

The Effects of Soy Isoflavones in Postmenopausal Women: Clinical Review

Eliana Aguiar Petri Nahas* and Jorge Nahas-Neto

Department of Gynecology and Obstetrics, Botucatu Medical School, UNESP- Sao Paulo State University, Brazil

Abstract: The hormonal therapy (HT) is recommended for postmenopausal women primarily for the relief of vasomotor symptoms, treatment of vaginal atrophy, and prevention of osteoporosis. Despite these important benefits, only 35% to 40% of the women ever start HT, and many do not continue it. The reasons for discontinuation include resumption of bleeding, perceived risks of breast cancer, unacceptable side effects and the belief that treatment is no longer necessary. As a result, there is an increasing interest in the use of plant-derived estrogens, also known as phytoestrogens, which seem to be very promising. Isoflavones is the most investigated subgroup of phytoestrogens. They are attenuated estrogens and behave both *in vivo* and *in vitro* as agonists and antagonists. The highest concentrations of isoflavones are found primarily in soy beans. In this study, the effects of soy isoflavones on postmenopausal women were reviewed.

Key Words: Soy isoflavone, postmenopausal women, clinical review.

INTRODUCTION

Some facts about menopause are applicable to all women: menopause is a natural event to be experienced by every woman. The common denominator is cessation of menstrual cycles. On the other hand, in relation to the symptoms and risks for osteoporosis, cardiovascular diseases, cancer and Alzheimer disease, every woman is unique. The notion that all postmenopausal women need hormonal therapy (HT) or can be treated with the same dose and type of therapy, is irrational and inappropriate [1]. HT is recommended for postmenopausal women primarily for the relief of vasomotor symptoms, treatment of atrophic vaginitis and preservation of bone mass [2]. Despite these well known benefits, approximately 70% of the women who start HT discontinue therapy within a year [3]. One of the main reasons for discontinuation is irregular bleeding. Others include mastalgia, nausea, migraine, weight gain and edema besides fear of breast cancer [4,5]. These effects have led many women to choose a "more natural" approach. Moreover, HT is contraindicated to some women to whom little may be offered for the relief of climacteric symptoms [6]. Therefore, there is an increasing interest in developing alternative therapies that show the benefits of HT without its side effects and contraindications [4,7]. Soy and isoflavone dietary supplements are regularly used by millions of North Americans for symptoms associated with menopausal transition or for other purported health benefits [8]. In a recent consensus, the North American Menopause Society recommends changes in lifestyle either alone or along with dietary isoflavones, black cohosh, or vitamin E. Estrogen-therapy, however, remains the standard treatment for severe symptoms in patients without contraindications to HT [9].

SOY ISOFLAVONE

Phytoestrogens are naturally occurring plant compounds that are structurally and functionally similar to estradiol [10].

There are four major classes of phytoestrogens: isoflavones, found in soy beans and soy products; lignans, found in whole grains, cereal and oilseeds; flavonoids, found in some fruits and legumes; and, coumestans found in bean and alfalfa sprouts [11,12]. Isoflavones are the phytoestrogens with the most potent estrogenic activity, being genistein, daidzein and glycitein, found in soy beans, their most active components [13] (Table 1). Soybeans are leguminous plants that have been cultivated in Asia, especially in China, Korea and Japan, for over 1000 years. However, due to their outstanding nutritional properties, they have been introduced to Western diets [14].

Soy isoflavones are non-steroidal compounds that bind poorly to estrogen receptors (100 times less affinity than estradiol) [10]. This binding ability is due to the fact that the distance between their hydroxyl groups is the same as that found in the 17 β -estradiol molecule. Thus, as isoflavones bind to estrogen receptors, a complex functionally equivalent to that formed by 17 β -estradiol is formed. Soy isoflavones (genistein and daidzein) preferentially bind to β -estrogen receptors that are found in the central nervous system, bones, vascular walls and the urogenital tract. Unlike estrogens, isoflavones have little affinity with the α receptors of breast and uterine tissues [15]. Depending on the concentrations of estradiol and the saturation of the receptors, they exert a selective action, i.e., in some tissues they display proestrogenic responses whereas in others they inhibit estrogenic action [16-18].

Soy isoflavones are naturally found as inactive glycosides. Following oral ingestion, complex enzymatic metabolic conversions take place in the gastrointestinal tract, resulting in the formation of heterocyclic phenols structurally similar to 17 β -estradiol. Absorbed by the enterocytes, isoflavone metabolites reach the peripheral circulation and are excreted in 7 to 8 hours [10,11,19]. Aglycones (genistein, daidzein and glycitein) are the active forms which are actually absorbed. Absorption rate ranges from 20% to 55% [18]. The concentrations of the different isoflavone metabolites, as well as their clinical effects, vary widely from individual to individual even when a controlled quantity is administered. Therefore, it is difficult to determine the ideal dosage. Some recommend from 30 mg to 100 mg/day [3,12,20,21].

