The Effect of Flaxseed and Wheat Bran Consumption on Urinary Estrogen Metabolites in Premenopausal Women[1]

Carol J. Haggans, Elsa J. Travelli, William Thomas, Margaret C. Martini, and Joanne L. Slavin[2]

Department of Food Science and Nutrition, University of Minnesota, St. Paul, Minnesota 55108 [C. J. H., E. J. T., M. C. M., J. L. S.], and Division of Biostatistics, School of Public Health, University of Minnesota, Minneapolis, Minnesota 55455 [W. T.]

Abstract

Estrogen is metabolized along two competing pathways to form the 2-hydroxylated and the 16α-hydroxylated metabolites. Based on proposed differences in biological activities, the ratio of these metabolites, 2-hydroxyestrogen:16α-hydroxyestrone ($2:16\alpha$-OHE1), has been used as a biomarker for breast cancer risk. Women with an elevated $2:16\alpha$-OHE1 ratio are hypothesized to be at a decreased risk of breast cancer. Flaxseed, the most significant source of plant lignans, and wheat bran, an excellent source of dietary fiber, have both been shown to have chemoprotective benefits. Some of these benefits may be attributable to their influence on endogenous sex hormone production and metabolism. We examined the effect of flaxseed consumption alone and in combination with wheat bran on urinary estrogen metabolites in premenopausal women. Sixteen premenopausal women were studied for four feeding treatments lasting two menstrual cycles each in a randomized cross-over design. During the four feeding treatments, subjects consumed their usual diets supplemented with baked goods containing no flaxseed or wheat bran, 10 g of flaxseed, 28 g of wheat bran, or 10 g of flaxseed plus 28 g of wheat bran/day. Urinary excretion of 2-hydroxyestrogen and 16α-hydroxyestrone, as well as their ratio, $2:16\alpha$-OHE1, were measured by enzyme immunoassay. Flaxseed supplementation significantly increased the urinary $2:16\alpha$-OHE1 ratio ($P = 0.034$), but wheat bran had no effect. These results suggest that flaxseed may be chemoprotective in premenopausal women.

Introduction

Strong evidence suggests that endogenous estrogens affect breast cancer risk (1–6). In recent years, estrogen metabolism has become a growing area of interest because of its proposed role in the etiology of breast cancer (7–9). Estradiol and its oxidative product, estrone, can be metabolized along two irreversible, mutually exclusive hydroxylation pathways, forming the metabolites 2-OHEstrogen[3] (including 2-OHE1 and 2-hydroxyestradiol) and 16α-OHE1 (Fig. 1; Ref. 10). These metabolites are proposed to have differences in biological activity, which may affect breast cancer risk.

The 2-OHEstrogen metabolites are proposed to be chemoprotective (7) because they have little estrogenic activity and may even act as antiestrogens (11). 2-OHE1 has been shown to be virtually devoid of uterotropic activity (12, 13), to suppress growth and proliferation of MCF-7 breast cancer cells (11), and to stimulate SHBG production in human hepatoma HepG2 cells (14).

In contrast, the 16α-OHE1 metabolite is proposed to be associated with increased breast cancer risk attributable to its significant estrogenic activity (9) and its low binding affinity for SHBG (15). In addition to being elevated in women with breast cancer (16, 17), the 16α-OHE1 metabolite has been shown to cause cell proliferation and DNA damage in mouse mammary cells (18), to have significant uterotropic activity (15), and to be positively correlated with mammary tumor incidence in mice (19). Recent studies that showed that urinary 16α-OHE1 is positively correlated with bone mass density in postmenopausal women (20, 21) further support its proposed estrogenic activity.

Because of the competing nature of the 2-hydroxylation and 16α-hydroxylation pathways, a ratio of the metabolites 2-OHEstrogen and 16α-OHE1, $2:16\alpha$-OHE1, has been used as a biomarker for breast cancer risk. Although the optimal level for the metabolite ratio is not known, an increase in the ratio is considered protective. Recent studies showing that the ratio is significantly decreased in women with breast cancer (22–25) further support this hypothesis.

