

Supplementation with flaxseed alters estrogen metabolism in postmenopausal women to a greater extent than does supplementation with an equal amount of soy¹⁻³

Jennifer D Brooks, Wendy E Ward, Jacqueline E Lewis, John Hilditch, Leslie Nickell, Evelyn Wong, and Lilian U Thompson

ABSTRACT

Background: Phytoestrogens, which are abundant in flaxseed and soy, have chemical structures resembling those of endogenous estrogens and have been shown to exert hormonal effects, thereby affecting chronic diseases.

Objective: We compared the effects of consuming equal amounts of flaxseed or soy on estrogen metabolism and biochemical markers of bone metabolism in postmenopausal women.

Design: In a parallel design, the diet of postmenopausal women ($n = 46$) was supplemented with either a placebo, soy (25 g soy flour), or flaxseed (25 g ground flaxseed) muffin for 16 wk. Blood and 24-h urine samples were collected at baseline and at the endpoint. Urine samples were analyzed for phytoestrogens, estrogen metabolites (2-hydroxyestrone, 16 α -hydroxyestrone), and serum hormones (estradiol, estrone, estrone sulfate). Serum and urine samples were also analyzed for biochemical markers of bone metabolism.

Results: Urinary concentrations of 2-hydroxyestrone, but not of 16 α -hydroxyestrone, increased significantly in the flaxseed group ($P = 0.05$). In the flaxseed group, the ratio of 2-hydroxyestrone to 16 α -hydroxyestrone was positively correlated with urinary lignan excretion ($r = 0.579$, $P = 0.02$). In the soy and placebo groups, no significant correlation was observed. No significant change in serum hormones or biochemical markers of bone metabolism was observed within or between the treatment groups.

Conclusions: Supplementation with flaxseed modifies urinary estrogen metabolite excretion to a greater extent than does supplementation with an equal amount of soy. This modification by flaxseed is associated with an increase in urinary lignan excretion. Despite the shift in estrogen metabolism to favor the less biologically active estrogens, a negative effect on bone cell metabolism was not observed. *Am J Clin Nutr* 2004;79:318–25.

KEY WORDS Flaxseed lignans, soy isoflavones, estrogen metabolism, 2-hydroxyestrone, 16 α -hydroxyestrone, biochemical markers of bone metabolism, postmenopausal women

INTRODUCTION

Flaxseed and soy are rich sources of lignans and isoflavones, respectively (1). Lignans and isoflavones are phytoestrogens with diphenolic ring structures resembling those of endogenous estrogens (2, 3) and have been shown to exert hormonal effects (4–6).

Estradiol is the biologically active estrogen that is most often associated with mammary tumorigenesis and maintenance of skeletal homeostasis (7–10). The metabolism of estrogen is primarily oxidative and occurs predominantly in the liver (11). Estradiol is first oxidized to estrone and then hydroxylated at either the A ring (C2 position) or the D ring (C16 α position) by the cytochrome P450 enzymes 2-hydroxylase or 16 α -hydroxylase (11, 12). This leads to the formation of the 2 major metabolites of estradiol, 2-hydroxyestrone (2OHE1) and 16 α -hydroxyestrone (16 α OHE1) (13), which are excreted in either the urine or the feces (14) and have distinct biological properties. Although hydroxylation of estradiol and estrone can also occur at multiple sites (carbons 1, 2, 4, 6, 7, 11, and 14–18), the 2- and 16 α -hydroxylated metabolites are the most abundant (15).

2OHE1 has shown little biological activity, with some antiestrogenic action in vitro (16–18). Conversely, 16 α OHE1 has shown estrogen agonistic activity, including increased cell proliferation of human breast cancer cell lines in vitro (17–19), and an uterotrophic effect comparable with that of estrogen in vivo (20, 21). Therefore, persons who have an increased proportion of 16 α -hydroxylation (a low ratio of 2OHE1 to 16 α OHE1) are suggested to have an increased risk of breast cancer (17, 22, 23). With respect to bone, 16 α OHE1 is suggested to be an estrogen agonist in ovariectomized rats (24) and is associated with increased bone mineral density (BMD) in postmenopausal women (25).

Consumption of flaxseed and soy influences estrogen metabolism, as indicated by both urinary metabolite excretion (26–28) and serum hormone concentrations (3, 29). Furthermore, in vitro studies showed that flaxseed lignans moderately inhibit the cytochrome P450 enzyme aromatase, which cata-

¹ From the Department of Nutritional Sciences, University of Toronto (JDB, WEW, and LUT), and the Sunnybrook Health Sciences Centre, Toronto (JEL, JH, LN, and EW).

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³ Reprints not available. Address correspondence to LU Thompson, Department of Nutritional Sciences, University of Toronto, 150 College Street, Toronto, Ontario M5S 3E2, Canada. E-mail: lilian.thompson@utoronto.ca.

