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Effects of dietary phytoestrogens in postmenopausal women

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ABSTRACT

The aim of this study was to test the hypothesis that increased dietary intake of phytoestrogens reduces the health impact of the menopause. To test this hypothesis, a double-blind, randomized, entry–exit, cross-over study was conducted to assess the effects of three dietary manipulations – soy and linseed diets (high in phytoestrogens) and a wheat diet (low in phytoestrogens). Postmenopausal women were recruited and randomly assigned to one of the three dietary regimens. Urinary phytoestrogen concentrations, hot flush rate, vaginal smears, bone mineral density and bone mineral content were assessed for two 12-week periods. Comparative analysis showed no significant differences, but, when analyzed separately, groups consuming high phytoestrogen diets had between 10 and 30 times higher urinary excretion of phytoestrogens compared to those consuming the low phytoestrogen diet ($p < 0.01$). Study participants consuming soy, linseed and wheat diets had a 22% (not significant, n.s.), 41% ($p < 0.009$) and 51% ($p < 0.001$) reduction in hot flush rate; a 103% ($p < 0.04$), 5.5% (n.s.) and 11% (n.s.) increase in vaginal cytology maturation index; and a 5.2% ($p < 0.04$), 5.2% (n.s.) and 3.8% (n.s.) increase in bone mineral content, respectively. No changes were detected in bone mineral density. The differential effects of high phytoestrogen dietary manipulations on outcomes may represent tissue-specific responses to isoflavones and lignans contained in soy and linseed, respectively. Whilst health outcome measures were not significantly different between groups, the data obtained from separate analysis suggest that phytoestrogens in soy and linseed may be of use in ameliorating some of the symptoms of menopause. Furthermore, the significant decrease in hot flush rate in the wheat group cannot be attributable to phytoestrogens measured in this study. Due to subject variability, larger studies are still needed to evaluate population benefit.

INTRODUCTION

Phytoestrogens are naturally occurring compounds that have a structure very similar to human endogenous estrogen[1]. There are three main classes of phytoestrogens: isoflavones, coumestans and lignans. They are found in a variety of plants ranging from legumes to cereals, with soy having the highest concentration of isoflavones and linseed (flaxseed) having the highest concentration of lignans[2,3]. This study assessed food products, concentrating on the isoflavones, daidzein and genistein, and the mammalian lignans, enterodiol and enterolactone. Daidzein and genistein are found in soy but are also derived from their respective precursors, formononetin and biochanin A,

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which also have estrogenic activity. Both lignans are formed from precursors in plants; enterodiol is formed from secoisolariciresinol, which can also then be oxidized to enterolactone, and enterolactone is formed from matairesinol. Isoflavones and lignans are formed from their precursors by the action of the gut microflora[4].

Phytoestrogens display pleiotropic actions and have been implicated in both physiological and pathological processes. With respect to their effects on menopausal women, Adlercreutz and colleagues were the first to measure phytoestrogen concentrations in postmenopausal women[5] and later Japanese women[6], which led them to hypothesize that the low frequency of hot flushes in Japanese women was due to the high excretion of phytoestrogens in the latter population[7]. At the same time, Wilcox and colleagues demonstrated that dietary supplementation with soy flour, linseed and red clover significantly improved vaginal cytology (i.e. had a more estrogenic profile) in postmenopausal women[8]. Murkies and colleagues showed that dietary supplementation of soy or wheat flour decreased hot flushes by 40% and 25%, respectively, with a significant decrease in the soy group within 6 weeks of supplementation[9]. Baird and colleagues also supplemented the diet of postmenopausal women with soy for a period of 4 weeks, but did not show any significant estrogenic effect[10]. More recently, Brzezinski and colleagues compared diets high and low in phytoestrogens and demonstrated more significant improvement in reductions in hot flushes and vaginal dryness in the phytoestrogen-rich diets[11].

