42-59 years of age were randomly assigned to receive twice a day either isoflavone tablet (n:45) that provided 29.8 mg genistein, 7.8 mg daidzein, and 2.4 mg glycitein per tablet or placebo tablets (n:45) containing 250 mg starch. BMD was measured both in lumbar spine and hip, and bone biomarkers of serum osteocalcin, serum alkaline phosphatase (ALP) and serum C-terminal telopeptide (CTX) were measured at baseline and after six months of therapy at the end of the study.

Results: Isoflavone treatment after six months significantly increased BMD of L2-4 T score (+19%, p=0.000) and Ward triangle T score (+20%, p=0.000); femur neck T score (+4%, p=0.06) was also increased but this change did not reach a level of statistical significance. Serum CTX level (-25%, p=0.047) decreased in the isoflavone group, while osteocalcin (-8.3%, p=0.23) and ALP levels (+ 4.5%, p=0.43) showed no change.

Conclusions: Isoflavone increases BMD of L2-4 and Ward triangle T scores at a dosage of 59.6 mg genistein with 15.6 mg daidzein and reduces bone resorption in early postmenopausal women.

Key Words: Isoflavone, genistein, bone mineral density, osteocalcin, serum C-terminal telopeptides

Postmenopausal Kadınlarda Isoflavonların Kemik Kitle ve Döngü Belirteçleri Üzerine Etkisi

Amaç: Son zamanlarda isoflavonlar östrojen ilişkili osteoporozun tedavi ve korunmasındaki potansiyel rolleri nedeniyle dikkatleri üzerlerine çekmiştir. Ancak, insanlarda isoflavonların kemik üzerine hangi doz ve tipte en uygun etkiyi gösterdiği hala tam olarak açık değildir. Bu çalışmada erken postmenapozal kadınlarda düşük doz isoflavonların kemik mineral yoğunluğu ve kemik döngüsünün biyokimyasal belirteçleri üzerine olan etkileri araştırıldı.

Yöntemler: Yaşları 42-59 arasında değişen 90 hasta günde 2 kez, içerisinde 29.8 mg genistein, 7.8 mg daidzein ve 2.4 mg glycitein bulunan isoflavon (n: 45) tabletleri veya içerisinde 250 mg nişasta bulunan tabletleri (n: 45) alacak şekilde rastgele belirlendi. Lomber omur ve kalça kemik mineral yoğunluğu ile serum osteokalsin, serum alkalen fosfataz (ALP) ve serum C terminal telopeptid (CTX) gibi kemik belirteçleri çalışmanın başlangıcında ve 6. ayında ölçüldü.

Bulgular: İsoflavon tedavisi 6 ayın sonunda L2-4 T skoru (+ % 19, p=0.000) ve Wards triangle T skorunu anlamlı olarak arttırmıştır, femur boynu T skorunda da artış gözlenmiştir fakat bu artış istatistiksel olarak anlamlı düzeye ulaşmamıştır. İsoflavon grubunda serum CTX (- % 25, p=0.047) düzeyi azalırken, serum osteokalsin (- % 8.3, p= 0.23) ve serum ALP düzeylerinde (+ % 4.5, p=0.43) bir değişiklik olmamıştır.

Sonuç: İsoflavon, 59.6 mg genistein ile 15.6 mg daidzein dozlarıyla erken postmenopozal kadınlarda L2-4 T skoru ve Wards üçgeni T skorunu anlamlı ölçüde arttırırken kemik rezorpsiyonunu azaltmaktadır.