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TABLE 1
Demographic characteristics of the subjects by treatment group¹

Characteristic	Placebo (<i>n</i> = 15)	Soy (<i>n</i> = 13)	Flaxseed (<i>n</i> = 16)
Age (y)	52.7 ± 1.0	54.1 ± 0.6	52.6 ± 1.0
Height (cm)	163.7 ± 1.1	162.2 ± 2.0	163.2 ± 1.7
Weight (kg)			
Week 0	74.0 ± 4.5	71.5 ± 3.9	67.0 ± 2.9
Week 16	74.8 ± 4.5	72.0 ± 3.9	66.7 ± 3.0
BMI (kg/m ²)			
Week 0	27.6 ± 1.6	27.1 ± 1.2	25.1 ± 0.8
Week 16	27.9 ± 1.5	27.3 ± 1.2	24.9 ± 0.8
Age at menopause (y)	49.7 ± 0.9	50.1 ± 0.8	50.0 ± 0.8

¹ Arithmetic $\bar{x} \pm$ SEM. There were no significant differences between the treatment groups in age, height, or age at menopause (ANOVA). For weight and BMI, two-factor ANOVA showed no significant effect of time or group and no significant group \times time interaction.

lyzes the conversion of androgens to estrogens (30, 31). In addition, flaxseed lignans and soy isoflavones modulate the activity of 17 β -hydroxysteroid dehydrogenases (32), enzymes involved in the balance between estradiol and estrone (33, 34).

In previous human studies on estrogen and bone metabolism, the diet of postmenopausal women was supplemented with ground flaxseed in the amounts of 5, 10 (3, 28), and 40 (35) g. Soy has been given as isolated soy protein (ISP) (36–39) or soy milk (26, 29), with various amounts of isoflavones. Isoflavones have also been administered in tablet form (40). Studies comparing the effects of equal amounts by weight of flaxseed and soy in amounts and forms that may be encountered in a habitual diet have not been conducted. Therefore, the specific objective of the present study was to compare the effects of consuming a moderate amount (25 g) of ground flaxseed or ground soy flour incorporated into a muffin on the metabolism of estrogen [ie, urinary estrogen metabolites (2OHE1 and 16 α OHE1) and serum hormones] and biochemical markers of bone metabolism in postmenopausal women. The results will suggest whether these phytoestrogen-rich foods favorably modulate estrogen and bone metabolism.

SUBJECTS AND METHODS

Subjects

A randomized, double-blind, parallel, placebo-controlled study of postmenopausal women was designed. The healthy postmenopausal women included in the present study (*n* = 46) were a subsample (selected for compliance) of those (*n* = 99) who participated in a study examining the effects of flaxseed and soy supplementation on symptoms of menopause. Natural menopause had been achieved ≥ 1 y before the start of the study. The exclusion criteria were as follows: active bowel disease; malabsorption syndrome; use of exogenous estrogens within the past 3 mo; use of phytoestrogen supplements within the past 1 mo; any thyroid disorder (treated or untreated); use of oral or parenteral corticosteroids; antibiotic use 4 wk before the study; any serious, active medical condition; a known allergy or intolerance to study ingredients; and anticipated absence for >4 wk during the study period. The demographic characteristics of the subjects in each treatment group are shown in **Table 1**. There were no significant differences in the selected variables (age, height, weight, body mass index, or age at menopause) between the treatment groups at baseline or at

the endpoint or within the treatment groups over time. All subjects gave written informed consent, and the study protocol was reviewed and approved by the Sunnybrook Research Ethics Board and the University of Toronto Human Ethics Committee.

Study design

The women were randomly assigned to 3 treatment groups in which the daily diet was supplemented with either a placebo muffin (*n* = 15), a flaxseed muffin (*n* = 16), or a soy muffin (*n* = 15) for 16 wk. Fasting blood samples and 24-h urine samples were collected, and 3-d food records were recorded at baseline (week 0) and at the endpoint (week 16). The subjects were asked to record their muffin ingestion on daily diary cards and to return uneaten muffin portions. Compliance measured by this means was similar for all the treatment groups. The muffins were well tolerated, with compliance calculated to be 96%. Compliance was also monitored through measurement of urinary phytoestrogen excretion.

