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EFFECTS OF SOY PROTEIN AND SOYBEAN ISOFLAVONES ON BONE HEALTH

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Abstract

Soybeans and soyfoods are for practical purposes, the only nutritionally relevant dietary sources of isoflavones. Soybean isoflavones are structurally similar to estrogen, bind to estrogen receptors, and exhibit weak estrogenic activity. Soyfoods and soybean constituents have come under investigation for their role in chronic disease prevention, especially for heart disease and cancer. Two factors, in particular, provided the basis for initial speculation that soy might contribute to bone health. These are the estrogenic properties of soybean isoflavones and the effectiveness of the synthetic isoflavone, ipriflavone, in reducing bone loss in perimenopausal and postmenopausal women. In addition, several human studies have shown that soy protein, when substituted for animal protein, decreases calcium excretion. This review will focus on some of the clinical researches relevant to the effects of both soy protein and soybean isoflavones on bone health.

Keywords:

Isoflavones**,** Soy Protein, Bone Health, Soybeans

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Introduction

Soybeans have been consumed in Asia since ancient times. Compared with Caucasians, the low incidence of heart disease, reproductive cancers, hip fracture, and climacteric symptoms in Asians has been considered to be associated with their high intake of soy foods. Recent growing interest in health and diet has led to an increased focus on soy foods and their functional components, e.g. isoflavones. Soybean isoflavones are structurally similar to estrogen, bind to estrogen receptors, and exhibit weak estrogenic activity. Isoflavones exert beneficial health effects by acting as antioxidants, tyrosine kinase and topoisomerase inhibitors as well as estrogenic activity. It has been reported that they play an important role in the prevention of chronic diseases, including osteoporosis, cardiovascular diseases, hormone-dependent cancer, and postmenopausal syndrome (Adlercreutz, 2002; Lampe, 2003; Magee and Rowland 2004; Messina et al., 2006).

During the past several years, soyfoods and soybean constituents have come under investigation for their role in chronic disease prevention, especially for heart disease and cancer. (Lampe, 2003; Magee and Rowland 2004; Messina et al., 2006). In fact, recently, the US Food and Drug Administration approved a health claim for the cholesterol lowering properties of soy protein. In addition to heart disease and cancer, there is limited research indicating that soy protein favorably affects renal function. For example, several studies have found that unlike animal protein, soy protein does not increase postprandial glomerular filtration rates or renal blood flow (Kontessis et al., 1990; Pecis et al., 1994; Nakamura et al., 1989). Also, some research indicates that soy protein, when substituted for animal protein, decreases proteinuria in individuals with chronic renal disease (Chan et al., 1988; Barsotti et al., 1988; D'amico and Gentile, 1993) It can be argued, however, that despite the many potential health benefits, the most natural role for soy may be its role in reducing risk of osteoporosis. Two factors, in particular, provided the basis for initial speculation that soy might contribute to bone health. These are the estrogenic properties of soybean isoflavones (Markiewicz et al., 1993; Mayr et al., 1992) and the effectiveness of the synthetic isoflavone, ipriflavone, in reducing bone loss in perimenopausal and postmenopausal women (Valente et al., 1994; Brandi, 1992; Civitelli, 1997). In addition, as discussed below, several human studies have shown that. soy protein, when substituted for animal protein, decreases calcium excretion (Breslau et al., 1988; Anderson et al., 1987). This review will focus on some of the clinical researches relevant to the effects of both soy protein and soybean isoflavones on bone health.

Soy Isoflavones

Isoflavones are a subclass of the more ubiquitous flavonoids and have a similar chemical structure to estrogen. However, unlike flavonoids, isoflavones have an extremely limited distribution in nature. Only in soybeans are isoflavones present in nutritionally relevant amounts. The primary isoflavones in soybeans are genistein and daidzein, although a third isoflavone, glycitein, is also present, but in much smaller amounts.

The United States Department of Agriculture as well as several other groups have published values for the isoflavone content of a variety of soy products (Coward et al., 1993; Reinli and Block, 1996; Murphy et al., 1999). One serving of a traditional soyfood, such as a cup of soymilk or 3 to 4 oz of tofu, contains about 30 mg of isoflavones. Isolated soy protein (90% protein) and defatted soy flour (about 50% protein) contain about 0,5 to 2 mg and 2 to 3 mg isoflavones/gram protein, respectively. Isoflavones are quite heat stable. Baking or frying does not alter total isoflavone content (Coward et al., 1998).