Anahtar Sözcükler: İsoflavon, genistein, kemik mineral yoğunluğu, osteokalsin, serum C terminal telopeptidler

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Introduction

Osteoporosis is now a major public health threat and its prevalence is expected to rise dramatically in the coming decades. Estrogen deficiency is generally not listed as one of the main risk factors for osteoporosis, but it is indirectly and strongly associated with the many recognized risk factors: female gender, thin stature, advanced age,

postmenopausal, amenorrhea and excessive alcohol intake. Although attainment of peak bone mass is in the late second decade, gradual bone loss occurs with aging and this loss is accelerated in menopause. The loss may initiate for the first time when menstrual cycles become irregular in the perimenopause period. From 1.5 years before to 1.5 years after menopause, spine bone mineral density (BMD) has been shown to reduce 2.5% per year, compared with a premenopausal loss rate of 0.13% per year (1,2). Innumerable studies attest to the importance of estrogen in bone remodeling, evident from the fact that hormone therapy administered in a dose-dependent manner effectively prevents bone loss in postmenopausal women and reduces the incidence of fractures. However, various reports about the adverse effects of hormone therapy including increasing incidence of endometrial disease, breast cancer and thromboembolic events (3-5) will undoubtedly serve to increase the search for alternative and natural strategies for menopausal estrogen deficiency, including ways of managing the prevention of osteoporosis with aging.

Recent studies have focused on natural alternatives, such as phytoestrogens, that exert estrogen activity on several tissues. In the past few years, isoflavones have attracted attention for their potential roles in the prevention and treatment of estrogen-related osteoporosis (6). The chemical structures of the phytoestrogens are similar to that of 17 β-estradiol, the most potent naturally occurring estrogen. There are three types of phytoestrogens, such as isoflavones, coumestans and lignans, but only the first two types of non-steroidal estrogen molecules own the skeletal effects. The main isoflavones found in soy proteins and soy foods are genistein and daidzein. Morabito et al. (7) showed that genistein prevents bone loss caused by estrogen deficiency. They are found in abundance in soybeans and their derivative foods. Epidemiological studies suggest that the low incidence of osteoporosis and heart diseases caused by estrogen deficiency in Asian women is attributable to their high intake of soy foods, compared with American and Finnish women (8).

It has been demonstrated that genistein has a direct effect on bone resorption and a stimulatory effect on bone formation in tissue culture system in vitro (9,10). Several animal studies have shown that soy protein or isolated isoflavone-enriched soy extract supplementation improves bone mass or other endpoints (11-13). Several human studies in postmenopausal women showed that dietary supplement of phytoestrogens improved spine BMD and reduced urinary excretion of bone resorption markers (7,14,15). However, in contrast to these studies, Kreijkamp-Kaspers et al. (16) reported that isoflavones 99 mg/day did not show any effect on bone health of postmenopausal women. The optimal dosage and the components of isoflavones with respect to bone health are still inconclusive.

The inconsistent and limited number of studies about the effect of phytoestrogens on bone mass led us to conduct a new prospective randomized study to provide insight into this question. In this study, we aimed to investigate the effects of isoflavones on bone mass and on biochemical markers of bone turnover in postmenopausal women.

Materials and Methods

In this study, 90 healthy postmenopausal women were randomly selected from the Menopause Clinics of the Department of Obstetrics and Gynecology of Fatih University Medical School. This was a single center, single-blind, placebo-controlled randomized study. The volunteers received written and verbal information on the purpose and the procedures of the study, and informed written consent was obtained from all. Approval for this study was obtained from the Institutional Review Board of Fatih University.

Women were invited to participate if they had not undergone surgically induced menopause, had not had a menstrual period in the preceding year and had a follicle stimulating hormone (FSH) level greater than 40 IU/L and a serum estradiol (E_2) level of 30 pg/ml or less. All women had incapacitating hot flashes and other climacteric symptoms.

Women with malignant disease, metabolic bone diseases, current steroid treatment, cardiovascular, hepatic, or renal disorders, coagulopathy, family history of estrogen-dependent cancer, usage of hormone therapy in the preceding year, or smoking habit of more than 10 cigarettes per day, and those receiving any medication that could affect the skeleton or for the relief of climacteric symptoms were excluded. The women were not using statins, natural products with presumed estrogenic activity, or drugs possibly affecting climacteric symptoms or metabolism and absorption of

phytoestrogens (e.g. antibiotics during the previous 3 months).