*Address correspondence to this author at the Department of Gynecology and Obstetrics, Botucatu Medical School - UNESP- Sao Paulo State University, Rubiao Junior - Botucatu- ZIP 18618-970 - Sao Paulo, Brazil; Tel: +55 14 38116227; Fax: +55 14 3882 1933; E-mail: epetri@fmb.unesp.br

Table 1. Total Values of Isoflavones in Foods

Foods	Total (µg/g)	Genistein (µg/g)	Daidzein (µg/g)	Glycitein (µg/g)
Roasted Soybeans	2661	1426	941	294
Textured Soy Protein	987	640	191	156
Tempeh	865	422	405	38
Tofu	532	245	238	49
Soy Drink	28	21	7	–

Values in the table are expressed as µg/g. (Modified from Wang & Murph, 1994 [94]).

SOY ISOFLAVONE AND CLINICAL EVIDENCE

Vasomotor Symptoms

There is evidence that isoflavones reduce both the severity and the frequency of menopause-related vasomotor symptoms [22]. Most observations on the use of phytoestrogens are epidemiologic, based on studies conducted in regions where soy is highly consumed [17]. Fewer than 25% of Japanese and 18% of Chinese women experience hot flushes compared with 85% of North American and 70%-80% of European women. These differences have been partially attributed to phytoestrogen consumption [11,23-25]. Even though some clinical studies have demonstrated the efficacy of isoflavones in reducing the frequency and severity of hot flushes [6,23,24,26-31], others have not found differences between treated and non-treated groups [32-34]. Albertazzi *et al.*, in a double-blind, placebo-controlled study, found that 60g/day of isolated soy protein (food supplementation) was superior to placebo, reducing vasomotor symptoms in 50% [6]. Faure *et al.* reported a 61% reduction in the frequency of hot flushes with 70 mg of soy genistein versus a 21% reduction with placebo [29]. Albert *et al.*, in multicentric, observational, no-randomized trial, observed a significant reduction in climacteric symptoms with the use of 35 mg of a phytoestrogen preparation derived from *Glycine max* (L.) [28]. Nahas *et al.*, in a double-blind, placebo-controlled study demonstrated the alleviation of vasomotor symptoms in 44% of the post-menopausal women using soy germ versus a 10% decrease with placebo [31]. Crisafulli *et al.*, in a double-blind, randomized study, evaluated 90 women aged 47-57 years for 12 months and observed that hot flushes were reduced in 24% of the women on genistein (54 mg) and 54% in those on estradiol (1 mg) combined with norethisterone (0.5 mg) as compared to placebo [30]. However, Van Patten *et al.*, in a double-masked, randomized clinical trial, studying the effectiveness of soy beverage (90 mg of isoflavone) in the treatment of hot flushes in postmenopausal women with breast cancer, found no reduction in the number or severity of hot flushes between the treated group and the placebo group [33]. Burke *et al.*, in randomized clinical trial, also found no reduction in hot flushes when 42 mg or 58 mg of isoflavone were used [34]. In 2005, Kok *et al.*, in randomized, placebo-controlled trial, evaluated the quality of life of 202 women aged 60-75 years and observed no marked effect of soy protein supplementation on health status, life satisfaction and depression [35].

Vaginal Atrophy

In a pioneer study, Wilcox *et al.*, in observational, no-randomized trial, demonstrated that dietary supplementation with soya flour (45g) improved vaginal cell maturation in 25 postmenopausal women [36]. However, results are controversial, making it difficult to determine whether phytoestrogens have a beneficial effect on the vaginal epithelium. In randomized clinical trials, Dalais *et al.*, Chiechi *et al.*, and Uesugi *et al.* observed a significant increase in the index of vaginal maturation in postmenopausal women receiving soy supplementation [26,37,38]. In 2004, Nahas *et al.*, in a double-blind, placebo-controlled study, found that the average vaginal maturation rate remained unchanged for 6 months in women using 60 mg of isoflavone, suggesting the maintenance of vaginal trophism in this group whereas those in the placebo group experienced worsening [31]. However, other studies failed to demonstrate a significant improvement in the vaginal epithelium [24,39-41]. In a recent randomized study, Nikander *et al.* evaluated 64 women with breast cancer and observed that isoflavone (114mg/day for 3 months) did not alleviate vaginal dryness while maturation of the vaginal epithelium remained unchanged [42]. Dietary soy supplementation seems to exert minimal effects on the female reproductive tract [39].