Recent data indicate that the isoflavone intake of Japanese adults in Japan is about 30 mg/d (Nagata et al., 1998). Not unexpectedly, intake in United States is likely no more than a few miligrams per day, with the exception of certain subpopulations such as vegetarians and Asian-Americans (Juturu et al., 1999).

Human Studies

A. Bone Turnover

Neverthless, studies are somewhat consistent in showing that soy inhibits bone turnover in post-menopausal women. The first group to examine this issue, Murkies and colleagues(1995), conducted a short-term study (12 weeks) in which diets of postmenopausal women were supplemented with either

wheat or soyflour (45 g/day, 52 mg isoflavones). Although the primary end point of this study was hot flashes, urinary hydroxyproline, a nonspecific marker of bone resorption, increased significantly in the women in the wheat group but not in the soy flour group, although there were no statistically significant differences between groups.

Three other studies also found that soy feeding was associated with a decrease in bone resorption. Postmenopausal women fed 40 g soy protein (76 mg of isoflavones) per day for 12 weeks exhibited decreases in urinary d-pyridinoline ($P<0.05$) and decreases in urinary N-telopeptide (NTx) concentrations ($P<0.001$), whereas there were no changes in these bone- specific markers in the placebo (casein) group (Pansini et al., 1997; Albertazzi et al., 1998). In another study, soyfood (60 to 70 mg isoflavones/day) consumption was found to decrease urinary excretion of NTx by 13,9% ($P<0.02$) and to increase serum osteocalcin by 10,2% ($P<0.03$), suggesting an increase in osteoblastic activity (Scheiber et al., 1999). However, there was no control group in this study. Interestingly though, there was a significant negative correlation between urinary NTx and serum isoflavone concentrations. Finally Wong, (1999) reported that isoflavone supplementation (160 mg/day) decreased urinary concentrations of deoxypyrindinoline (Dpd) and increased serum concentrations of osteocalcin and bone-specific alkaline phosphatase activity, although differences were not statistically significant. However, since the magnitude of the changes were similar to those seen with estrogen administration, the lack of statistical significance was likely due to the very small sample size (N=6).

The final study in postmenopausal women was by Washburn and colleagues (1999), who reported the effects of soy protein on alkaline phosphatase activity, a nonspecific marker for bone formation, in a double-blind randomized crossover study in which three different diets were fed to subjects for 6 weeks each. One of the diets was supplemented with 20 g carbohydrate and the other two were supplemented with 20 g soy protein (34 mg of isoflavones); one of these groups consumed soy protein once per day and the other twice per day. Alkaline phosphatase activity decreased significantly in women on either soy diet compared with the carbohydrate-supplemented diet ($P<0.05$), suggesting that bone formation declined. However, since bone resorption markers were not examined, the significance of this finding, if any, is difficult to determine.

In contrast to the above studies, Alekel and colleagues (2000) recently completed a trial a in peri-menopausal women (N=69) randomly assigned (double-blind) to one of three treatment groups who did not exhibit any decline in bone resorption during the course of treatment. These women entered the study in four waves or cohorts with approximately equal presentation from each of the three treatments (isoflavone-rich soy protein isolate, or SPI+; isoflavone- deficient soy protein isolate, SPI-; and whey protein, control)in each cohort. Subjects consumed 40 g of protein (soy or whey) for 24 weeks. Repeated measures of ANCOVA indicated that both time ($P\leq0,005$) and baseline value ($P\leq0,0001$) were very significant, whereas treatment had no significant effect on either NTx ($P= 0,12$) or bone alkaline phosphatase ($P= 0,32$). Interestingly, cohort had a significant effect on NTx ($P=0,0089$), but not phosphatase ($P=0,56$), suggesting that cohort may reflect a seasonal effect on bone resorption.

B. Bone Mineral Density

Dalais and colleagues (1998) fed postmenopausal women 45 g of soy grits (flour)/day (52 mg of isoflavones) for 12 weeks and found that bone mineral content (BMC) significantly increased 5,2% ($P<0.03$) although there was no change in bone mineral density (BMD). However, not only is the magnitude of this increase surprising, but there were also increases in BMC, albeit not significant, in both the control group who were fed wheat flour and a group of woman fed flaxseed. Clearly, these results need to be followed up to draw meaningful conclusions.