Phytoestrogenic effects on bone metabolism have not yet been clearly established. In human subjects, calcium loss was used as an end-point to test the action of three protein sources (animal, dairy and soy). The group consuming animal protein lost 50% more calcium than those in the soy group, with the dairy group falling in between the animal and soy groups[12]. Soy and genistein have been demonstrated to prevent loss in bone density and bone mass, respectively, in an ovariectomized rat model[13,14]. Since hormone replacement therapy is protective against postmenopausal bone loss, estrogen-like compounds may confer similar effects[15,16].

Based upon the available evidence supporting a role for dietary phytoestrogens in the management of menopausal symptoms, a double-blind, randomized, entry–exit, cross-over study was designed to test the hypotheses that dietary supplementation with phytoestrogens decreases hot flushes, improves vaginal cytology and prevents reduction in postmenopausal bone mineral density and loss of bone mineral content.

SUBJECTS AND METHODS

Subjects and study design

In this study, the effects of phytoestrogens contained in soy (the isoflavones, genistein and daidzein), linseed (mammalian lignan precursors, secoisolariciresinol and matairesinol) and wheat (low in isoflavones and lignans) have been evaluated. Fifty-two postmenopausal women were recruited to the study. This number of subjects was calculated from previous studies[8,9] to give a 60% chance of detecting a 40% increase in vaginal cytology maturation index and an 80% chance of detecting a 40% decrease in hot flush rate. The entry criteria were age 45–65 years, follicle stimulating hormone (FSH) level greater than 40 IU/ml, hot flush rate greater than 14 per week, 12 months of amenorrhea, no antibiotic or hormone replacement therapy (HRT) use for the preceding 3 months, non-smoker and non-vegetarian. Prior to randomization, the subjects underwent a general physical examination and completed a food frequency questionnaire and a 2-week food diary. They were asked to repeat the same 2-weekly diet throughout the study. If they diverged from that diet, a note was made on the hot flush diary provided. The subjects were randomized to a soy (high phytoestrogen content), linseed (high phytoestrogen content) or wheat (low phytoestrogen content) diet in order to compare high and low phytoestrogen diets (Table 1). The dietary treatments were then applied for a period of 12 weeks. In order to minimize interindividual variation, by using subjects twice, and, in this event, to negate the possible confounding effect of time on study end-points, a cross-over design was applied (Figure 1). Blood and spot urine samples were collected prior to the commencement of each dietary manipulation period (weeks 0 and 16) and at 4-weekly intervals throughout the study. Urinary excretion of phytoestrogens was normalized to creatinine excretion. Hot flush rate was measured on a daily basis using a hot flush diary throughout the whole study, excluding the 4-week break. Vaginal smears were collected by a clinical research nurse and cytology assessment was performed by the Victorian Cytology Service. Results from vaginal smears were expressed as a maturation index (0 X% basal cells + 0.5 X% intermediate cells + 1.0 X% superficial cells). Total body bone mineral density and bone mineral content

Table 1 Subject data at study entry (mean ± standard error)

	Soy	Linseed	Wheat
Age (years)	53.6 ± 1.7	54.6 ± 1.2	53.7 ± 1.2
Weight (kg)	71.3 ± 2.4	69.2 ± 2.7	74.0 ± 3.9
Body mass index (kg/m^2)	26.5 ± 0.8	25.3 ± 0.8	28.2 ± 1.4
Time since menopause (years)	4.5 ± 1.1	4.5 ± 1.1	4.1 ± 1.2