Cardiovascular Effects

Dyslipidemia, arterial hypertension and central obesity have been associated with a higher risk for cardiovascular disease (CVD) in postmenopausal women. Klejn *et al.* evaluated the diet of the 939 women participating in the Framingham Offspring Study, and demonstrated that waist/hip ratio and triglycerides were significantly lower in the women on an isoflavone-rich diet than in those who were not. Furthermore, they suggested that a high postmenopausal intake of soy derivatives is associated with a favorable metabolic profile for CVD [43]. In 2003, Goodman-Gruen & Kritz-Silverstein studied body fat in 208 women aged 45-74 years and observed that usual dietary isoflavone was associated with reduced total body fat, suggesting that isoflavone may play a role in the prevention of obesity-related chronic diseases [44]. In a recent cohort study, 16,165 European women aged 49-70 years were assessed with regard to their dietary phytoestrogen intake and its association with CVD. During an average follow-up of 75 months, 372 women experienced a coronary event and 147 a

stroke. No correlation between phytoestrogen intake and risk for CVD was observed [45].

A soy-rich diet may benefit the cardiovascular system due to its favorable effect on vascular reactivity and lipid profile [17,46,47]. Some studies have associated isoflavone with a significant reduction in LDL and triglycerides, and increase in HDL [22]. In 1995, Anderson *et al.*, in a meta-analysis of 38 controlled clinical trials, examined the relation between soy protein consumption and serum lipid concentrations. They found an average reduction of 13% in LDL and no change in HDL [48]. However, others have demonstrated average HDL increases ranging from 3.7% to 28.6% [31,41,49-51]. In recent meta-analysis of the effects of soy protein on lipid profile, Zhan & Ho reviewed 23 trials published from 1995 to 2002, and reported that isoflavone was associated with significant decreases in serum total cholesterol (3.77%), LDL (5.25%) and triglycerides (7.27%) and increases in HDL (3.03%) [52]. These changes were related to the level and duration of intake. Some studies have shown that intakes greater than 80 mg/day are more beneficial to lipid profile. The effects on total cholesterol and LDL can be observed in a short term while those on HDL are seen after 12 weeks of treatment. The benefits on lipid profile led The United States Food and Drug Administration to conclude that "a diet low in saturated fat and cholesterol, including 25 grams/day of soy protein may reduce the risk for coronary heart disease" [53].

Osteoporosis

Over the first years following menopause, due to hypoestrogenism, bone mass loss rapidly occurs and the risk for osteoporosis largely increases. Only 8% to 10% of the women undergo HT to preserve bone mass. Phytoestrogens have been proposed as an alternative to HT to prevent osteoporosis and show beneficial effects on bone health [5,54-56]. In the skeleton, isoflavones interact directly with bone cell estrogenic receptors. *In vitro*, daidzein and genistein promote the growth and the differentiation of osteoblasts besides stimulating bone formation [57,58]. Blum *et al.* and Li & Yu demonstrated that, in ovariectomized rats, dietary soy had a beneficial effect on bone health, sustaining bone formation and preventing bone resorption [59,60]. On the other hand, Cai *et al.*, in a similar study, observed that isoflavone, in comparison with estradiol, did not prevent trabecular bone loss without affecting calcium balance [61].

Research on the effects of phytoestrogens on bone after menopause is at a relatively early stage [62]. The results of clinical studies, though variable, have been promising [58]. Upon clinical observation postmenopausal women with a high dietary consumption of isoflavone showed greater bone mineral density values at both the spine and the hip [54,63-65]. In 2003, Chen *et al.*, in a double-blind, placebo-controlled study, evaluated 203 postmenopausal women and observed that the hip bone mineral density was higher in the group using 80 mg of isoflavone [66]. In 2004, these same investigators observed that the effect of soy on bone mass was more marked in women in later menopause or lower body weight [67]. On the other hand, some investigators detected no effect on bone mineral density after 6 to 12 months of soy isoflavone supplementation [68-70]. Morabito

et al., in a double-blind, placebo-controlled clinical trial, studying bone markers in women at early menopause, demonstrated that genistein reduced bone resorption and increased bone formation [71]. Similarly, Arjmandi *et al.* reported that soy supplementation positively influenced the insulin-like growth factor (IGF-I), known to correlate with bone formation, and reduced urinary deoxypyridinoline, a biomarker of bone resorption, in 71 postmenopausal women [70]. IGF-I exerts a strong effect on cell proliferation and differentiation. Although IGF-I declines with aging, there is evidence that its synthesis and activity are affected by nutritional status as well as by insulin and gonadal steroids [72]. Adams *et al.*, observed no changes in serum IGF-I in soy isoflavone users [73]. The majority of the studies of the effects of isoflavone on bone mass is of short duration, with relatively small sample sizes making it difficult to determine the significance of the results [58]. There are no studies available about bone fractures. The collective data suggest that diets rich in isoflavone have bone-sparing effects although the magnitude of such effects and the exact mechanism of action are speculative. To Weaver & Cheong, the beneficial effects of isoflavone on bone health are still inconclusive [74].