The subjects were asked to maintain their habitual diet and to avoid foods containing flaxseed and soy during the study. The subjects were counseled by the research assistant to maintain their prestudy weight throughout the trial because changes in body weight may influence endogenous hormone concentrations (41, 42). The study muffins for all 3 treatment groups contained similar ingredients and were prepared from either white flour (20.7 g; flaxseed and soy groups) or whole-wheat flour (20.7 g; placebo group) by using traditional methods. The flaxseed muffin contained 25 g ground flaxseed, which supplied 50 mg of the mammalian lignan precursor secoisolariciresinol diglycoside/d (26.4 mg secoisolariciresinol/d). Soy muffins contained 25 g soy flour, which supplied 41.9 mg isoflavones/d (15.5 mg daidzein/d, 25.7 mg genistein/d, 0.7 mg glycitein/d). The placebo muffin was prepared with whole-wheat flour, instead of white flour, to raise the fiber content of the placebo muffin closer to that of the other muffins. Wheat fiber has been shown to have no significant effect on urinary estrogen metabolites (43). All muffins were formulated in an attempt to make them isocaloric and equivalent in macronutrients (fat, protein, and fiber). Hence, additional canola oil was added to the placebo (10 g) and soy muffins (4 g) but not to the flaxseed muffin. Muffins were also flavored with nutmeg, cinnamon, and vanilla extract to help maintain subject blindness. To maintain the double-blind status of the study, muffins

TABLE 2
Macronutrient content of the muffins

Macronutrient	Placebo	Soy	Flaxseed
Energy (kJ)	1121	1134	1075
Protein (g)	7.5	15.2	9.6
Fat (g)	8.8	7.5	8.9
Total carbohydrates (g)	44.3	40.1	41.2
Total dietary fiber (g)	4.6	4.3	6.6

were packaged in opaque wrappings with 7 muffins to a tray so that the different muffins could not be visually distinguished, and the muffins were labeled with a unique 4-digit number before delivery to the research assistant. For each subject visit, the research assistant received a list indicating which 4 trays of prewrapped muffins were to be dispensed to the subject for that 4-wk period.

The macronutrient content of the muffins is shown in **Table 2** (Association of Official Analytical Chemists, *Official Methods of Analysis*, 16th ed, Washington, DC: AOAC, 1997). Three-day food records were analyzed and averaged by using the NUTRIWATCH nutrient analysis program (version 6.1.22E Delphi 1, based on the 1997 Canadian Nutrient File; Elizabeth Warwick, PEI, Cornwall, Canada).

Urinary phytoestrogen analysis

Urinary phytoestrogens were analyzed by gas chromatography–mass spectrometry, which is routinely used in our laboratory (44). Briefly, mammalian lignans and isoflavones were extracted from the urine samples on a reversed-phase octadecylsilane bonded silica cartridge (C₁₈; Applied Separations, Allentown, PA). Phytoestrogens adsorbed to the silica particles were eluted with 4 mL methanol and evaporated to dryness. The residue was treated overnight with β -glucuronidase at 37 °C. The unconjugated phytoestrogens were extracted from the hydrolysate by being passed through another C₁₈ column and were further purified and isolated on a DEAE Sephadex ion-exchange column that had been prepared in the alcohol form (methanol). Phytoestrogens were then derivatized (Tri-Sil Reagent; Pierce, Rockford, IL), dissolved in 100 μ L hexane, and analyzed by gas chromatography–mass spectrometry (GC 5890 Series II, MS 5971; Hewlett-Packard, Avondale, PA). The oven temperature began at 100 °C and was programmed to increase to 250 °C at a rate of 30 °C/min. An HP-1 capillary column (25 m \times 0.2 mm \times 0.11 μ m; Agilent Technologies Inc, Wilmington, DE), which consisted of a cross-linked methyl silicone gum phase, was used with helium as the carrier gas. Total analysis time was 15 min.

Enzyme-linked immunoassay for 2OHE1 and 16 α OHE1

ESTRAMET (ImmunaCare, Bethlehem, PA) is a competitive, solid-phase enzyme immunoassay for the quantification of the urinary estrogen metabolites 2OHE1 and 16 α OHE1. Values obtained from this method correlate highly with those obtained by using gas chromatography–mass spectrometry (45). Analysis of samples was carried out with kits from the same lot and performed within 2 wk of delivery. Baseline and follow-up samples for each subject were analyzed within the same plate. A laboratory control (24-h postmenopausal urine sample) was also included within each assay. Standards, con-

trols, and samples, all of which were run in triplicate, were first deconjugated of glucuronic acid and sulfate through the addition of a mixture containing β -glucuronidase and arylsulfatase enzymes isolated from the snail *Helix Pomatia*, and concentrations are expressed in μ g/24 h. The interassay coefficients for 2OHE1 and 16 α OHE1 were 9.02% and 6.85%, respectively, and the intraassay coefficients were 2.99% and 4.53%, respectively.

Serum hormones

Serum estradiol, estrone, and estrone sulfate concentrations were determined by using a double-antibody ¹²⁵I radioimmunoassay (DSL-4800, 8700, and 5400, respectively; Diagnostic Systems Laboratories Inc, Webster, TX). Analysis was conducted as described by the manufacturer. All samples were run in duplicate with the same kit lot, and samples from each treatment group were included in each assay. Baseline and follow-up samples from each subject were analyzed within the same assay. The interassay coefficients for estradiol, estrone, and estrone sulfate were 8.97%, 11.83%, and 23.58%, respectively, and the intraassay coefficients were 10.76%, 4.47%, and 8.11%, respectively.

