Isoflavone supplementation reduced serum sex hormone-binding globulin concentration in postmenopausal women

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ABSTRACT

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INTRODUCTION
The bone loss that occurs with ageng in postmenopausal women is related to a decrease in serum levels of bioavailable estrogen and testosterone, which are mainly bound to sex hormone-binding globulin (SHBG) and albumin. Phytoestrogens are thought to exert hormonal effects in the body due to their structural resemblance to 17â-estradiol. The objective of this study was to evaluate the effect of isoflavone supplementation on levels of SHBG in postmenopausal women aged 47- 60 years.

METHODS
A study of pre and post test design with controls was conducted in 70 women aged 47- 60 years. Subjects were randomly divided into 2 groups, the isoflavone group received 100 mg isoflavones/day + calcium 500 mg/day and the control group calcium 500 mg/day for 6 months. Measurement of bone mineral density was performed prior to supplementation, and serum SHBG levels before and after supplementation.

RESULTS
Supplementation of isoflavones for 6 months reduced the SHBG levels by 31.1% in the isoflavone group ($p=0.000$), whereas supplementation of calcium for 6 months did not affect the levels of SHBG in the control group ($p=0.359$). Supplementation of isoflavones for 6 months reduced SHBG levels of postmenopausal women in the isoflavone group with either osteopenia ($p=0.028$) or osteoporosis ($p=0.008$).

CONCLUSION
Supplementation of isoflavones for 6 months decreased the SHBG levels of postmenopausal women in the isoflavone group with osteopenia and osteoporosis. Our findings suggest that phytoestrogens may significantly decreased SHBG levels in postmenopausal women.

Keywords: Isoflavones, calcium, sex hormone-binding globulin, postmenopausal women

Suplementasi isoflavon menurunkan kadar sex hormone-binding globulin *pada perempuan pascamenopause*

ABSTRAK

LATAR BELAKANG
Osteoporosis pada perempuan pasca menopause umumnya terjadi akibat kekurangan estrogen dan progesterone. Sex hormone-binding globulin (SHBG) berperan dalam aksi estrogen maupun progesteron terhadap sel sasaran. Phytoestrogens – isoflavon berefek serupa dengan 17â – estradiol. Tujuan penelitian ini adalah untuk menilai pengaruh suplementasi isoflavon terhadap kadar SHBG pada perempuan pascamenopause yang berusia 47–60 tahun.

METODE
Sebuah rancangan eksperimental secara buta ganda menggunakan kontrol mengikutsertakan sebanyak 70 perempuan berusia 47–60 tahun. Subjek penelitian secara acak menggunakan blok sebesar 4 dibagi dalam 2 kelompok perlakuan, yaitu diberikan isoflavon 100 mg/hari + kalsium 500 mg/hari (kelompok isoflavon) dan diberikan kalsium 500 mg/hari (kelompok kontrol). Pemberian suplemen dilakukan selama 6 bulan. Pengukuran densitas massa tulang (BMD) terhadap subjek dilakukan sebelum pemberian suplemen. Pengukuran kadar SHBG serum dilakukan sebelum dan sesudah pemberian suplemen. Untuk menguji pengaruh pemberian suplemen terhadap kadar SHBG digunakan uji t-independen.

HASIL
Suplementasi isoflavon 100 mg/hari + kalsium 500 mg/hari selama 6 bulan menurunkan kadar SHBG pada perempuan pascamenopause (p=0,000), sedangkan suplementasi kalsium 500 mg/hari selama 6 bulan tidak mempengaruhi kadar SHBG (p=0,359). Suplementasi isoflavon 100 mg/hari + kalsium 500 mg/hari selama 6 bulan menurunkan kadar SHBG baik pada perempuan pascamenopause penderita osteopenia (p=0,028) maupun osteoporosis (p=0,008).