Certain factors have been shown to influence estrogen metabolism and the $2:16\alpha$-OHE1 ratio. High-protein diets (26), low-fat diets (27), consumption of broccoli (28), and consumption of indole-3-carbinol (29–31), a compound found in broccoli and other cruciferous vegetables, have all been shown to increase the $2:16\alpha$-OHE1 ratio. Similarly, omega-3 fatty acid consumption has been shown to decrease excretion of the 16α-hydroxylated metabolites (32), suggesting it may also have protective effects.

F is the richest known source of lignans, one of the main classes of phytoestrogens (33–35). On consumption, the main plant lignans, secoisolariciresinol and matairesinol, are metabolized by the intestinal microflora into the main mammalian

[3] The abbreviations used are: 2-OHEstrogen, 2-hydroxyestrogen; 2-OHE1, 2-hydroxyestrone; 16α-OHE1, 16α-hydroxyestrone; SHBG, sex hormone-binding globulin; $2:16\alpha$-OHE1, 2-hydroxyestrogen:16α-OHE1; LH, luteinizing hormone; BMI, body mass index; Ctrl, control; F, flaxseed; WB, wheat bran; WBF, WB F.
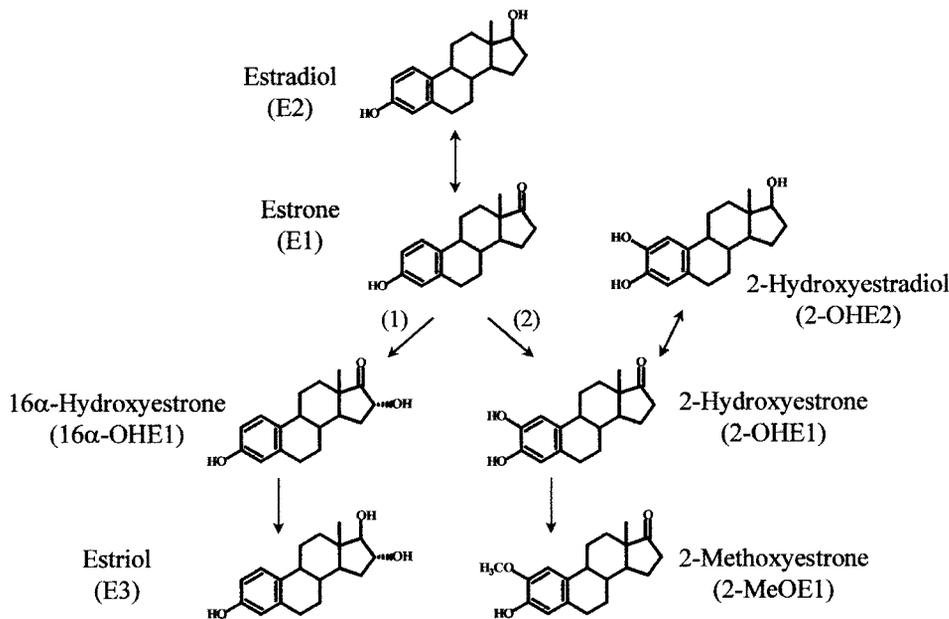
Fig. 1. Oxidative metabolism of estradiol, 16α-hydroxylase (1), and 2-hydroxylase (2). Adapted from Michnovicz *et al.* (80).

lignans, enterodiol and enterolactone (36). After absorption, lignans are conjugated with glucuronic acid and sulfate, are excreted in urine (37) and bile, and undergo enterohepatic recirculation (38).