The next two studies provide support for he hypothesis that isoflavones are the component of soy responsible for the protective effects on bone. In the study by Alekel and colleagues (2000) previously described, percentage change in lumbar spine BMD or BMC did not decline in the SPI+ or SPI- groups; however, significant loss occurred in the control group. Absolute values for bone at baseline and post-treatment were not statistically different among the three groups. Results of ANCOVA indicated that treatment has a significant effect on percentage change in BMC ($P=0.021$), but not on percentage change in BMD ($P=0.25$). However, when various contributing factors were taken into account using multiple-regression analysis, SPI+ had a significant positive treatment effect on the percentage change in both BMD (5,6%, $P=0.023$) and BMC (10,1%, $P=0.0032$), while the other treatments had no effect. There was no effect of any treatment on bone sites other than the lumbar spine.

In agreement with the study by Alekel and colleagues (1999) are results from Potter and colleagues (1998), who found that in postmenopausal women consuming 40 g of soy protein/day (90 mg of isoflavones) for 6 months there was a statistically significant increase in lumbar spine BMD, whereas there were decreases in BMD in the woman fed 40 g of soy protein containing a lesser amount of isoflavones (56 mg) or 40 g of casein-based milk protein. However, as was also observed by Alekel and colleagues (1999), there were no differences among treatments at the other bone sites measured. The findings by Alekel and colleagues and Potter and colleagues, which suggest that isoflavones are responsible for the bone-sparing effects of soy protein, are consistent with those of Arjmandi and colleagues (1998) in animals and Scheiber and colleagues (1999) in women.

In contrast to the three previously cited studies, Gallagher and colleagues (1999) found that soy protein, regardless of isoflavone content, had no effect on BMC. Over a 9-month period, early postmenopausal women were fed 40 g soy protein containing one of three levels of isoflavones: (1) little or no isoflavones, (2) 52 mg, or (3) 96 mg per day. There were no significant differences among the three groups in spinal, femoral neck, or trochanteric BMD during the intervention phase. Gallagher has commented that the lack of effect may have been because women in the study were 1 to 5 years postmenopausal (personal communication). During this period of time twice the dose of estrogen may be needed compared with that required by older women to reduce bone loss. Another consideration is that if components in soy other than isoflavones are responsible for the purported effects on bone, this study would not have been able to identify protective effects since soy protein was fed to each group.

Effects of Soy on Bone Health: Possible Mechanisms

A variety of mechanisms have been proposed for the favorable effects of soy protein/isoflavones on bone health. The effects of soy protein on renal function may have been responsible for the higher BMD in soy-fed rats in a long-term study (Kalu et al., 1988). Additionally, when substituted for animal protein, soy protein has been shown to result in lower urinary calcium excretion. However, these effects are likely not responsible for the favorable effects observed in clinical and epidemiological studies since both isoflavone-poor and isoflavone-rich soy protein would be expected to have similar effects on renal function and calcium excretion. In contrast, two human studies found that isoflavone-rich but not isoflavone-poor soy protein favorably affected BMD (Alekel et al., 1999; Potter et al., 1998). Furthermore, Scheiber and colleagues (1999) found there was a significant negative correlation between urinary NTx and serum isoflavone concentrations. Thus, although the effects of soy protein on calcium excretion, and to a lesser extent renal function, may be clinically relevant, there is considerable evidence indicating that isoflavones have direct beneficial skeletal effects. In addition to the three human studies cited above, supporting evidence emerges from work in cells, (Anderson and Garner, 1997; Yoon et al., 1998; Stephan and Dziak, 1994; Williams et al., 1998; Blair et al., 1996; Gao and Yamaguchi, 1999; Yamaguchi and Gao, 1998) organ culture, (Yamaguchi et al., 2000; Yamaguchi and Gao, 1998; Gao and Yamaguchi, 1998; Gao and Yamaguchi 1999) and animal models (Blair et al., 1996; Ishimi et al., 1999; Fanti et al, 1998; Ishida et al., 1998; Anderson et al., 1998) in which isolated isoflavones have been employed.

The estrogenic effects of isoflavones are well established. Yet it is not clear that isoflavones exert their effects on bone by binding to estrogen receptors. Of interest are the results from several studies which found that soy protein or isolated isoflavones exerted favorable effects on BMD with either minimal or no increase in uterine weight in contrast to the markedly increased uterine weight in response to estrogen administration (Blair et al., 1996; Ishimi et al., 1999; Fanti et al., 1998; Ishida et al., 1998, Arjmandi et al., 1996). These data do not in any way preclude the possibility that isoflavones exert estrogenic effects on bone tissue. In fact, in culture, Yamaguchi and Gao (1998) reported that the antiestrogen tamoxifen blocks the ability of genistein to inhibit parathyroid-induced bone resorption. However, they do indicate that isoflavones are tissue selective. Many researches in this area, thus, consider soy isoflavones as naturally occurring selective estrogen receptor modulators (SERMs).