KESIMPULAN
Suplementasi isoflavon 100 mg/hari + kalsium 500 mg/hari selama 6 bulan mampu menurunkan kadar SHBG pada perempuan pascamenopause yang mengalami osteopenia dan osteoporosis. Implikasi klinik dari hasil studi ini memerlukan konfirmasi lebih lanjut.

Kata kunci*: Isoflavon, kalsium,* sex hormone-binding globulin, *perempuan pascamenopause*

INTRODUCTION

In post menopausal women, estrogen deficiency causes reduced bone mineral density (BMD), resulting in osteoporosis.[1] In addition to reduced BMD, patients with osteoporosis also have bone marrow degeneration.[2] According to the World Health Organization (WHO), individuals have osteoporosis if their BMD value expressed as a T score is ≤ -2.5 and osteopenia if the T score is within the range of -1 up to $-$ 2.5, while those with a T score from $T -1$ to 0 or more have normal bone mineral density.[3]

Estrogens and progesterones are frequently associated with osteoporosis. Estrogens are steroid hormones produced by women for their menstrual cycle. Estrogens are also produced by men, but at a low level. Menopausal women experience a reduction in estrogen levels that subsequently stabilize during postmenopause. High levels of estrogens indicate an increased risk of endometrial and mammary cancers,

whereas low estrogen levels increase the risk of bone fracture. Women with low levels of estradiol have a twofold higher risk of fracture, which is independent of the BMD.[4]

Progesterone is a steroid hormone with androgenic properties and is produced by both men and women. Progesterone is synthesized from cholesterol as pregnenolone, which is subsequently converted into progesterone. Progesterone synthesis needs steroid hormone precursors, including cortisol, estrogen and testosterone. In women, progesterone is synthesized by the corpus luteum, adrenal glands, and placenta during pregnancy. In adults, progesterone levels decrease with age. In the menopausal phase, progesterone levels decrease drastically to nearly undetectable levels. As with estrogen, low progesterone levels reduce bone density and cause osteoporosis.[5]

In women, estrogen and progesterone levels decrease at menopause, and subsequently both hormones decrease progressively in postmenopause. At menopause, estrogen concentrations decrease by approximately 60%, whereas estrone concentrations decrease by approximately 40%, leading to increased bone resorption. As is the case with estrogen, progesterone and progesterone receptors are decreased in menopausal women, thus reducing new bone formation. Progesterone deficiency is the major early cause of osteoporosis, while a reduction in estrogen increases bone resorption by osteoclasts. In postmenopausal women serum estradiol concentrations are lower than before menopause and serum estradiol concentrations in postmenopausal women with osteoporosis are lower than in those with osteopenia.[6]

Sex hormone-binding globulin (SHBG) is produced by hepatocytes and is then secreted into the circulatory system. Besides by hepatocytes, SHBG is also produced by target cells. Within the circulatory system, SHBG binds to estrogen or testosterone. Free SHBG functions as a regulator of steroid activity on target cells. In healthy adult women, both estrogen and progesterone are positively correlated with SHBG.[7,8] Isoflavones are phytoestrogens similar to 17â–estradiol and are also called estrogen-like molecules or non-steroidal estrogens. Phytoestrogens are categorized as isoflavones (genistein, daidzein, biochanin A and formonetin), lignans (matairesinol, secoisolariciresinol diglucosides), coumestans (coumestrol, 4–metoxy coumestrol) and stilbens (resveratrol). Currently more than 100 molecules have been found to belong to the phytoestrogen family.[9] In view of this, it is not surprising that isoflavone supplementation in postmenopausal women, for the purpose of increasing BMD, have shown variable results. In contrast, calcium and vitamin supplementation to postmenopausal women has been found to be beneficial for preventing loss of bone mass and decreased bone turnover, and for reducing nonvertebral fractures.[10]

Isoflavone supplementation have also been found to have variable effects on SHBG concentrations. Isoflavones do not affect SHBG levels in both premenopausal[11] and postmenopausal women.[11,12] Although isoflavone supplementation for a period of 6 months does not affect SHBG levels, isoflavone supplementation for 1 year increases SHBG concentrations.[13] The increase in SHBG concentrations by isoflavones has been shown to be inconsistent.[14] Another study showed that isoflavone supplementation decreases SHBG concentrations.[15] The purpose of the present study was to evaluate the effect of isoflavone supplementation on SHBG concentrations in postmenopausal women in the age range of 47–60 years.