Lignans have been proposed to be chemoprotective because they have been shown to stimulate SHBG synthesis (39), inhibit growth of human mammary tumor cells (40), reduce mammary tumor initiation (41), and inhibit aromatase (estrogen synthetase) activity (42, 43). Consumption of F has been found to inhibit mammary tumor growth (44) and to reduce early markers of risk for mammary (45) and colon carcinogenesis (46, 47). F also contains the omega-3 fatty acid α-linolenic acid (48), which has been shown to reduce mammary tumor growth (49, 50) and number (51) in animal studies.

Consumption of dietary fiber has also been proposed to be chemoprotective because of its influence on endogenous sex hormone levels (52). In premenopausal women, dietary fiber reduced serum estrone and estradiol (53) and was negatively correlated with urinary estrogen excretion (54) and serum estradiol (55). These effects may result from partial interruption of the enterohepatic circulation of estrogen (56). WB, which is a concentrated source of insoluble dietary fiber, was found to reduce serum estrone and estradiol in premenopausal women (57), increase serum LH in adult female rats (58), and reduce early biomarkers of colon cancer risk in male rats (59).

Although lignans and dietary fiber may influence endogenous sex hormone production, little is known about how they interact or whether they affect estrogen metabolism and the urinary $2:16\alpha$-OHE1 ratio in premenopausal women. Thus, we chose to examine the effects of F consumption alone and in combination with WB in premenopausal women. We hypothesized that consumption of these components would increase urinary 2-OHEstrogen excretion and the urinary $2:16\alpha$-OHE1 ratio, thereby possibly offering some chemoprotective benefits.

Materials and Methods

Subjects. Healthy, premenopausal women between the ages of 18 and 38 who were not pregnant or lactating were recruited for the study and screened with a detailed health and dietary questionnaire. After screening, 24 subjects were contacted and agreed to participate by providing informed written consent. All subjects were within 10% of ideal body weight, had regular menstrual cycles, and consumed a typical American diet with low to moderate fiber intake. To eliminate factors shown to affect estrogen metabolism or lignan absorption, exclusion criteria included cigarette smoking, use of oral contraceptives or antibiotics within 6 months, alcohol consumption greater than two drinks per day or five drinks per week, caffeine consumption greater than three caffeinated beverages per day, or regular consumption of products containing F or soy. Subjects were asked to maintain their body weights and usual exercise habits throughout the duration of the study. Eight of the 24 subjects who started the study withdrew for various reasons. The remaining 16 subjects ranged in age from 20 to 38 and included 1 African American, 1 Asian American, and 14 Caucasian women. Fourteen of the subjects were nulliparous and two were parous.

The mean values $\pm$ SD for age, height, weight, and BMI of the 16 subjects were 26.7 ± 5.7 years, 166.9 ± 6.0 cm, 63.3 ± 7.9 kg, and 22.6 ± 1.6 kg/m^2, respectively.

Experimental Design. The study design, approved by the Institutional Review Board: Human Subjects Committee at the University of Minnesota, was a randomized, cross-over trial consisting of four feeding treatments lasting two menstrual cycles each, with no wash-out between treatments. Subjects, who were randomly assigned to feeding treatments, consumed their habitual diets plus a supplement of two baked goods (cookies or muffins), which provided the following per day: Ctrl, no F or WB; F, 10 g; WB, 28 g; and WBF, 10 g of F plus 28 g of WB. Daily nutrient content of the baked goods are presented in Table 1. To maintain compliance, subjects were allowed to select their baked goods from several choices for each diet treatment. This is reflected by the macronutrient ranges in Table 1.

The baked goods were prepared in small batches from commercially available mixes or from scratch. Ground F

Table 1 Daily nutrient content of supplemental baked goods

	Ctrl	F (10g)	WB (28g)	WBF (28g of W + 10g of F)
Energy (kcal)[a]	306–453	289–330	204–531	241–540
Carbohydrate (g)[a]	67.8–95.2	41.6–62.5	50.8–116.8	51.5–112.1
Protein (g)[a]	4.5–5.7	5.3–10.0	6.0–10.2	7.6–11.5
Fat (g)[a]	2.0–6.1	7.2–9.7	2.0–7.5	5.3–10.4
Total dietary fiber (g)[b]	4.8	5.5	14.8	20.7
Soluble fiber (g)[b]	0.6	1.3	0.0	2.5
Insoluble fiber (g)[b]	4.2	4.3	14.8	18.2
Lignans[c] (mg)[b]		79.6		70.6

[a] Range.
[b] Mean.
[c] Secoisolariciresinol.