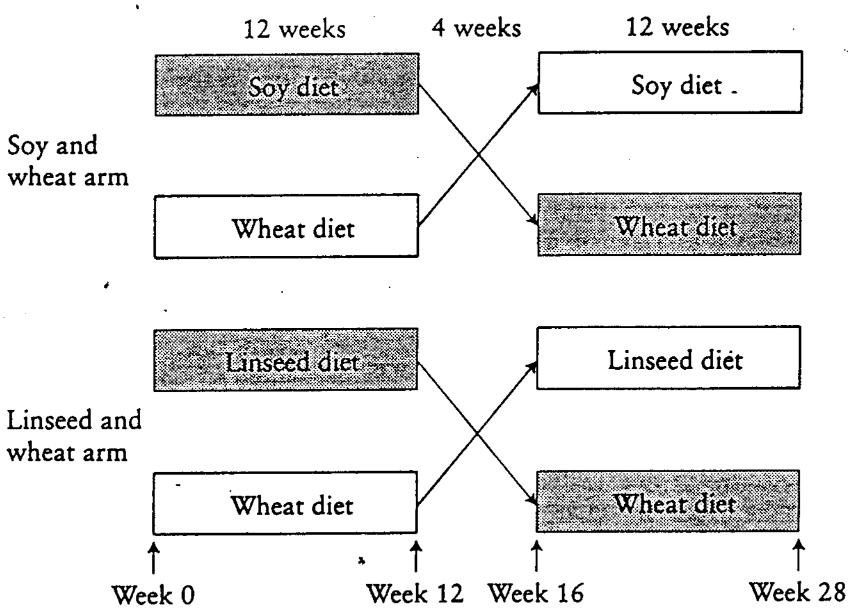

Figure 1 Double-blind, randomized, cross-over study design

were also assessed by The Body Composition Laboratory at Monash Medical Centre, using dual energy X-ray absorptiometry (DEXA) model DPX-L 3.6Y (Lunar Corporation, Madison, WI, USA).

The dose of phytoestrogen-containing foods chosen for this study was based on the results of previous studies[8,9]. Forty-five grams per day of soy grits, linseed or wheat kibble was achieved by using three different types of bread with the same background composition. The subjects ate four slices, two rolls or two slices and one roll, substituting for their daily intake of bread. The dosage of isoflavones (daidzein, genistein and their respective glycosides) achieved in the soy bread was 52.64 ± 8.68 mg per day (mean ± standard deviation).

Analysis of phytoestrogens

Extraction of phytoestrogens followed a previously described method[17]. Phytoestrogens were isolated by reverse-phase high-performance liquid chromatography (HPLC) (Shimadzu system LC 10A) following a method developed by Eldridge[18]. Detection was by dual wavelength ultraviolet absorbance at 230 nm and 254 nm. The intra-assay coefficient of variation for the HPLC analysis of the samples was 1.7%. All the urine samples were processed in one assay.

Statistical analysis

The effects of dietary manipulation on hot flush rate, vaginal cytology and bone mineral content were assessed within treatment groups using entry–exit data. Data were expressed as percentage change – as each subject served as her own control. The data were analyzed using Mann–Whitney non-parametric comparison of two samples on a commercially available statistical package (Statsgraphics version 4.0, STSC, Rockville, MD, USA). As there were no statistical differences between wheat groups, they were pooled for analyses.

RESULTS

Forty-four of the 52 women completed the study; seven study participants discontinued the dietary