METHODS

Design of the study

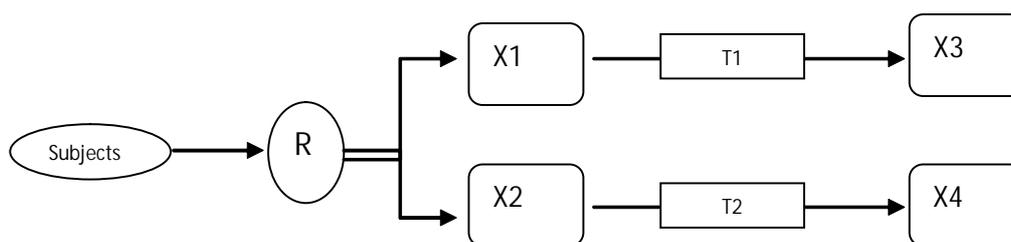This study was a pre and post test experimental design with controls, as diagrammed in Figure 1.

Figure 1. Diagram of study design

Legend: R = randomization, X1 and X2 = presupplementation observations, X3 and X4 = postsupplementation observations, T1 = supplementation of isoflavones + calcium (isoflavone group), T2 = calcium supplementation (control group).

Study subjects

The subjects of the study were women with minimally one year of menopause but not exceeding 10 years, who resided at Mampang subdistrict, South Jakarta. Recruitment of subjects was by cluster random sampling at the villages *(kelurahan)* of Kuningan Barat, Mampang Prapatan, Tegal Parang and Pela Mampang. Inclusion criteria were: postmenopausal women, aged 47–60 years, pronounced healthy by a physician, comunicative, not taking hormonal drugs in the last 3 months and agreeing not to consume vitamins and minerals during the study period, never having undergone hysterectomy and/or oophorectomy. Exclusion criteria were: patients with diabetes, renal, hepatic and cardiovascular diseases, and hypertension.

Sample size

The number of subjects in each of the two groups was calculated using the formula:

$$n = \frac{2\,SD^2(Z\alpha + Z\beta)^2}{(\overline{X}1 - \overline{X}2)^2}$$

Notes: n = sample size; $\overline{X}1$ = mean SHBG concentration (72.2 nmol/L) from a preliminary study on 5 Indonesian postmenopausal women given a supplement of isoflavone + calcium; $\overline{X}2$ = mean SHBG concentration (58.6 nmol/L) from a preliminary study on 5 Indonesian postmenopausal women given a supplement of calcium. SD = pooled standard deviation = 13.3 nmol/L. α = 5%; Z_α = 1.96 (two-sided test); β = 10%; power of the test 1 - β = 0.90, $Z\beta$ = 1.645.

Intervention

There were two treatments administered to the subjects. The first used a combination of isoflavones 100 mg + calcium (Ca) 500 mg, given daily in one caplet for 6 months (isoflavone group). The second treatment used Ca 500 mg 1 caplet daily for 6 months (control group). The supplements were given to the subjects by specially trained personnel. Supplementation was administered in a double blind manner. To determine subject compliance in consuming the supplements, the remaining caplets were counted every other day by the personnel entrusted with this task.

Analysis of serum

Blood samples were drawn from the left cubital vein of each subject. Serum was obtained from the blood by centrifugation at 2000 x g for 20 minutes, then stored at -20°C. Measurement of serum SHBG concentrations by immunoradiometric assay was performed in the Laboratory for Immunoendocrinology *(Makmal Terpadu Imunoendokrinologi),* Faculty of Medicine, University of Indonesia, Jakarta. The reagents for the assay IRMA-

Count SHBG from Diagnostic Products Corporation (DPC). Serum SHBG concentrations were expressed in nmol/L. The normal value of serum SHBG is 55 nmol/L.