Participants were randomly assigned to receive twice daily either isoflavone tablet (n:45) that provided 29.8 mg genistein, 7.8 mg daidzein, and 2.4 mg glycitein per tablet (Isoflavin®; Mikrogen) or placebo tablets (n:45, 250 mg starch per tablet) of identical appearance. Placebo pills were prepared with the same appearance by the manufacturer of the isoflavone tablets. The manufacturing company was not involved in the study design and did not participate in any further part of the trial. A block randomization was used in order to ensure an equal number of patients in each group. Blocks were numbered, all numbered papers were placed in a bag and a staff who was blinded to the research protocol selected the patients into the treatment groups.

Before initiation of therapy, a detailed personal and family history was taken and all patients underwent gynecologic and physical examination that included: Pap smear, transvaginal ultrasonography (TVS), breast examination and mammography, thyroid, renal and liver function tests, and blood coagulation tests. Laboratory tests including serum ALP, osteocalcin and CTX were conducted before the initiation and after six months of therapy. Fasting blood samples were collected at baseline and at the sixth month of the therapy to measure bone markers. Serum was separated by centrifugation and serum aliquots kept frozen at -80 °C for subsequent analysis. Serum ALP was measured by using the Metra kit (Quidel Ltd, Oxford, United Kingdom; 35). Serum CTX was measured with electrochemiluminescence immunoassay "ECLIA" kits (β-CrossLaps, English) according to the manufacturer's instruction. For the analysis of osteocalcin, electrochemiluminescence immunoassay "ECLIA" kits (N-MID, English) were used.

During the study, the women were encouraged to lead normal lives, with no changes that might influence the outcome in dietary habits, alcohol consumption, or physical activity, which were all recorded by means of questionnaires before initiation of study, at the third month and at the end of the treatment period. Participants were asked to complete a food-frequency questionnaire (FFQ) at baseline at the third month and at the end of the treatment period after having been given verbal and written instructions on how to complete it. Dietary calcium and vitamin D intakes were estimated based on published food-composition tables by means of the FFQ. Women with a calcium intake of <500 mg/day were advised to increase their intake. Dietary isoflavone and lignan intake was assessed with the FFQ covering habitual diet during the year preceding enrollment.

Patient compliance was reinforced by a physician by means of a questionnaire that was completed by the subjects before initiation of the study, at the third month and at the end of the treatment period. All unfavorable effects were accepted as adverse effects, such as breast tenderness, vaginal bleeding, abdominal distention and constipation.

BMDs of the anteroposterior lumbar spine (L2-L4), femur neck and Ward triangle were measured by dual-energy X-ray absorptiometry (DXA) by one expert technician at baseline and after six months of treatment. The instrument was calibrated on a daily basis according to the manufacturer's instructions. All subjects were subclassified by the World Health Organization (WHO) criteria according to the lowest BMD in the lumbar spine and the femoral neck as: normal controls (T-score >–1), osteopenic (– 2.5 < T-score ≤ –1) and osteoporotic (T-score ≤ – 2.5).

Statistical Analysis

Analysis of data was performed by SPSS package program for Windows (11.5 SPSS Inc, Chicago, US). Student's t-test, paired-t test and chi-square test were used when appropriate. A p value of <0.05 was accepted as significant.

Based on the literature, we expected genistein to reduce the markers of bone resorption. We considered a 20% decrease in six months as acceptable and power analysis identified 90 patients (45 for each group) as the total sample size required to detect the difference in therapeutic success with a power of 80% at 5% significance level.

Results

In total 90 subjects who met the inclusion criteria were eligible for the study and received study medication. The clinical data of the two randomized groups are shown in Table 1. The 90 subjects were between 42-59 years of age (1 to 6 years of menopause). All the groups had a similar age, body mass index, menopause age and years postmenopausal. Education levels of groups, food,

Table 1. Baseline characteristics of participants in the trial.

Characteristics	Placebo (n=45)	Isoflavone (n=45)
Age (year)	51.0 ± 4.94	52 ± 5.43
BMI (kg/m^2)	27.4 ± 3.23	26.6 ± 3.04
Menopause age	46.71 ± 2.72	47.21 ± 3.76
Years after menopause	4.0 ± 1.66	3.3 ± 1.89
Tea consumption	3.28 ± 1.58	4.66 ± 3.29

Values are mean ± SD.
BMI: Body mass index.

vegetable, coffee, tea and soy-related food consumption, exercise and smoking habits were similar.