Biochemical markers of bone turnover

Bone-specific alkaline phosphatase (AP) was measured in fasting serum samples by using an enzyme-linked immunoassay (Metra BAP; Quidel Corporation, San Diego). Free deoxypyridinoline (DPD) was measured in 24-h urine samples by using an enzyme-linked immunoassay (Metra DPD; Quidel Corporation), and concentrations are expressed as a function of creatinine. Creatinine was measured by using a colorimetric assay (Kit 555-A; Sigma Chemical Co, Mississauga, Canada). All samples were run in duplicate with the same kit lot, and baseline and follow-up samples for each subject were analyzed within the same batch. The interassay coefficients for AP and DPD were 7.40% and 3.19%, respectively, and the intraassay coefficients were 9.27% and 6.92%, respectively.

Statistical analyses

Urinary estrogen metabolites and phytoestrogens, serum hormones, biochemical markers of bone metabolism, dietary intakes, and weight and body mass index data were analyzed by using two-factor analysis of variance followed by Tukey's multiple comparison test. The ratio of 2OHE1 to 16 α OHE1 within each group was also examined by using a paired *t* test. Demographic data were compared between treatment groups by using one-factor analysis of variance followed by Tukey's multiple comparison test. Regression analysis was used to examine the association between urinary concentrations of estrogen metabolites and phytoestrogens (lignans and isoflavones). Where necessary, data were log transformed to satisfy the normality assumptions of the statistical tests. Results were converted back to the original scale for reporting purposes. Two subjects were excluded from the soy group because of a missing urine volume; thus, the total number of subjects used for statistical analysis was 44. Urinary metabolite concentrations that were <0.625 ng/mL (the lower detection limit of the kit) were assigned values of 0.625 ng/mL, as done by others (28). All of the treatment groups had a similar number of samples with concentrations <0.625 ng/mL (3, 2, and 2 for the



TABLE 3

Macronutrient intakes at baseline (week 0) and at the endpoint (week 16) by treatment group¹

Macronutrient	Placebo (n = 15)		Soy (n = 13)		Flaxseed (n = 16)	
	Week 0	Week 16	Week 0	Week 16	Week 0	Week 16
Energy (kJ/d)	6933 ± 428	8219 ± 484	7326 ± 234	7161 ± 276	7778 ± 518	7916 ± 404
Protein (g/d)	81.0 ± 5.3	84.4 ± 4.8	67.7 ± 4.6	79.0 ± 4.4	72.6 ± 6.4	75.2 ± 5.4
Fat (g/d)	52.8 ± 4.5	68.1 ± 9.2	58.0 ± 5.0	59.0 ± 3.0	66.8 ± 6.1	71.7 ± 6.2
Total carbohydrates (g/d)	240.2 ± 17.8	269.7 ± 21.6	242.9 ± 17.5	219.6 ± 16.5	242.1 ± 15.8	234.9 ± 12.9
Total dietary fiber (g/d)	16.5 ± 2.0	21.8 ± 3.0	18.3 ± 1.6	17.5 ± 3.2	22.1 ± 3.5	22.4 ± 2.1

¹ Arithmetic $\bar{x} \pm$ SEM. Values are based on data from 3-d food records. There were no significant effects of time or group and no significant group \times time interaction (two-factor ANOVA).

placebo, soy, and flaxseed groups, respectively). In all cases, $P \leq 0.05$ was considered statistically significant. All statistical analyses were conducted by using SIGMASTAT 2.0 (Jandel Corporation, San Rafael, CA).

RESULTS

Subjects and diet

The mean macronutrient intake of each treatment group is shown in **Table 3**. No significant changes in macronutrient intake were observed with any of the 3 treatments, nor were significant differences observed between the groups either at baseline or at the endpoint.

Urinary phytoestrogens

There were no significant differences in total urinary phytoestrogen (lignans + isoflavones) excretion at baseline (**Figure 1**). After the 16-wk study period, total urinary phytoestrogen excretion values (arithmetic $\bar{x} \pm$ SEM) in the placebo, soy, and flaxseed groups were 3.24 ± 0.71 , 21.84 ± 4.37 , and 41.05 ± 8.49 $\mu\text{mol/d}$, respectively. Both the soy and the flaxseed groups had significantly higher urinary phytoestrogen excretion than did the placebo group. Although the flaxseed group tended to have higher total urinary phytoestrogen excretion than did the soy group, the difference was not significant.