Body Composition

Sarcopenia, the loss of muscle mass that is associated with aging, is the direct cause of muscle strength reduction [75]. After the age of 50 years, muscle mass is reduced in approximately 6% every ten years while muscle strength decreases 15% between the age of 60 and 70, 30% thereafter [76]. Sarcopenia and physical inactivity positively correlate with reduction in bone density in postmenopausal women. However, adequate nutrition and physical exercises can delay this process and improve the life quality of aged women [77]. Wu *et al.*, examined the effect of soy isoflavone combined with moderate physical exercise on body composition in ovariectomized mice. By using whole body densitometry, they demonstrated that this combination prevented body fat accumulation and bone mass loss as well as increased muscle mass in the treated group [78]. Deibert *et al.*, conducted a six-month study with 83 postmenopausal women divided into three groups: control, soy-rich diet with physical activity and soy-rich diet without physical activity. They concluded that a soy-rich diet along with physical activity can improve body composition, promote fat loss, and preserve muscle mass [79]. However, Möeller *et al.*, who followed up 69 women at perimenopause for 24 weeks on isoflavone-rich soy protein (n=24) or isoflavone-poor soy protein (n=24) or placebo (n=21), did not observe changes in body composition [80].

Cancer Risk/Benefit

The incidence of breast, colon, endometrial and ovarian cancer in Asian countries is lower than in Western countries [11]. Isoflavones may influence cell proliferation, playing an inhibitory role during the initiation and promotional stages of cancer [18,81]. In breast cancer cultured cells, isoflavones show a dose-dependent antiproliferative effect [25,82]. Experimental and observational studies provide evidence on the inverse association between breast cancer risk and phytoestrogen intake [81]. Maskarinec *et al.*, found no

changes in mammographic density among postmenopausal women using isoflavone compared with placebo, and thus suggested that no estrogenic effect was exerted on the breast tissue [83]. The proposed mechanisms of phytoestrogen-containing foods as they relate to the prevention of malignant tumors include inhibition of tyrosine kinases, suppression of angiogenesis, and antioxidant effects [7,84]. The antioxidant properties of isoflavones seem to be independent of receptor binding [8]. Dietary phytoestrogens that play an immuno-modulating role may interfere with the gene expression of interleukin-6 (IL-6) levels that progressively increase with age. Pharmacological modulations of IL-6 may favor the prevention of cancer progression, aging diseases, inflammatory disorders and immune homeostasis restoration [85]. Jenkins *et al.* observed elevated IL-6 concentrations following soy-protein intake (73 mg) suggesting that the estrogen-like effect of isoflavone can modulate immune response and thus provide an explanation for the lower incidence of cancer in regions where soy consumption is high [86].

Studies on the effects of isoflavone on the endometrium have demonstrated that the use of supplementation in postmenopausal women does not increase endometrial thickness when measured by ultrasound [87]. Kayisly *et al.*, studying the effects of genistein and daidzein on cultured human endometrial cells, found that isoflavones showed a weak estrogenic activity (39%-67% less than estradiol) [88]. However, Unfer *et al.*, in a randomized, double-blind, placebo-controlled study, followed-up 298 women who were randomly assigned to either 150 mg/day of isoflavone or placebo for 5 years and found that the occurrence of endometrial hyperplasia was significantly higher in the isoflavone group (3.37% versus 0.0%). Thus, these authors called into question the long-term safety of isoflavones with regard to the endometrium [89].

Adverse Effects

Although it is true that isoflavone have thus far exhibited few negative effects, very little is known about dose-response relationships, toxicity, synergistic effects, drug interactions, and effects of ingesting concentrated forms of isoflavone extracts [81]. Soy isoflavone is generally well tolerated, with the most common side effects being mild gastrointestinal upset (constipation, flatulence, nausea) [90]. Soybeans have been found to contain various allergenic proteins. Women with allergies, asthma or severe peanut allergy may experience an anaphylactic reaction to soy [91]. A final endocrine tissue sensitive to genistein is the thyroid [92]. Use of soy isoflavone has been found to affect concentrations of thyroxine [4]. Hypothyroidism may be observed in adults who preferentially consume soy products. Genistein and daidzein can block thyroid peroxidase and can thus inhibit thyroxine synthesis [93]. Additionally, women using levothyroxine in combination with soy foods should stagger the time of administration by at least 2 hours because soy protein can decrease levothyroxine absorption in the gastrointestinal tract [90]. Despite these concerns, there is no definitive evidence that the consumption of phytoestrogens is likely to be harmful in adults [4]. In a recent review of the current literature, Barnes concluded that isoflavones

consumed orally and in doses below 2mg/kg body weight per day should be considered safe for most population groups [5].

CONCLUSIONS

Soy isoflavones have been the focus of research over the past 10 years because of their potential roles in women's health. However, the body of published literature presents conflicting data. This may be attributed to the differences found among isoflavones, amounts used, estrogen status, and environment-genetic interactions, and individual absorption ability. The intestinal metabolism plays a key role in the determination of soy isoflavone potency. To date, even though some epidemiologic data and clinical studies suggest potential protective effects on specific target organs, further controlled, randomized studies are necessary to determine the real benefits of soy isoflavone to postmenopausal women.