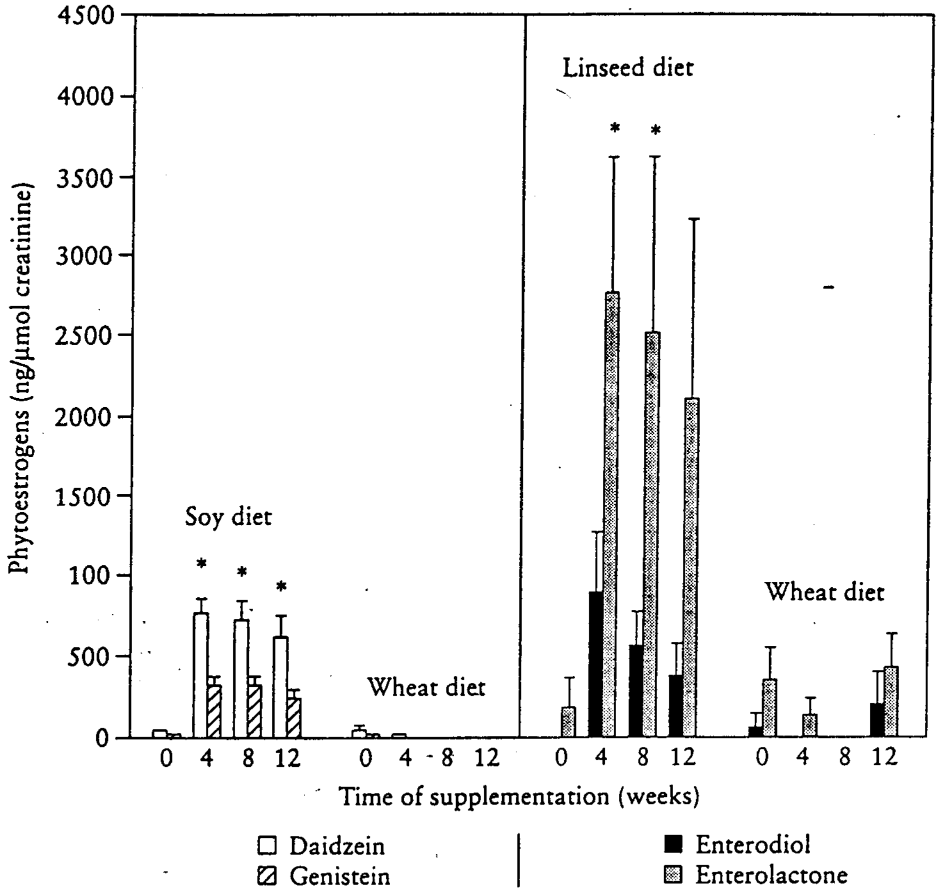

Figure 2 Urinary phytoestrogen excretion (mean + SEM) on soy, linseed and wheat diets. *$p < 0.01$

manipulation due to personal reasons and one due to lack of compliance to the study demands.

Urinary excretion of daidzein, genistein, enterodiol and enterolactone increased significantly (10–30-fold) during soy and linseed ingestion, respectively ($p < 0.01$) (Figure 2). There were no significant changes in phytoestrogens during wheat ingestion.

In women consuming linseed and wheat, a significant decrease in hot flush rate was observed following 12 weeks of ingestion (41% decrease, $p < 0.009$; 51% decrease, $p < 0.001$, respectively), compared to baseline (Figure 3). No statistically significant decrease in hot flush rate was detected during soy ingestion.

Women consuming soy showed a statistically significant increase of 103% in vaginal cytology maturation index pattern following 12 weeks of the diet compared to baseline ($p = 0.03$), but there were no significant cytological changes following linseed or wheat ingestion (Figure 4).

There were no significant bone mineral density changes associated with soy, linseed or wheat ingestion. A significant increase of 5.2% in bone mineral content was detected following 12 weeks

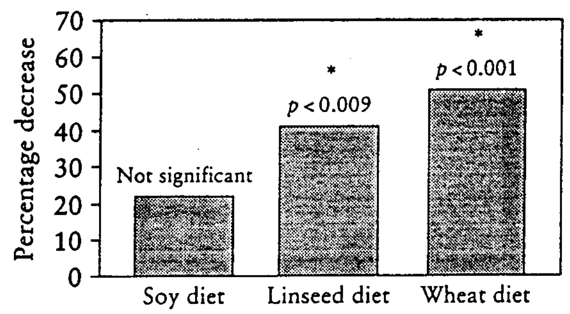

Figure 3 Percentage decrease in hot flush rate on soy, linseed and wheat diets

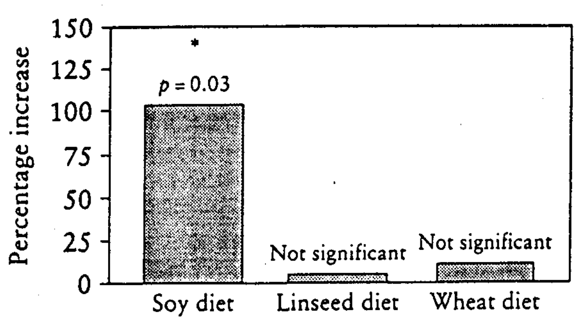

Figure 4 Percentage increase in vaginal cytology maturation index on soy, linseed and wheat diets