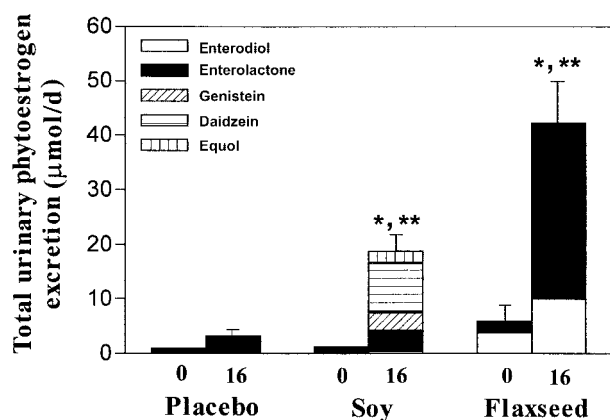


FIGURE 1. Mean (\pm SEM) total urinary phytoestrogen excretion at baseline (week 0) and at the endpoint (week 16) by treatment group (placebo, $n = 15$; soy, $n = 13$; flaxseed, $n = 16$). A significant group \times time interaction was observed ($P < 0.05$). *Significantly different from the placebo group at week 16, $P < 0.05$ (two-factor ANOVA followed by Tukey's test). **Significantly different from week 0 within the same group, $P < 0.001$ (two-factor ANOVA followed by Tukey's test).

However, the flaxseed group excreted primarily enterodiol and enterolactone, whereas the soy group excreted primarily genistein, daidzein, and equol.

Urinary estrogen metabolites and sex hormone analyses

Baseline values for both estrogen metabolites and their ratio (2OHE1:16 α OHE1) were not significantly different between the treatment groups (7.93 ± 1.96 , 4.19 ± 0.46 , and 5.60 ± 0.93 μg 2OHE1/24 h and 5.97 ± 1.03 , 4.64 ± 0.89 , and 4.78 ± 0.81 μg 16 α OHE1/24 h in the placebo, soy, and flaxseed groups, respectively) (**Figure 2**). Two-factor analysis of variance showed a group \times time interaction for both 2OHE1 and 2OHE1:16 α OHE1. Supplementation with flaxseed but not soy or placebo significantly increased urinary 2OHE1 concentrations (7.25 ± 1.48 , 6.15 ± 0.97 , and 11.36 ± 1.93 $\mu\text{g}/24$ h in the placebo, soy, and flaxseed groups, respectively). No significant differences in 16 α OHE1 concentrations after 16 wk were observed in any of the treatment groups (6.87 ± 1.32 , 6.24 ± 1.05 , and 5.07 ± 0.79 $\mu\text{g}/24$ h in the placebo, soy, and flaxseed groups, respectively). Tukey's multiple comparison test did not show significant differences between the groups in 2OHE1:16 α OHE1. However, when the ratios within each group were examined by using a paired t test, a significant increase in the ratio was observed in the flaxseed group but not in the soy or placebo groups.

Linear regression showed a significant positive correlation between changes in urinary lignans and changes in 2OHE1:16 α OHE1 within the flaxseed group (**Figure 3A**). When a similar regression was conducted for the soy group, no significant correlation was observed (**Figure 3B**). There were no significant differences in serum estradiol, estrone, and estrone sulfate concentrations between the treatment groups and no significant differences within any of the treatment groups between the concentrations at baseline and those at the endpoint (**Table 4**).

Biochemical markers of bone metabolism

The results of serum AP and urinary DPD analyses are shown in **Table 4**. There were no significant differences in AP or DPD concentrations between the groups or within the groups over time. A significant inverse correlation between the change in serum AP and the change in total urinary lignan excretion was observed in the flaxseed group only (**Figure 4**).

DISCUSSION

This study showed that dietary supplementation with 25 g ground flaxseed but not with 25 g soy flour significantly alters