During the 6 months period, there were 10 dropouts; 2 (4.4 %) dropouts in the isoflavone group, and eight (17.7 %) dropouts in the placebo group, which left 43 and 37 participants, respectively, for the per protocol analysis. In the isoflavone group one patient dropped out because of being out of control and one patient for personal reasons. In the placebo group two patients dropped out because of taking the drugs irregularly and 6 patients because of lack of effect. Breast tenderness occurred in 2 women during isoflavone treatment, and in one women taking placebo-therapy.

In placebo group, baseline levels were as follows; L2-L4 T score=-1.79 ± 0.47, Wards tringle T score= -1.19 ± 0.98, femur neck T score = -1.38 ± 0.87, serum osteocalcin = 22.92 ± 6.61, serum CTX=0.39 ± 0.17 and serum ALP=205.1 ± 43.9.

After 6 months in placebo group, L2-L4 T score= -1.97 ± 0.5, Wards tringle T score= -1.18 ± 0.95, femur neck T score = -1.45 ± 0.99, serum osteocalcin= 25.47 ± 4.65, serum CTX= 0.59 ± 0.26 and serum ALP= 207.1 ± 46 (Table 2).

In isoflavone group, baseline levels were; L2-L4 T score= -1.76 ± 0.79, Wards tringle T score= -2.14 ± 0.77, Femur neck T score = -1.81± 0.81, serum osteocalcin= 23.14 ± 5.67, serum CTX= 0.50 ± 0.21 and serum ALP= 211.5 ± 57.3.

After 6 months therapy in isoflavone group, L2-L4 score T score= -1.47 ± 0.89, Wards tringle T score= -1.69 ± 0.77, Femur neck T score=-1.51 ± 1.00, serum osteocalcin 19.05 ± 6.17, serum CTX= 0.36 ± 0.15 and serum ALP= 221.2 ± 56.4 (Table 2).

At baseline L2–L4 T score (p= 0.879), serum osteocalcin (p= 0.274), serum CTX (p= 0.159), serum ALP (p= 0.708) and femur neck (p= 0.310) levels were similar in both groups but Wards Triangle T score was statistically lower in isoflavone group (p= 0.005).

After six months, Wards T score (p=0.104), femur neck (p=0.07), serum CTX (p=0.035) and serum ALP levels (p=0.418) were similar in both groups. However, L2–L4 T score (p=0.04) was statistically increased in isoflavone group and serum osteocalcin levels (p=0.012) were also increased in placebo groups (Table 2).

Isoflavone treatment significantly increased BMD of L2-4 T score and Wards triangle T score (L2-L4 T score = + 19 %, p=0.000; Wards triangle T score = + 20 %, p=0.000) but femur neck T score (+7 %, p=0.06) did not change. Serum CTX levels after six months (CTX= -25 %, p=0.047) significantly reduced. However, isoflavone treatment did not change serum osteocalcin (Osteocalcin= -8.3 %, p=0.066) and ALP levels (ALP= + 4.5 %, p= 0.389) after six months (Table 2).

In placebo group, significantly decreased BMD scores (L2-4 T score=-9.5 %, p=0.008), increased serum levels of CTX (CTX= + 34.8 %, p=0.028) and osteocalcin (osteocalcin= + 10.7 %, p=0.013) were observed. Serum ALP levels (+ 0.9 %, p=0.867) did not change (Table 2).

Discussion

In this study, we examined the effects of isoflavones on BMD and biochemical markers of bone formation (osteocalcin, ALP) and bone resorption (serum CTX). At the sixth month of the treatment, we observed that isoflavone treatment significantly reduced bone loss of the lumbar spine and Wards triangle. In addition, it reduced the bone resorption marker (CTX) in early postmenopausal women, but bone formation markers (osteocalcin, ALP) did not change.