REFERENCES

- [1] Notelovitz M. Guest Editorial. The clinical practice of the Women's Health Initiative: political vs biological correctness. *Maturitas* 2003; 44: 3-9.
- [2] The North American Menopause Society (NAMS). Recommendations for estrogen and progestogen use in peri- and postmenopausal women: October 2004 position statement of NAMS. *Menopause* 2004; 11: 589-600.
- [3] Elkind-Hirsch K. Effect of dietary phytoestrogens on hot flashes: can soy-based proteins substitute for traditional estrogens replacement therapy? *Menopause* 2001; 8: 154-6.
- [4] Glazier M, Gina MB, Bowman MA. A review of the evidence for the use of phytoestrogens as a replacement for traditional estrogen replacement therapy. *Arch Int Med* 2001; 161: 1161-72.
- [5] Barnes S. Phyto-oestrogens and osteoporosis: what is a safe dose? *Br J Nutr* 2003; 89(suppl. 1): S101-8.
- [6] Albertazzi P, Pansini F, Bonaccorsi G, Zanotti L, Forini E, De Aloysio D. The effects of dietary soy supplementation on hot flashes. *Obstet Gynecol* 1998; 91: 6-11.
- [7] Kass-Annese B. Alternative therapies for menopause. *Clin Obstet Gynecol* 2000; 43: 162-83.
- [8] Henderson V. Isoflavones: food for thoughtful consideration. *Menopause* 2003; 10: 189-90.
- [9] The North American Menopause Society (NAMS). Treatment of menopause-associated vasomotor symptoms: position statement of NAMS. *Menopause* 2004; 11: 11-33.
- [10] Mackey R, Eden J. Phytoestrogens and the menopause. *Climacteric* 1998; 1: 302-8.
- [11] Murkies AL, Wilcox G, Davis SR. Phytoestrogens. *J Clin Endocrinol Metab* 1998; 83: 297-303.
- [12] Branca F, Lorezenti S. Health effects of phytoestrogens. *Forum Nutr* 2005; 57: 100-11.
- [13] Sutar AC, Banavalikar MM, Biyani MK. Pharmacological activities of genistein, an isoflavone from soy (*Glycine max*): part II – anti-cholesterol activity, effects on osteoporosis & menopausal symptoms. *Indian J Exper Biol* 2001; 39: 520-5.
- [14] Golbitz P. Traditional soy foods: processing and products. *J Nutr* 1995; 125: 570S-2S.
- [15] Morito K, Hirose T, Kinjo J, *et al.* Interaction of phytoestrogens with estrogen receptors α and β . *Biol Pharm Bull* 2011; 24: 351-6.
- [16] Baker VL, Leitman D, Jaffe RB. Selective estrogen receptor modulators in reproductive medicine and biology. *Obstet Gynecol Surv* 2000; 55(suppl 2): S21-S47.
- [17] Lissin LW, Cooke JP. Phytoestrogens and cardiovascular health. *J Am Coll Cardiol* 2000; 35: 1403-10.
- [18] Wolters M, Hahn A. Soy isoflavones: a therapy for menopausal symptoms? *Wien Med Wochenschr* 2004; 154: 334-41.
- [19] Setchell KD, Brzezinski A, Brown NM, *et al.* Pharmacokinetics of a slow-release formulation of soybean isoflavones in health postmenopausal women. *J Agric Food Chem* 2005; 53: 1938-44.