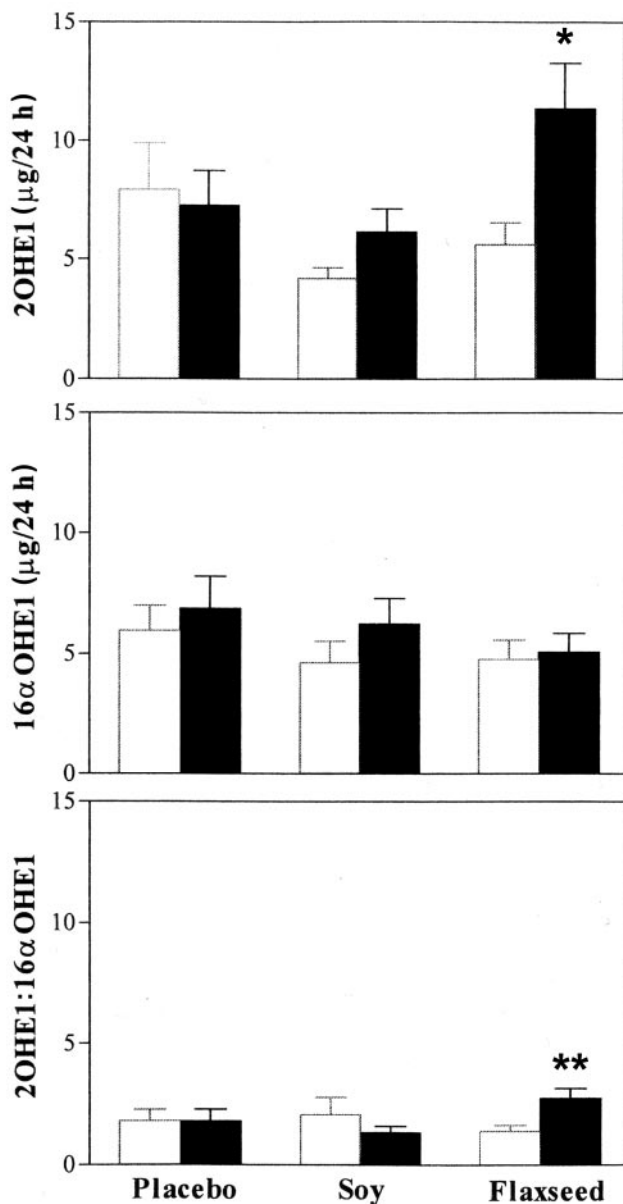


FIGURE 2. Mean (\pm SEM) urinary excretion of 2-hydroxyestrone (2OHE1) and 16 α -hydroxyestrone (16 α OHE1) and mean (\pm SEM) ratio of 2OHE1 to 16 α OHE1 at baseline (\square , week 0) and at the endpoint (\blacksquare , week 16) by treatment group (placebo, $n = 15$; soy, $n = 13$; flaxseed, $n = 16$). Two-factor ANOVA followed by Tukey's test showed a significant group \times time interaction for both 2OHE1 and 2OHE1:16 α OHE1. *Significantly different from week 0, $P = 0.05$ (post hoc Tukey's test). **Significantly different from week 0, $P = 0.005$ (paired t test).

the metabolism of estradiol in favor of the less biologically active estrogen metabolite (2OHE1) in postmenopausal women. Our study showed for the first time that changes in urinary lignan excretion with flaxseed supplementation are positively related to significant changes in 2OHE1:16 α OHE1.

In support of our results, Haggans et al (28) reported that supplementation with 10 g ground flaxseed/d for 7 wk significantly increased the urinary excretion of 2OHE1 (34%) as well as 2OHE1:16 α OHE1 (21%) in postmenopausal women, although no correlation between these markers and urinary lignans was reported. Our study showed a further increase in

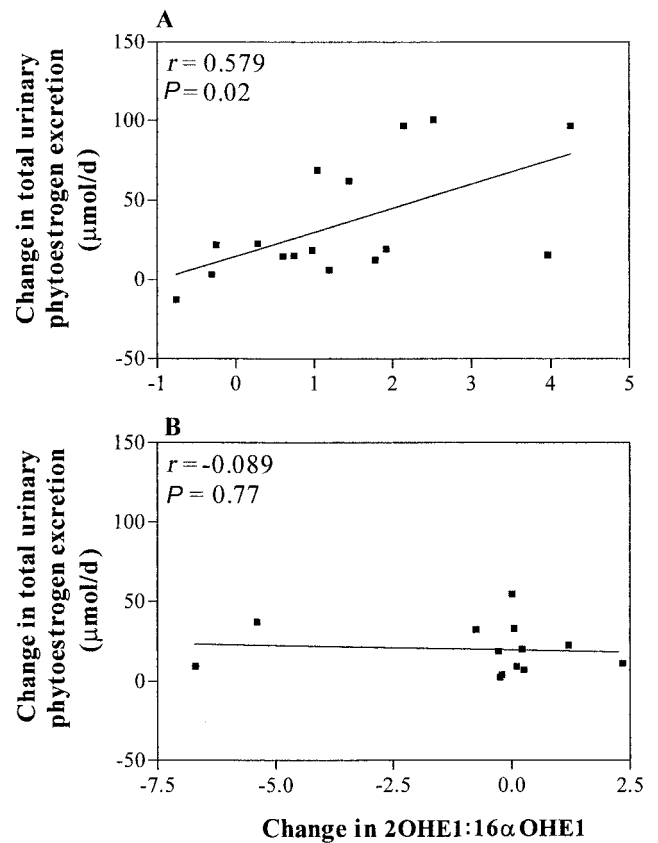


FIGURE 3. Correlation between the change in total urinary phytoestrogen (primarily enterolactone and enterodiol) excretion and the change in the ratio of 2-hydroxyestrone (2OHE1) to 16 α -hydroxyestrone (16 α OHE1) in the flaxseed treatment group (A) and correlation between the change in total urinary phytoestrogen (primarily genistein, daidzein, and equol) excretion and the change in 2OHE1:16 α OHE1 in the soy treatment group (B).