Several human studies found that bone resorption markers were decreased relative controls; (Murkies et al., 1995; Pansini et al., 1997; Albertazzi et al., 1998; Scheiber et al., 1999; Wong, 1999) however, other findings suggest soy may also stimulate bone formation (Scheiber et al., 1999; Wong, 1999). Interestingly, in rodents, Ishida and colleagues (1998) found that while both daidzin and genistin retarded done loss, daidzin but not genistin inhibited bone turnover induced by ovariectomy. Thus, genistin

appeared to retard bone loss by increasing bone formation. Fanti and colleagues (1998) suggested that genistein stimulates bone formation by suppressing the activity of one or more cytokines, whereas Blair and colleagues (1996) suggested that genistein may suppress osteoclastic activity through its ability to inhibit tyrosine kinases.

Potentially important insight into the action of genistein comes from a study that found that, in culture, blocking the action of transforming growth factor-factor β (TGF-β) has been shown to prevent the inhibitory effects of estrogen on bone breakdown (Hughes et al., 1996). Kim and colleagues (1998) have found that, at least in breast cells, genistein increases TGF-β levels.

Finally, as noted at the outset, the soybean isoflavones have a similar chemical structure to the syntehetic ipriflavone, which has been shown to be quite effective in inhibiting bone loss in perimenopausal and postmenopausal women (Valente et al., 1994; Brandi, 1992; Civitelli, 1997). In fact, daidzein is a metabolite of ipriflavone. There are data indicating that ipriflavone favorably affects both bone resorption and bone formation (Ohta et al., 1999; Arjmandi et al., 2000). However, the standard dose of ipriflavone is 600 mg/day and daidzein does not appear to be one of the metabolites of ipriflavone responsible for its effects on bone (Cheng et al., 1994; Petilli et al., 1995). Interestingly, Ishida and colleagues (1998) found that while both genistin and daidzin retarded bone loss in ovariectomized rats, ipriflavone was without effect. Thus, the extent to which insight on the mechanism of action of isoflavones on bone tissue can be gained by looking at ipriflavone is not clear. Clearly though, there are data indicating that isoflavones may both inhibit bone resorption and stimulate bone formation and that the proposed mechanisms for the effects of isoflavones on bone tissue include both estrogenic and nonestrogenic effects.

Conclusions and Public Health Implications

Overall, the evidence that soy protein and/or its isoflavones favorably affect BMD is promising. However, because of the limited data, no firm conclusions can be made at this time. Fortunately, several human studies are under way. Therefore, considerably more knowledge about this area of research will be available within just a few years.

Thus far, with only one exception, the existing human studies are supportive of protective effects of soy with studies having only been conducted in women. Neverthless, the strength of the evidence clearly justifies conducting long-term human studies using either isolated isoflavones or soyfoods. Isoflavone supplements may allow for better compliance and for less confounding by other dietary variables as when soyfoods are added to the diet. Data clearly suggest isoflavones are the primary components of soy acting on bone tissue. However, to date, human studies have used only soy protein. Moreover, the use of soy protein allows for the possibility that nonisoflavone components of soy, although unlikely, contribute to the bone-related effects. Therefore, before conducting long-term human trials using isolated isoflavones, the effects of using isoflavone supplements vs. soy protein on bone turnover and BMD should be directly compared in short-term studies.

Soyfoods cannot be recommended at the present time as a substitute for estrogen replacement. But soyfoods can be strongly recommended for those women who choose not to use estrogen. How much soy should be consumed? Few dose-response studies have been conducted. Neverthless, human data suggest 60 to 90 mg of isoflavones per day may be needed, or about two to three servings of traditional soyfoods. From a practical perspective, incorporating an amount of soy into the diet that will provide this level of isoflavones will be a challenge for many consumers. However, the food industry is responding with new soy products that should make such dietary changes possible. Furthermore, it may be that lesser amounts of soy protein or soy isoflavones consumed over the course of a lifetime will exert favorable effects on bone tissue. This remains to be determined.

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