- [20] Brouns F. Soy isoflavones: a new and promising ingredient for the health foods sector. *Food Res Int* 2002; 35: 187-93.
- [21] Messina M, Messina V. Provisional recommended soy protein and isoflavone intakes for health adults: rationale. *Nutr Today* 2003; 38: 100-9.
- [22] The North American Menopause Society (NAMS). The role of isoflavones in menopausal health: consensus opinion of NAMS. *Menopause* 2000; 7: 215-29.
- [23] Nagata C, Shimizu H, Takami R, Hayashi M, Takeda N, Yasuda K. Hot flushes and other menopausal symptoms in relation to soy product intake in Japanese women. *Climacteric* 1999; 2: 6-12.
- [24] Scambia G, Mango D, Signorile PG, *et al.* Clinical effects of a standardized soy extract in postmenopausal women: a pilot study. *Menopause* 2000; 7: 105-11.
- [25] Davis SR. Phytoestrogen therapy for menopausal symptoms: there's no good evidence that it's any better than placebo. *BMJ* 2001; 323: 354-5.
- [26] Dalais FS, Rice GE, Wahlqvist ML, *et al.* Effects of dietary phytoestrogens in postmenopausal women. *Climacteric* 1998; 1: 124-9.
- [27] Upmalis DH, Lobo R, Bradley L, Warren M, Cone FL, Lamia CA. Vasomotor symptom relief by soy isoflavone extracts tablets in postmenopausal women: a multicenter, double-blind, randomized, placebo-controlled study. *Menopause* 2000; 7: 236-42.
- [28] Albert A, Altabre C, Baro F, *et al.* Efficacy and safety of soy a phytoestrogen preparation derived from *Glycine max* (L.) Merr in climacteric symptomatology: a multicentric, open, prospective and non-randomized trial. *Phytomedicine* 2002; 9: 85-92.
- [29] Faure ED, Chantre P, Mares P. Effects of a standardized soy extract on hot flushes: a multicenter, double-blind, randomized, placebo-controlled study. *Menopause* 2002; 9: 329-34.
- [30] Crisafulli A, Marini H, Bitto A, *et al.* Effects of genistein on hot flushes in early postmenopausal women: a randomized, double-blind EPT and placebo-controlled study. *Menopause* 2004; 11: 400-4.
- [31] Nahas EP, Nahas-Neto J, De Luca L, Traiman P, Pontes A, Dalben I. Benefits of soy germ isoflavones in postmenopausal women with contraindication for conventional hormone replacement therapy. *Maturitas* 2004; 48: 372-80.
- [32] St Germain A, Peterson CT, Robinson JG, Alekel DL. Isoflavone-rich or isoflavone-poor soy protein does not reduce menopausal symptoms during 24 weeks of treatment. *Menopause* 2001; 8: 17-26.
- [33] Van Patten CL, Olivotto IA, Chambers GK, *et al.* Effects of soy phytoestrogens on hot flashes in postmenopausal women with breast cancer: a randomized, controlled clinical trial. *J Clin Oncol* 2002; 20: 1449-55.
- [34] Burke GL, Legault C, Anthony M, *et al.* Soy protein and isoflavone effects on vasomotor symptoms in peri- and postmenopausal women: the Soy Estrogen Alternative Study. *Menopause* 2003; 10: 147-53.
- [35] Kok L, Kreijkamp-Kaspers S, Grobbee DE, Lampe JW, van der Schouw YT. A randomized, placebo-controlled trial on the effects of soy protein containing isoflavone on quality of life in postmenopausal women. *Menopause* 2005; 12: 56-62.
- [36] Wilcox G, Wahlqvist ML, Burger HG, Medley G. Oestrogenic effects of plant foods in postmenopausal women. *BMJ* 1990; 301: 905-6.
- [37] Chiechi LM, Putignano G, Guerra V, Schiavelli MP, Cisternino AM, Carriero C. The effect of a soy rich diet on the vaginal epithelium in postmenopause: a randomized double-blind trial. *Maturitas* 2003; 45: 241-6.
- [38] Uesugi T, Toda T, Okuhira T, Chen JT. Evidence of estrogenic effect by the three-month-intervention of isoflavone on vaginal maturation and bone metabolism in early postmenopausal women. *Endocrine J* 2003; 50: 613-9.
- [39] Cline JM, Paschold JC, Anthony MS, Obasanjo IO, Adams MR. Effects of hormonal therapies and dietary soy phytoestrogens on vaginal cytology in surgically postmenopausal macaques. *Fertil Steril* 1996; 65: 1031-5.
- [40] Duncan AM, Underhill KE, Xu X, *et al.* Modest hormonal effects of soy isoflavones in postmenopausal women. *J Clin Endocrinol Metab* 1999; 84: 3479-84.