2OHE1 concentration (103%) and in 2OHE1:16 α OHE1 (98%) with an increase in dose from 10 (28) to 25 g. However, in our study, only the change in 2OHE1 concentration was significant.

Some studies showed that supplementation with soy as ISP providing 56–132 mg isoflavones/d influences urinary estrogen metabolite excretion in postmenopausal women (46), whereas other studies did not (36). In comparison, our study used soy flour containing 42 mg isoflavones. Results in premenopausal women appear to be more consistent. Supplementation with soy as ISP in similar amounts was found to influence premenopausal urinary estrogen metabolite excretion (26, 27), which suggests that the effect of dietary soy may be dependent on estrogen status (47).

Results concerning the effects of phytoestrogen supplementation on serum hormones are conflicting. Although the present study reports no change in serum hormone concentrations, another study reported that consumption of 5 or 10 g ground flaxseed/d for 7 wk significantly reduced serum estradiol concentrations in postmenopausal women (3). The 10-g dose also reduced serum estrone sulfate concentrations (3). In support of our results, Lucas et al (35) found that supplementation with 40 g flaxseed/d for 3 mo had no effect on serum estradiol or estrone concentrations in postmenopausal women.

TABLE 4

Concentrations of serum hormones and biochemical markers of bone metabolism at baseline (week 0) and at the endpoint (week 16) by treatment group¹

	Placebo (n = 15)		Soy (n = 13)		Flaxseed (n = 16)	
	Week 0	Week 16	Week 0	Week 16	Week 0	Week 16
Estradiol (pg/mL)	20.11 ± 3.55	15.14 ± 2.11	10.69 ± 1.00	20.27 ± 9.92	14.09 ± 1.70	14.04 ± 1.53
Estrone (pg/mL)	29.88 ± 3.81	31.45 ± 6.58	23.24 ± 3.58	32.53 ± 5.02	29.95 ± 4.22	28.79 ± 3.82
Estrone sulfate (ng/mL)	2.76 ± 0.48	3.12 ± 0.70	2.87 ± 0.36	3.49 ± 0.79	2.93 ± 0.53	3.17 ± 0.51
AP (U/L)	15.62 ± 3.20	16.38 ± 3.22	15.89 ± 2.73	15.16 ± 2.27	14.83 ± 1.99	14.64 ± 1.91
Deoxyypyridinoline (nmol/mmol creatinine)	9.01 ± 1.10	9.32 ± 1.30	9.52 ± 1.23	8.98 ± 1.19	9.30 ± 1.44	10.44 ± 1.92

¹ Arithmetic $\bar{x} \pm$ SEM. AP, bone-specific alkaline phosphatase. There were no significant effects of time or group and no significant group \times time interaction (two-factor ANOVA).

The effect of soy supplementation on serum hormones also varies and, like the effect on urinary metabolites, appears to be dependent on estrogen status. In agreement with our results, both Persky et al (36), in whose study postmenopausal women consumed ISP supplying 56 or 90 mg isoflavones/d for 3 or 6 mo, and Petrakis et al (48), in whose study postmenopausal women consumed ISP with 38 mg genistein for 6 mo, observed no effects on serum hormones in postmenopausal women. Studies in premenopausal women report conflicting results, with some showing no effect (40, 49), and others showing a moderate reduction (29, 39).

Despite increased metabolism to the less estrogenic metabolite (2OHE1) (with no change in 16 α OHE1 concentration) in the flaxseed group, a corresponding change in biochemical markers of bone metabolism was not observed. Lim et al (25) found that postmenopausal women with osteopenia had significantly lower urinary 16 α OHE1 excretion than did healthy control subjects. 2OHE1:16 α OHE1 was also found to be negatively correlated with spinal BMD in these women (25). Supporting this apparent estrogenicity of 16 α OHE1 with respect to bone, 16 α OHE1 treatment in ovariectomized, growing rats resulted in bone measurements that did not differ from those after estradiol treatment (24).

Few studies relating flaxseed consumption to markers of bone metabolism have been conducted. Our results are supported by those of Lucas et al (35), who found that flaxseed supplementation had no effect on postmenopausal markers of bone metabolism. It is important to note the inverse correlation

between urinary lignan excretion and serum bone-specific alkaline phosphatase that was observed in the flaxseed group in the present study. This correlation suggests a potentially anti-estrogenic effect of flaxseed supplementation on bone, although the correlation did not translate to changes in the concentrations of the biochemical markers measured.