Measurement of BMD

BMD was measured by dual-energy X-ray absorptiometry (DXA), using a Lunar DPX Bravo Nomusa densitometer (GE Medical Systems). Measurement of BMD was performed on the lumbar vertebrae, femoral neck and distal radius. The results of BMD measurement were expressed as the T score. T scores between -1 and 0 signify normal bone mineral density, T scores from -1 up to -2.5 signify osteopenia and T scores $\leq$ - 2.5 indicate osteoporosis.[3] Measurement of BMD was performed at Budi Jaya Hospital, South Jakarta.

Data analysis

Comparison of demographic, physical and laboratory variables, including mean SHBG concentratiions, between the isoflavone group and the control group was by means of independent sample t-tests. Comparison of mean SHBG concentrations before and after supplementation in the isoflavone group and the control group was done with the paired t-test. The level of significance used was 0.05.

Ethical clearance

This study was approved by the Commission on Research Ethics, Faculty of Medicine, Trisakti University. Before participating in the study, all subjects gave written informed consent.

RESULTS

Subject characteristics

Postmenopausal women meeting the inclusion criteria were 96 in number, among whom 20 subjects did not attend meetings and 6 subjects withdrew from the study. The remaining seventy subjects were divided into two groups of 35 subjects each. The first group was the isoflavone group, who were given isoflavone + Ca supplementation, and the second group was the control group, who were given Ca supplemention (Figure 2).
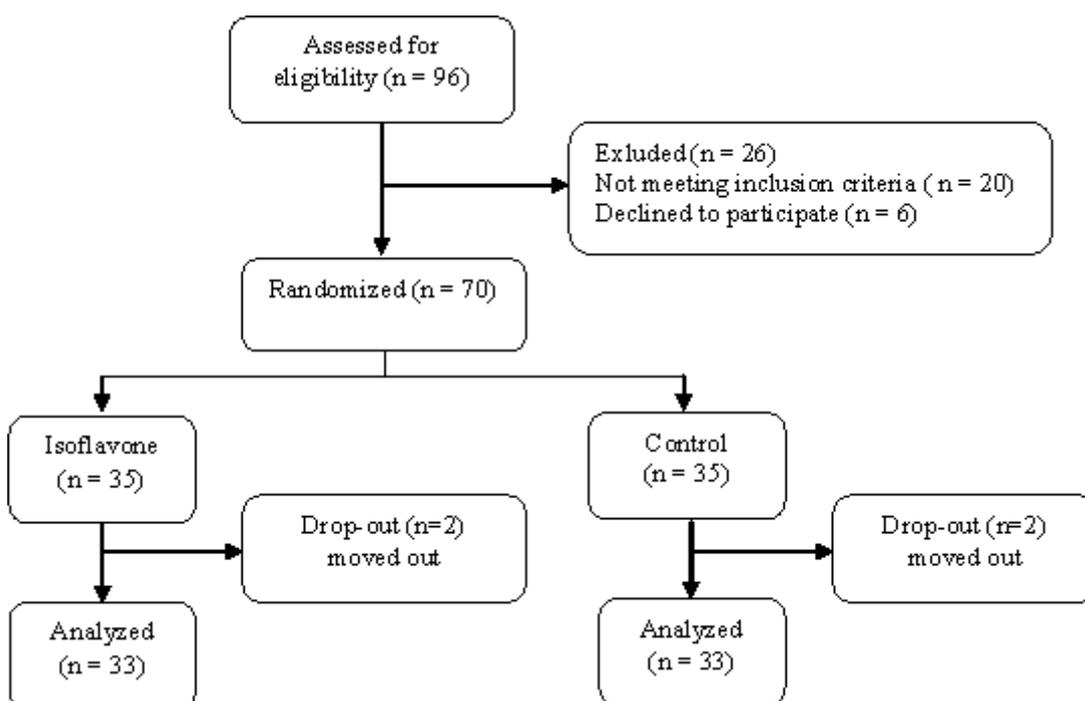