- [41] Han KK, Soares JM Jr, Haidar MA, de Lima GR, Baracat EC. Benefits of soy isoflavone therapeutic regime on menopausal symptoms. *Obstet Gynecol* 2002; 99: 389-94.
- [42] Nikander E, Rutanen EM, Nieminen P, Wahlstrom T, Ylikorkala O, Tiitinen A. Lack of effect of isoflavonoids on the vagina and endometrium in postmenopausal women. *Fertil Steril* 2005; 83: 137-42.
- [43] Kleijn MJ, van der Schouw YT, Wilson PW, Grobbee DE, Jacques PE. Dietary intake of phytoestrogens is associated with a favorable metabolic cardiovascular risk profile in postmenopausal US women: the Framingham study. *J Nutr* 2002; 132: 276-82.
- [44] Goodman-Gruen D, Kritiz-Silverstein D. Usual dietary isoflavone intake and body composition in postmenopausal women. *Menopause* 2003; 10: 427-32.
- [45] van der Schouw YT, Kreijkamp-Kaspers S, Peeters P, Keinan-Boker L, Rimm EB, Grobbee DE. Prospective study on usual dietary phytoestrogen intake and cardiovascular disease risk in western women. *Circulation* 2005; 111: 465-71.
- [46] Nestel P. Isoflavones: their effects on cardiovascular risk and functions. *Cur Opin Lipidol* 2003; 14: 3-8.
- [47] Colacurci N, Chiantera A, Fornaro F, *et al.* Effects of soy isoflavones on endothelial function in healthy postmenopausal women. *Menopause* 2005; 12: 299-307.
- [48] Anderson JW, Johnstone BM, Cook-Newell ME. Meta-analysis of effects of soy protein intake on serum lipids. *N Engl J Med* 1995; 333: 276-82.
- [49] Potter SM, Baum JA, Teng H, *et al.* Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am J Nutr* 1998; 68: 1375S-9S.
- [50] Dewell A, Hollenbeck CB, Bruce B. The effects of soy-derived phytoestrogens on serum lipids and lipoproteins in moderately hypercholesterolemic postmenopausal women. *J Clin Endocrinol Metab* 2002; 87: 118-21.
- [51] Dalais FS, Eberling PR, Kotsopoulos D, McGrath BP, Teede HJ. The effects of soy protein containing isoflavones on lipids and indices of bone resorption in postmenopausal women. *Clin Endocrinol* 2003; 58: 704-9.
- [52] Zhan S, Ho SC. Meta-analysis of the effects of soy protein containing isoflavones on the lipid profile. *Am J Clin Nutr* 2005; 81: 397-408.
- [53] Food and Drug Administration. United States FDA approves new health claim for soy protein and coronary heart disease. Available at: <http://www.fda.gov/bbs/topics/ANSWERS/ANS00980.html>.
- [54] Ho SC, Woo J, Lam S, Chen Y, Sham A, Lau J. Soy protein consumption and bone mass in early postmenopausal Chinese women. *Osteoporosis Int* 2003; 14: 835-42.
- [55] Coxam V. Prevention of osteopaenia by phyto-oestrogens: animal studies. *Br J Nutr* 89 (suppl. 1): S75-S85.
- [56] Morin S. Isoflavones and bone health. *Menopause* 2004; 11: 239-41.
- [57] Migliaccio S, Anderson JB. Isoflavones and skeletal health: are these molecules ready for clinical application? *Osteoporosis Int* 2003; 14: 361-8.
- [58] Setchell KD, Lydeking-Oslen E. Dietary phytoestrogens and their effect on bone: evidence from *in vitro* and *in vivo*, human observational and dietary intervention studies. *Am J Clin Nutr* 2003; 78(suppl. 3): 593S-609S.
- [59] Blum SC, Heaton SN, Bowman BM, Hegsted M, Miller SC. Dietary soy protein maintains some indices of bone mineral density and bone formation in aged ovariectomized rats. *J Nutr* 2003; 133: 1244-9.
- [60] Li B, Yu S. Genistein prevents bone resorption diseases by inhibiting bone resorption and stimulating bone formation. *Biol Pharma Bull* 2003; 26: 780-6.
- [61] Cai DJ, Zhao Y, Glasier J, *et al.* Comparative effects of soy protein, soy isoflavones, and 17beta-estradiol on bone metabolism in adult ovariectomized rats. *J Bone Miner Res* 2005; 20: 828-39.
- [62] Valtuena S, Cashman K, Robins SP, Cassidy A, Kardinaal A, Branca F. Investigating the role of natural phyto-oestrogens on bone health in postmenopausal women. *Br J Nutr* 2003; 89(suppl. 1): S87-S99.
- [63] Mei J, Yeung SC, Kung A. High dietary phytoestrogen intake is associated with higher bone mineral density in postmenopausal but not pre menopausal women. *J Clin Endocrinol Metab* 2001; 86: 5217-21.