Studies in postmenopausal women have shown conflicting results concerning the influence of soy isoflavones on BMD. Significant relations between habitual dietary isoflavone intake and BMD in postmenopausal Asian women have been observed (50, 51). However, intervention studies in postmenopausal women that examined both biochemical markers of bone metabolism and BMD yielded inconsistent results. Some studies showed a beneficial effect (37, 52), but another study showed none (38). These studies have generally involved ISP supplementation (56–90 mg isoflavones/d) over the short term (3–6 mo). With respect to bone, there is some question of whether the beneficial agent is the soy protein component rather than the isoflavones in soy (53, 54). Further studies investigating the effect of long-term supplementation with dietary phytoestrogens on BMD and, ultimately, the incidence of fracture are needed to fully understand this relation.

Estrogen is involved in the development and progression of several chronic diseases, including osteoporosis and hormone-sensitive cancers such as breast cancer. The structural similarity of the phytoestrogens enterolactone and enterodiol from flaxseed and genistein and daidzein from soy suggests that they may interfere with estrogen metabolism. Studies, including our own, suggest that flaxseed lignans and soy isoflavones interfere with the normal physiologic activity and metabolism of estrogens (4, 30–32, 55, 56). The ability to modulate estrogen metabolism and thereby affect tissue exposure to biologically active estrogens (ie, estradiol and 16 α OHE1) may influence disease.

From the present study, we conclude that flaxseed supplementation in the amount of 25 g/d modifies estrogen metabolism, as indicated by changes in urinary metabolite excretion. Consumption of 25 g flaxseed/d significantly increased urinary 2OHE1 excretion, whereas consumption of 25 g soy/d did not. The increase in 2OHE1:16 α OHE1 in the flaxseed group was not significant after a post hoc Tukey's test but was significant after a paired *t* test. A similar significant effect was not seen in the soy or placebo groups. Thus, these results should be interpreted cautiously and suggest that perhaps with a larger sample size, a significant effect may be achieved by using Tukey's test. The positive correlation between 2OHE1:16 α OHE1 and uri-

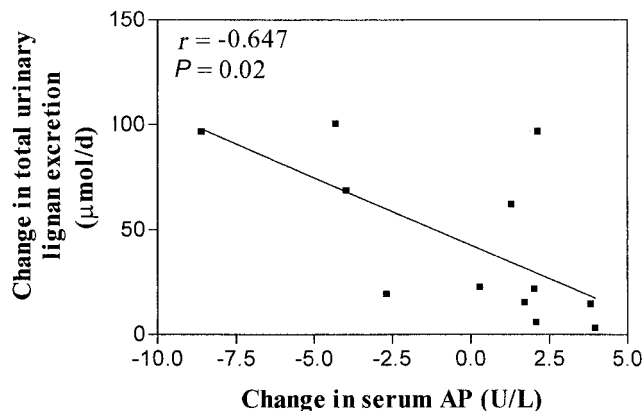



FIGURE 4. Correlation between the change in total urinary lignan excretion and the change in serum bone-specific alkaline phosphatase (AP) in the flaxseed treatment group.

nary phytoestrogens in the flaxseed group (primarily lignans) but not in the soy group (primarily isoflavones) suggests that changes in metabolite excretion may be related to the higher activity and availability of lignans than of isoflavones. This suggestion is supported by the higher total phytoestrogen excretion in the flaxseed group than in the soy group (although not significant) despite the lower phytoestrogen intake of the flaxseed group.

The ability of phytoestrogens (in the present study, those from flaxseed most notably) to modify estrogen metabolism suggests a mechanism through which these compounds may be involved in both disease prevention and treatment strategies. However, flaxseed is also a very rich source of α -linolenic acid (ALA) (57). Flaxseed oil has been shown to reduce mammary tumor growth (58), and ALA has been shown to alter the growth of breast cancer cell lines *in vitro* (59). Although the mechanism involving the effect of ALA, which may include an effect on estrogen metabolism, remains controversial, ALA should not be ruled out as a contributor to the effects seen with dietary flaxseed.

Modulation of estrogen metabolism has the capacity to influence tissue estrogen exposure and therefore breast cancer and osteoporosis (38, 60, 61). The present study suggests no negative effect of changing estrogen metabolism on biochemical markers of bone metabolism; however, the study was limited by the short treatment time and the small number of subjects. This suggests the need for long-term studies in larger treatment groups to examine the effect of whole soy and flaxseed, as well as their isolated components (eg, secoisolariciresinol diglycoside), on estrogen and bone metabolism to further understand their role as alternatives to traditional hormone replacement therapy. With the findings of the Women's Health Initiative study raising serious concerns about the safety of pharmacologic estrogen therapy (62), the potential of natural alternatives becomes more attractive. 

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JDB drafted the manuscript and did the analysis for sex hormones, estrogen metabolites, and nutrient intakes. WEW did the bone marker analysis and helped write the manuscript. JH, JEL, LN, and EW coordinated the subject recruitment and the sample and data collection for the original menopausal symptom study. LUT was the principal investigator for the estrogen metabolite and bone marker component of the study and helped write the manuscript. None of the authors had any conflicts of interest.

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