Although in some studies daidzein was reported to be more bioavailable (17) than genistein in rats and humans, the anabolic effect of genistein on bone metabolism is equal to that of daidzein (18). When daidzein is hydroxylated, its chemical structure is similar to that of genistein. The soy isoflavone tablets used in this study have a higher proportion of genistein (29.8 mg) and lower dose of daidzein (7.8 mg) and with this proportion we observed a bone-sparing effect.

Table 2. Effects of isoflavone and placebo on BMD and remodeling markers.

Variable	Baseline	After 6 months therapy	% change	P value for changes compared with		
				Baseline	Isoflavone-Placebo Baseline	Isoflavone-Placebo After
L2–L4 T score g/cm^2						
Isoflavone	-1.76 ± 0.79	-1.47 ± 0.89	+ 19	0.000	0. 879	0.040
Placebo	-1.79 ± 0.47	-1.97 ± 0.5	- 9.5	0.008		
Wards T score g/cm^2						
Isoflavone	-2.14 ± 0.77	-1.69 ± 0.77	+ 20	0.000	0.005	0.104
Placebo	-1.19 ± 0.98	-1.18 ± 0.95	- 0.3	0.954		
Femur neck T score g/cm^2						
Isoflavone	- 1. 81 ± 0.81	-1.51 ± 1.00	+7	0.06	0.310	0.07
Placebo	-1.38 ± 0.87	-1.45 ± 0.99	-3	0.35		
Osteocalcin (ng/ml)						
Isoflavone	23.14 ± 5.67	19.05 ± 6.17	- 8.3	0.066	0.274	0.012
Placebo	22.92 ± 6.61	25.47 ± 4.65	+ 10.7	0.013		
Serum CTX (ng/ml)						
Isoflavone	0.50 ± 0.21	0.36 ± 0.15	- 25	0.047	0.159	0.035
Placebo	0.39 ± 0.17	0.59 ± 0.26	+ 34.8	0.028		
Serum ALP (U/L)						
Isoflavone	211.5 ± 57.3	221.2 ± 56.4	+ 4.5	0. 389	0.708	0.418
Placebo	205.1 ± 43.9	207.1 ± 46.9	+ 0.9	0.867		

BMD: Bone mineral density. CTX: C-terminal telopeptide. ALP: Alkaline phosphatase.

The relationships between phytoestrogens and bone tissue have been studied for less than a decade. Therefore, the potential use of phytoestrogens to preserve bone tissue and delay or prevent the onset of osteoporosis in humans is only now being addressed by researchers. The mechanism of the action of phytoestrogens on bone metabolism are not yet clear and still have to be elucidated. The beneficial effects of soy isoflavones on bone tissue can result from either increased bone formation by osteoblasts or decreased bone resorption by osteoclasts. Several experimental evidences suggest that the phytoestrogen effect on bone formation may be a consequence, at least in part, of a genomic and estrogen receptor-mediated effect involving the activation or inhibition of the nuclear estrogen receptor (19,20). Alternatively, a variety of non-genomic mechanisms, including the inhibition of tyrosine kinase (21), inhibition of topoisomerase II (22), or activation of a putative membrane-bound receptor for estrogenic molecules (23), have been proposed as the mechanisms of action of genistein and other phytoestrogens. The inhibition of osteoclastic bone resorption may result from a direct action of phytoestrogens, presumably via non-genomic mechanisms, since mammalian osteoclasts appear to lack estrogen receptors. Previous studies in cell culture systems have shown that genistein has a direct suppressive effect on osteoclasts by a number of possible mechanisms, including induction of apoptosis through the pathway of intracellular Ca+2 signalling, activation of protein tyrosine phosphatase, and inhibition of cytokines (24).