(Linola, United Grain Growers Ltd., Winnipeg, Manitoba, Canada) and WB were weighed out and added to the batches to achieve the final concentrations per baked good. The dietary fiber content of the baked goods was measured by Medallion Laboratories (Minneapolis, MN) and is presented in Table 1. The approximate plant lignan content of the baked goods was determined by Kenneth D. R. Setchell, Ph.D., at the Children's Hospital Medical Center (Cincinnati, OH) by measuring the secoisolariciresinol-diglycoside content via high-performance liquid chromatography (60). The other primary plant lignan, matairesinol, was not measured because it represents only about 0.3% of the total lignans in F (61). Analyses showed that the 10 g of ground F in diets of F and WBF supplied 79.6 mg and 70.6 mg of the lignan secoisolariciresinol, respectively (Table 1). Differences in the measured lignan content may have been caused by the baking process, the interaction of F with other ingredients, or variability in the lignan analyses. Lignan content of the WB was assumed to be biologically insignificant because WB contains very low levels of lignans (35, 62).

Subjects picked up their baked goods weekly from the University of Minnesota Clinical Research Center and kept them frozen until just before consumption. They were instructed to consume them daily during at least two different sittings. Subject compliance was monitored by collecting and measuring any uneaten baked goods. Because of the high kilocalorie content of the baked goods, the subjects were encouraged to substitute them for similar items in their habitual diets to maintain their body weights.

Fourteen of the 16 subjects completed all four dietary treatments. One subject completed only dietary treatments of the Ctrl, F, and WBF and another completed only dietary treatments of the F, WB, and WBF because of personal schedule conflicts. Data for the feeding treatment these two subjects completed were included in analyses.

The subjects' food intake was monitored during the urine collection times as described below by self-reported 3-day diet records. Body weights were also measured during these times. Diet analyses were performed with the Minnesota Nutrition Data System software, which was developed by the Nutrition Coordinating Center, University of Minnesota (Minneapolis, MN; Food Database version 6A, Nutrient Database 21, 1992; Ref. 63).

Sample Collection and Analysis. During both menstrual cycles of each diet treatment, subjects performed LH surge testing to detect ovulation using a commercial kit (OvuQuick One-Step, Quidel Corp., San Diego, CA). Ovulation was defined as 1 day after a LH surge. During the midluteal phase (days 6, 7, and 8 after ovulation) of the second menstrual cycle of each diet treatment, subjects completed three consecutive 24-h urine collections. Midluteal phase collections were used because estrogen levels are higher during this phase of the menstrual cycle (64–67), possibly enhancing the ability to detect differences between study treatments. Urine was collected into 1-liter bottles containing 1 g of ascorbic acid to preserve the estrogen metabolites (65, 68). Urine was stored at 4°C until processed. Before processing, the final volume of each 24-h urine collection was measured, and an aliquot was frozen for subsequent creatinine analysis to monitor the completeness of the collections. The three 24-h urine collections were then pooled, and an aliquot was stored at −20°C until analyzed.