- [64] Somekawa Y, Chiguchi M, Ishibashi T, Aso T. Soy intake related to menopausal symptoms. Serum lipids and bone mineral density in postmenopausal Japanese women. *Obstet Gynecol* 2001; 97: 109-15.
- [65] Greendale GA, Fitts Gerald G, Huang MH, *et al.* Dietary soy isoflavones and bone mineral density: results from the Study of Women's Health Across the Nation. *Am J Epidemiol* 2002; 155: 746-54.
- [66] Chen Y, Ho SC, Lam SS, Ho SS, Woo JLF. Soy isoflavone have a favorable on bone loss in Chinese postmenopausal women with lower bone mass: a double-blind, randomized controlled trial. *J Clin Endocrinol Metab* 2003; 88: 4740-7.
- [67] Chen Y, Ho SC, Lam SS, Ho SS, Woo JLF. Beneficial effect of soy isoflavones on bone mineral content was modified by years since menopause, body weight and calcium intake: a double-blind, randomized controlled trial. *Menopause* 2004; 11: 246-54.
- [68] Gallager JC, Satpathy R, Rafferty K, Haynatzka V. The effect of soy protein isolate on bone metabolism. *Menopause* 2004; 11: 290-98.
- [69] Krejnkamp-Kaspers S, Kok L, Grobbee DE, *et al.* Effect of soy protein containing isoflavones on cognitive function, bone mineral density and plasma lipids in postmenopausal women: a randomized controlled trial. *JAMA* 2004; 292: 65-74.
- [70] Arjmandi BH, Lucas EA, Khalil DA, *et al.* One year soy protein supplementation has positive effects on bone formation markers but not bone density in postmenopausal women. *Nutr J* 2005; 4: 8-14.
- [71] Morabito N, Crisafulli A, Vergara C, *et al.* Effects of genistein and hormone-replacement therapy on bone loss in early postmenopausal women: a randomized double-blind placebo-controlled study. *J Bone Miner Res* 2002; 17: 1904-12.
- [72] Kamel HK, Maas D, Duthie EH. The role of hormones in the pathogenesis and management of sarcopenia. *Drugs Aging* 2002; 19: 865-77.
- [73] Adams KF, Newton KM, Chen C, *et al.* Soy isoflavone do not modulate circulating insulin-like growth factor concentrations in an older population in an intervention trial. *J Nutr* 2003; 133: 1316-9.
- [74] Weaver CM, Cheong JM. Soy isoflavones and bone health: the relationship is still unclear. *J Nutr* 2005; 135: 1243-7.
- [75] Evans WJ. Effects of exercise on senescent muscle. *Clin Orthop* 2002; 1: S211-S220.
- [76] Hurley BF, Roth SM. Strength training in the elderly: Effects on risk factors for age-related diseases. *Sports Med* 2000; 30: 249-68.
- [77] Haub MD, Wells AM, Tarnaopolsky MA, Campbell WW. Effect of protein source in resistive-training-induced changes in body composition and muscle size in older men. *Am J Clin Nutr* 2002; 76: 511-7.
- [78] Wu J, Wang X, Chiba H, *et al.* Combined intervention of soy isoflavone and moderate exercise prevents body fat elevation and bone loss in ovariectomized mice. *Metabol Clin Exp* 2004; 53: 942-8.
- [79] Deibert P, Konig D, Schmidt-Trucksaess A, Zaenker KS, Landmann U, Berg A. Weight loss without losing muscle mass in pre-obese and obese subjects induced by a high-soy-protein diet. *Int J Obes* 2004; 28: 1349-52.
- [80] Moeller LE, Peterson CT, Hanson KB, Dent SB, Lewis DS, King DS, Alekel DL. Isoflavone-rich soy protein prevents loss of hip lean mass but does not prevent the shift in regional fat distribution in perimenopausal women. *Menopause* 2003; 10: 322-31.
- [81] Mishra SI, Dickerson V, Najm W. Phytoestrogens and breast cancer prevention: What is the evidence? *Am J Obstet Gynecol* 2005; 188(suppl 5): S66-S70.
- [82] Liu J, Chang SK, Wiesenbron D. Antioxidant properties of soybean isoflavone extract and tofu *in vitro* and *in vivo*. *J Agric Food Chem* 2005; 53: 2333-40.
- [83] Maskarinec G, Williams AE, Carlin L. Mammographic densities in a one-year isoflavone intervention. *Eur J Cancer Prev* 2003; 12: 165-9.
- [84] Macdonald RS, Guo J, Copeland J, *et al.* Environmental influences on isoflavones and saponins in soybeans and their role in colon cancer. *J Nutr* 2005; 135: 1239-42.
- [85] Dijsselbloem N, Vanden BW, De Naeyer A, Haefeman G. Soy isoflavone phyto-pharmaceuticals in interleukin-6 affections. *Biochem Pharmacol* 2004; 68: 1171-85.
- [86] Jenkins DJ, Kendall CW, Connelly PW, *et al.* Effects of high and low isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. *Metabol Clin Exp* 2004; 51: 919-24.
- [87] Hale GE, Hughes CL, Cline JM. Endometrial Cancer: hormonal factors, the perimenopausal "window of risk", and isoflavones. *J Clin Endocrinol Metab* 2002; 87: 3-15.
- [88] Kayisli UA, Asku CAH, Berkkanoglu M, Arici A. Estrogenicity of isoflavones on human endometrial estromal and glandular cells. *J Clin Endocrinol Metab* 2002; 87: 5539-44.
- [89] Unfer V, Casini ML, Costabile L, Mignosa M, Gerli S, Di Renzo GC. Endometrial effects of long term treatment with phytoestrogens: a randomized, double-blind, placebo-controlled study. *Fertil Steril* 2004; 82: 145-8.
- [90] Tsouronis C. Clinical effects of phytoestrogens. *Clin Obstet Gynecol* 2001; 44: 836-42.
- [91] Foucard T, Malmheden YI. A study on severe food reactions in Sweden – is soy protein an underestimated cause of food anaphylaxis? *Allergy* 1999; 54: 261-5.
- [92] Sirtori CR. Risks and benefits of soy phytoestrogens in cardiovascular disease, cancer, climacteric symptoms and osteoporosis. *Drug Saf* 2001; 24: 665-82.
- [93] Divi RL, Chang HC, Doerge DR. Anti-thyroid isoflavones from soybean. Isolation, characterization and mechanisms of action. *Biochem Pharmacol* 1997; 54: 1087-96.
- [94] Wang H, Murphy PA. Isoflavone content in commercial soybean foods. *J Agric Food Chem* 1994; 42: 1666-73.