Figure 2. Diagram of screening, recruitment, follow-up and data analysis of study subjects

Table 1. Demographic, physical, and laboratory characteristics of subjects at baseline

Variables	Supplementation group		p
	Isoflavone (n = 35)	Control (n = 35)	
Age (years)*	52.02 ± 3.40	53.08 ± 3.60	0.215
BMI (kg/m²)*	26.99 ± 4.29	25.86 ± 5.50	0.341
BMD: (T score)			
Lumbar*	−1.24 ± 1.10	−1.70 ± 0.97	0.056
Femoral*	−0.78 ± 1.11	−1.05 ± 0.70	0.239
Radial*	−1.57 ± 1.18	−1.90 ± 1.23	0.174
Glucose (mg/dL)*	96.48 ± 31.69	94.91 ± 39.31	0.854
Total cholesterol (mg/dL)*	201.45 ± 31.79	205.91 ± 36.17	0.587
HDL cholesterol (mg/dL)*	53.62 ± 9.35	57.82 ± 11.8	0.104
LDL cholesterol (mg/dL)*	129.42 ± 26.46	127.08 ± 30.60	0.733
Triglycerides (mg/dL)*	115.57 ± 67.95	107.40 ± 44.47	0.554
SHBG (nmol/L)*	74.30 ± 35.10	63.07 ± 36.59	0.195
SHBG ≥ 55	89.58 ± 29.28	90.26 ± 34.14	0.481

*(mean ± SD); p = significance level of independent sample t-test; BMI = body mass index; BMD = bone mineral density; HDL = high density lipoprotein; LDL = low density lipoprotein; SHBG = sex hormone-binding globulin

The youngest subjects participating in this study were 47 years old and the oldest subjects were 60 years old. In Table 1 are shown the results of comparing subject characteristics between the isoflavone group and the control group, indicating no differences in age, BMI, BMD and laboratory test results. In the group with SHBG concentrations of ≥ 55 nmol/L, the results of data analysis showed that mean SHBG concentrations between the isoflavone group and the control group were not significantly different (p=0.481). These results indicated that randomization had successfully achieved a uniform distribution of the non-treatment variables between both groups.

Effect of isoflavone supplementation on SHBG concentrations

The results of comparing SHBG concentrations between the isoflavone group and the control group after supplementation for 6 months are presented in Table 2.

There were no significant differences between SHBG concentrations in the isoflavone and control groups after 6 months of supplementation (p=0.177).

Effect of isoflavone supplementation on SHBG levels in each group

The effect of supplementation of isoflavones and calcium on SHBG concentrations in each intervention group of postmenopausal women is presented in Table 3.

Isoflavone supplementation for 6 months significantly reduced SHBG concentrations by 31.1% in the isoflavone group (p=0.000), whereas Ca supplementation for 6 months did not affect SHBG concentrations in the Ca group (p=0.059).

Table 2. Comparison of SHBG concentrations between isoflavone group (isoflavones 90 mg + Ca 500 mg/day) and control group (Ca 500 mg/day) after 6 months of supplementation

Variable	Isoflavone group (n=33)	Control group (n=33)	p
SHBG (nmol/L)	51.21 ± 19.15	58.84 ± 26.94	0.177

SHBG = sex hormone-binding globulin; nmol/L = nano mol per liter; p = level of significance

Table 3. Effect of supplementation of isoflavones and calcium on SHBG concentrations in postmenopausal women

Supplementation group	n	SHBG (nmol/L) before supplementation	SHBG (nmol/L) after 6 months of supplementation	p
Isoflavones	33	74.30 ± 35.15	51.20 ± 19.15	0.000
Ca	33	63.07 ± 36.50	58.80 ± 26.90	0.059