Commercially available enzyme immunoassay kits (Estramet 2/16 Enzyme Immunoassay Kit, Immuna Care, Bethlehem, PA; Ref. 69) were used to measure 2-OHEstrogen and 16α-OHE1 in the urine samples. These assays are competitive solid-phase enzyme immunoassays that use high affinity monoclonal antibodies. The monoclonal antibody to 2-OHEstrogen is specific for all 2,3-hydroxylated estrogen metabolites, showing 100% reactivity with 2-OHE1 and 2-hydroxyestradiol, and 68% reactivity with 2-hydroxyestriol. The monoclonal antibody to 16α-OHE1 shows 100% reactivity with 16α-OHE1 (69). Correlation with gas chromatography-mass spectrometry for premenopausal samples has produced Pearson correlations of 0.94, 0.96, and 0.93 for 2-OHEstrogen, 16α-OHE1, and the $2{:}16\alpha$-OHE1 ratio, respectively (70). To perform the assays, the estrogen metabolites were first deconjugated of glucuronic acid and sulfate by incubating the urine samples with β-glucuronidase and arylsulfatase. The samples, along with competitive alkaline phosphatase conjugates, were then added to the antibody-coated 96-well microtiter plates. Standards of 0.625–20 ng/ml were used in addition to in-house quality control samples. After the plates were incubated and washed with Tris-buffered saline (pH 7.4)/0.05% Tween-20, paranitrophenyl phosphate was added as the enzyme substrate, and the plates were read kinetically at 405 nm.

To eliminate interassay and lot-to-lot variability (65), one kit lot was used for the entire study and all samples from each subject were run in the same assay. All samples were run in triplicate, and the results were averaged. Intra-assay coefficients of variation based on in-house quality control samples were 6.7%, 5.0%, and 8.0% for 2-OHEstrogen, 16α-OHE1, and the $2{:}16\alpha$-OHE1 ratio, respectively. Interassay coefficients of variation were 8.9%, 15.0%, and 14.5% for 2-OHEstrogen, 16α-OHE1, and the $2{:}16\alpha$-OHE1 ratio, respectively.

Statistical Analysis. Statistical analyses were performed using the Statistical Analysis System (SAS Proprietary Software Release 6.12, SAS Institute Inc., Cary, NC). Data were normally distributed and were analyzed using a repeated measure ANOVA within subject. Analyses were conducted to determine differences between diet treatments as well as the main effects from F and WB, the two factors in the two-by-two factorial design. For all measurements, results were considered statistically significant at $P < 0.05$.

Results

Intakes of total energy, carbohydrate, protein, fat, and dietary fiber during each feeding treatment are presented in Table 2. There were no significant differences in carbohydrate or protein intake during any of the feeding treatments. Total energy intake was significantly different during feeding treatments mainly attributable to differences in fat intake. The feeding treatments also differed significantly in total dietary fiber intake, attribut-

Table 2 Nutrient intake during each feeding treatment (includes supplemental baked goods)[a]

	Ctrl (n = 15)	F (n = 16)	WB (n = 15)	WBF (n = 16)
Energy (kcal)	2272 ± 106[b,c]	2164 ± 110[b,c]	2091 ± 121[b]	2468 ± 108[c]
Carbohydrate (g)	352 ± 20	311 ± 21	321 ± 23	339 ± 20
Protein (g)	75 ± 5	78 ± 5	73 ± 5	85 ± 5
Fat (g)	65 ± 6[b]	68 ± 6[b,c]	66 ± 6[b,c]	84 ± 6[c]
Total dietary fiber (g)	17.4 ± 0.9[b]	18.2 ± 1.0[b]	26.4 ± 1.1[c]	31.4 ± 1.0[d]
Soluble fiber (g)	6.0 ± 0.4[b]	6.6 ± 0.4[b]	6.1 ± 0.4[b]	8.6 ± 0.4[c]
Insoluble fiber (g)	11.0 ± 0.7[b]	11.0 ± 0.7[b]	19.8 ± 0.8[c]	23.2 ± 0.7[d]

[a] LS mean ± SE.
[b,c,d] Where treatment means differ significantly ($P < 0.05$); the groups with the same letter are indistinguishable.