Several human studies have shown that isoflavones can reduce bone loss as measured by surrogate markers of bone turnover and changes in BMD in postmenopausal women. Morabito et al. (7) performed a randomized double blind placebo controlled study to evaluate and compare hormone therapy with the effect of the phytoestrogen genistein on bone metabolism and BMD in postmenopausal women. At the end of the treatment, they showed that genistein increased BMD in the femur and lumbar spine as effectively as hormone therapy, and in addition administration of genistein markedly increased markers of bone formation (serum bone-specific ALP and osteocalcin) and reduced bone resorption in postmenopausal women. In our study, in addition to lumbar spine and femur neck BMD, we also studied Ward triangle. After six months of therapy, only lumbar spine and Ward triangle BMD were increased significantly; femur neck BMD did not increase. Similarly, the bone resorption marker (CTX) was decreased in our study but bone formation markers (osteocalcin and ALP) showed no change. The probable cause of the discrepancy between Morabito's results and ours may be the difference in the periods of the studies. We used genistein for only six months while Morabito et al. used it for one year. Like our study, Mori et al. (25) reported that four-week supplementation of isoflavone 40 mg a day as a tablet does not influence the serum bone formation marker but inhibited bone resorption in postmenopausal women. Actually, if the objective is to monitor therapeutic efficacy, it seems most rational to use a resorption marker for drugs that act principally on osteoclasts, such as estrogens or bisphosphonates (26). Although several studies reported that isoflavone at 70-90 mg/day may be needed for its bone-protective effect, we observed a bone-protective effect of isoflavone at a dosage of 59.6 mg genistein and 15.6 mg daidzein. Mei et al. (27) similarly reported a bone-protective effect of isoflavone at 53.3 mg/day.

In another study, Chiechi et al. (28) randomized 187 healthy postmenopausal women into a soy-rich diet, hormone replacement therapy and a control group, and assessed bone biomarkers and BMD at baseline and after six months of intervention. No significant differences were demonstrated in either markers of bone resorption (N-telopeptide-C and hydroxyproline) or forearm BMD between those three groups. However, they studied only forearm BMD, though it is well known that lumbar spine and femur neck are more sensitive than forearm BMD to measurement in osteoporosis. More than half of the subjects in the study group discontinued the study mainly due to dislike of soy and difficulties in finding and cooking foods. As a result of this high dropout rate, data from only small number of subjects (n=24) was a disadvantage of these studies.

In 2003, Chen et al. (15) reported a favorable effect of soy isoflavone treatment on maintenance of hip bone mineral content (BMC) but not on BMD in postmenopausal women. The reasons for such different responses of BMC and BMD to isoflavone supplementation are unclear. The postmenopausal period of the patients was long (within 10 years of natural menopause) and this may have caused the different responses of BMC and BMD to isoflavone supplementation.

In our results, we clearly observed an increase in lumbar spine BMD, but for hip, while Ward triangle BMD demonstrated an increase, femur neck BMD did not change. It is well known that many drugs used for osteoporosis, such as estrogen and bisphosphonate, are more effective on spinal bone than hip. The spine is remodeled more rapidly than the hip, which contains a higher proportion of cortical bone (29,30). The spine is the area thought to be the most sensitive to estrogen because of its higher content of trabecular bone. The soy isoflavones used in this study have a higher proportion of genistein and we thought that the bone-sparing effect of isoflavone can be attributed to the similar effect of estrogen. Hence, the undetectable effect of isoflavone supplementation at the femur neck could be related to this issue and the increase in Ward triangle BMD might be accidental. The treatment period and the relatively small number of patients might be accepted as the disadvantages of our study. It is well known that bone remodeling is a relatively slow process and six months of treatment may not be adequate to predict the long-term effects of isoflavones on bone mass. Normally, 6–18 months are needed to reach a new equilibrium with a certain intervention (31,32). Hence, longer-term trials would be required to evaluate the effects of isoflavones on bone mass.

The unexpected results of our study were the increases in both bone formation (osteocalcin) and bone resorption (CTX) markers in the placebo group. This may be explained by a new onset of osteoporosis in the

placebo group. The increase in bone resorption stimulated bone formation. However, this is a short study with respect to bone and these findings need to be checked via longer studies.

In conclusion, our results show that low dose of isoflavone treatment improved BMD in the lumbar spine and reduced bone resorption in early postmenopausal women. Nevertheless, before recommendation of this low dose of isoflavones for the prevention of postmenopausal osteoporosis, further studies are needed to confirm the optimal dosages and treatment period.

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