SHBG=sex hormone-binding globulin; Ca=calcium; p=level of significance

DISCUSSION

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In the present study it was found that after 6 months isoflavone supplementation there was no significant difference in SHBG concentrations between the isoflavone group and the control group. Isoflavone supplementation for 6 months did not affect SHBG concentrations. Similar results were obtained in a meta-analytic study involving 35 studies of the effects of isoflavone supplementation for 4 weeks or longer on SHBG concentrations, indicating that isoflavone supplementation had no significant influence on SHBG concentrations.[11] The results of a randomized, placebo-controlled, double-blinded, crossover trial showed that administration of a supplementation of red clover-derived isolated isoflavones of 84 mg/day for 2 months did not affect serum SHBG concentrations.[16]

Other studies also found that isoflavones did not result in differences in SHBG and testosterone levels in postmenopausal women.[12] In postmenopausal women, estrogens and androgens are biosynthesized from dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S). DHEA and DHEA-S are steroidal precursors of estrogens and androgens produced by the adrenal glands.[17] the abovementioned biosynthesis of estrogens and androgens takes place in peripheral intracrine tissues.[18]

Our study results showed that isoflavone supplementation for 6 months reduced SHBG concentrations, both in patients with osteopenia and in those with osteoporosis, but that calcium supplementation over the same period had no effect on SHBG concentrations. Supplementation for 6 months in this study did not lead to differences in SHBG concentrations between the isoflavone group and the control group. These results differed those of other studies, in whom it was shown that although supplemenation of isoflavones 70 mg/day for 6 months did not affect SHBG concentrations, supplementation for 1 year did effect an increase in SHBG concentrations.[13] The increase in SHBG concentrations caused by isoflavones in postmenopausal women is inconsistent.[14]

Our study showed that isoflavone supplementation for 6 months was capable of significantly reducing SHBG concentrations by 31.1% only in the isoflavone group. Essentially similar results were shown by a study on postmenopausal women below 65 years of age, stating that daily consumption of isoflavones in the form of 25 g protein from soy products for one year decreased SHBG concentrations by 14.5% in the isoflavone group, but did not affect the control group.[15]

Several factors, such as age, body weight, sex steroids, and insulin, regulate plasma SHBG levels to a varying degree. SHBG levels have been associated with a number of diseases, including osteoporosis. In several studies, an inverse correlation was found between serum SHBG levels and BMD in both males and females. SHBG levels may be predictive of various macro-architectural features of cortical bone.[19]

High SHBG levels predict the occurrence of osteoporotic fractures of the vertebrae and peripheral bones, particularly the proximal femur. Estradiol and SHBG play an important role in bone loss and osteoporotic fractures. As

increased serum SHBG concentrations are associated with multiple fractures, measurement of serum SHBG level in the general practitioner's office may be useful for predicting the severity of osteoporosis.[20]

The variable effects of isoflavones on SHBG levels may be due to genetic factors, dosage differences, duration of supplementation, and type of isoflavone. The influence of genetic factors on SHBG concentrations is apparent from the positive correlation of isoflavone supplementation with SHBG concentrations found in women with the genetic SHBG N variant (p=0.006), but not in those with the D variant (p=0.999).[21] Isoflavones are steroids, and they exert their actions on target cells in two ways, i.e. activation of both intracellular and membrane ERâ, and interaction with steroidal hormone metabolism.[9] Regarding the reduction in SHBG concentrations by isoflavones, there are two possibilities. The first possibility is that isoflavones are bound by SHBG to form isoflavone-SHBG complexes, causing the isoflavones to become unavailable to the target cells. The second possibility is that isoflavones are bound by SHBG receptor-SHBG complexes (rSHBG – SHBG) on the surface of the cell membrane, subsequently activating their genes according to the signal tranduction theory.[22] These cell surface-bound isoflavone- rSHBG - SHBG complexes are then used for activating bone formation by osteoblasts, causing them to undergo proliferation and differentiation, and form mineral deposits (mineralization).[23] Studies on femoral-metaphyseal tissues of aged female rats demonstrated that genistein and genistin increased the osteoblastic calcium content, the activity of alkaline phosphatase as marker enzyme in osteoblasts, and their DNA content as an index of bone cell numbers.[24] Phosphogenistein and phosphodaidzein increased bone components in tissue culture. Phosphoisoflavones containing low levels of genistein and daidzein had no effect on bone components, such that it was assumed that the OH group at position 7 in genistein and daidzein plays a role in the anabolic effect of isoflavones on bone components.[25]