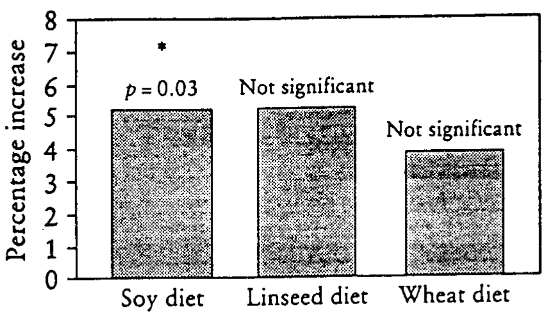

Figure 5 Percentage increase in bone mineral content on soy, linseed and wheat diets

of soy ingestion ($p = 0.03$), whereas no significant changes were detected with linseed or wheat ingestion (Figure 5).

DISCUSSION

This double-blind, randomized, entry–exit, crossover study was designed to evaluate the effects of a dietary phytoestrogen supplementation on hot flushes, vaginal cytology, bone mineral density and bone mineral content. The significant effects identified were an increase in urinary phytoestrogen excretion with soy and linseed ingestion, a decrease in hot flush rate with linseed and wheat ingestion, a shift to a more estrogenic epithelial profile and an increase in bone mineral content with soy ingestion.

The increase in urinary excretion of phytoestrogens illustrates subject compliance. This study confirms and extends the finding of our previous study by demonstrating that an increase in soy consumption alters the vaginal cytology maturation index to a more estrogenic epithelial pattern[8]. The baseline maturation index in this group was 16, indicating an atrophic smear, and the significant increase in maturation index with soy consumption is suggestive that phytoestrogens do have a physiological effect in postmenopausal women.

This study presents the first data on the effects of phytoestrogens on bone in postmenopausal women. The increase in bone mineral content demonstrated with soy ingestion over such a short period of time is of considerable interest. An increase in bone mineral content is consistent with an estrogenic effect of isoflavones in soy, but the magnitude of change demonstrated was certainly greater than expected. In bone studies looking at the effects of HRT on bone mineral content, it took 36 months of treatment to achieve an increase of just under 4%[19]. The double-blind, randomized, cross-over study design deals with systematic bias drift, for which evidence was provided by laboratory quality control. Nonetheless, the small study numbers must allow for the possibility of a chance effect. With a greater sample size and longer study time, it may be possible to demonstrate a more general effect of isoflavones, lignans and possibly an unknown factor in wheat, on bone.

The dietary manipulation used resulted in an improvement in estrogen-dependent end-points. The respective effects of soy and linseed consumption on such end-points may be attributed to the known phytoestrogens, genistein, daidzein, enterodiol and enterolactone. The effects of wheat on hot flush rate cannot be attributed to those phytoestrogens contained in soy and linseed. The putative estrogenic action of wheat consumption may be an effect of an as yet unidentified phytoestrogen-like compound. Also, possible mold contamination of wheat kibble in the wheat bread may have resulted in the production of another class of estrogenic compound, the resorcylic acid lactones, which were not measured in this study. Similar to previous studies, there was no difference between high and low phytoestrogen groups[9,11]. Both studies by Murkies and Brzezinski and colleagues demonstrated a more significant decrease in the high phytoestrogen group with regard to hot flush rate. The lack of effect in this study is possibly due to a phytoestrogen dose effect.

This study adds to the growing body of data on the physiological effects of dietary phytoestrogens in postmenopausal women. Longer-term, well-controlled, dose-ranging studies with adequate sample size are required to substantiate the efficacy of dietary phytoestrogens in the control of vasomotor symptoms and bone preservation.

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Conflict of interest Nil.

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