A study on postmenopausal women showed that the combination of genistein and daidzein increased lumbal BMD scores and decreased bone resorption.[26] Other phytoestrogens, such as resveratrol, may also be beneficial in osteoporosis.[27] Isoflavones also have direct effects on protein synthesis. Genistein enhances the capacity of osteoblasts for producing collagen and alkaline phosphatase, and even in nonosteogenic media mineralized bone nodules are formed.[28] Genistein, but not daidzein, is bound to estrogen receptors (á and â). Both genistein and daidzein are bound to transcription proteins within osteoblastic cells. There have been no studies demonstrating any effects of genistein or daidzein on various protein kinases and protein phosphatases that show a relationship between osteoblastic proliferation and DNA synthesis.[29] It has been demonstrated that genistein increases production of estrogens by increasing the activity of aromatases and mRNA. In estrogen production, genistein also activates several protein kinases and transcription factors, such as cyclic AMP response element (CRE)-binding protein (CREB) in gene regulation.[30]

SHBG concentrations can be affected by several factors, such as genetic factors, by pregnancy, exogenous steroidal sex hormones, and body weight. The genetic factor that has been most investigated is polymorphism of the SHBG gene located on chromosme 17. Recent studies have reported that several SHBG genotypic variants are strongly associated with SHBG concentrations in postmenopausal women, but have no effect on BMD.[31] However, several studies have shown that SHBG levels are negatively correlated with BMD.[32,33] SHBG genetic variants also influence estrogenic activity in normal as well as neoplastic tissues.[34,35]

Soy isoflavones are effective in raising lumbar BMD scores[26] and reducing bone

resorption in postmenopausal women.[36] Bone resorption is performed by osteoclasts, while osteoblasts are responsive to bone synthesis and mineralization, especially during bone formation and remodeling.[37] The isoflavones genistein and daidzein stimulate osteoblasts and inhibit osteoclasts.[38] These isoflavones decrease the number of osteoclasts, thus reducing bone resorption. In this connection, with a stable number of osteoblasts and an increase in bone components, bone BMD will of necessity also be increased. In addition, genistein also enhances mineralization of the extracellular matrix.[39]

Other studies show that calcium supplementation effected some increase in BMD, but did not reduce fractures and increased the risk of renal calculus.[40] Low calcium intakes result in increased bone resorption, decreased bone mass, and increased osteoporosis. In Japanese postmenopuasal women, low calcium intakes of less than 400 mg/day was associated with increased bone resorption but not with bone formation.[41]

One limitation in this study is that no investigations were conducted on SHBG polymorphism of the subjects, although SHBG polymorphism has been found to affect SHBG levels. Investigations of SHBG polymorphism should be done for accurate evaluation of the effect of isoflavone supplementation on SHBG concentrations in postmenopausal women. Another limitation is that the supplementation was conducted in the homes of the subjects and thus it was difficult to monitor whether or not the subjects took other supplements.

CONCLUSIONS

Isoflavone supplemenatation for 6 months decreased SHBG concentrations in osteopenic and osteoporotic postmenopausal women. Soy supplementation may have increased the availability of estrogens without affecting actual concentrations.

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