Table 3 Subject weight and BMI during each feeding treatment[a]

	Ctrl (n = 15)	F (n = 16)	WB (n = 15)	WBF (n = 16)
Weight (kg)	63.8 ± 9.9	63.9 ± 8.8	63.4 ± 9.1	63.5 ± 8.0
BMI (kg/m²)	22.8 ± 2.2	22.8 ± 1.9	22.6 ± 2.0	22.7 ± 1.7

[a] Mean ± SD. No significant differences.

able more to differences in insoluble fiber intake than to differences in soluble fiber intake. Fat and total dietary fiber intakes were highest during the WBF treatment, the latter attributable to the fiber content of the WB rather than the F.

Body weight measurements and BMI at the end of each feeding treatment are presented in Table 3. There were no significant differences in mean body weight or BMI among any of the treatments.

The effects of the feeding treatments on luteal phase total 24-h urinary excretion of 2-OHEstrogen, 16α-OHE1, and the 2:16α-OHE1 ratio are summarized in Tables 4 and 5. Analyses were conducted with and without adjustment for total energy and fat intake, but not for fiber intakes because these differences were inherent to the study design. Comparisons between diets with or without adjusting for energy or fat produced the same conclusions and led to similar estimates of effects. Therefore, for clarity, only adjusted data are presented in Tables 4 and 5.

The F treatment significantly increased urinary 2-OHEstrogen excretion by 30.7% compared with the Ctrl treatment ($P = 0.048$; Table 4). The F treatment also significantly increased the 2:16α-OHE1 ratio by 25.2% compared with the WB treatment ($P = 0.045$). Analyses of the main effects of F and WB showed that the two treatments containing F significantly increased the 2:16α-OHE1 ratio by 18.9% ($P = 0.034$; Table 5). These two treatments also increased 2-OHEstrogen excretion by 17.9%, but the differences were not significant ($P = 0.097$). There were no significant main effects of WB.

Discussion

This study showed that daily consumption of 10 g of ground F for two menstrual cycles significantly increased the 2:16α-OHE1 ratio in premenopausal women during the luteal phase of the menstrual cycle. It also showed that consumption of 28 g of WB did not significantly affect the luteal phase 2:16α-OHE1 ratio, nor did it interact with the effect from ground F. These results suggest that F contains components that may be protective against breast cancer in premenopausal women.

Consumption of 10 g/day of F powder has been investigated in previous human studies involving premenopausal women. It has been shown to significantly increase urinary (71) and fecal (72) excretion of lignans and to increase the length of the luteal phase of the menstrual cycle (73), suggesting that it has hormonal effects. Previous work in our laboratory (74) showed that the addition of 10 g of ground F to the daily diets of postmenopausal women significantly raised total urinary 2-OHEstrogen excretion and the 2:16α-OHE1 ratio. This suggests that there are compounds in F capable of influencing

estrogen metabolism, particularly in the 2-hydroxylation pathway. The results from the present study showed that F consumption resulted in a moderate but nonsignificant increase in 2-OHEstrogen excretion, as well as a significant increase in the 2:16α-OHE1 ratio during the luteal phase of the menstrual cycle in premenopausal women. These results also suggest F alters estrogen metabolism in the 2-hydroxylation pathway preferentially over the 16α-hydroxylation pathway.

The F used in this study, Linola, is a low α-linolenic acid variety (75). Because we fed F and not its isolated components, it is not possible to identify the specific compound(s) responsible for the observed results. However, our finding that Linola F influences estrogen metabolism suggests that lignans rather than α-linolenic acid may be the active component in F. This agrees with the findings from previous work in our laboratory using traditional F (74), which contains both lignans and α-linolenic acid and was also found to influence estrogen metabolism.

Multiple cytochrome P-450 enzymes can catalyze the estrogen 2-hydroxylation step in mammals (76). Certain dietary compounds have been shown to induce these enzymes, resulting in increased production of estrogen metabolites along the 2-hydroxylation pathway. In humans, consumption of 500 g/day of broccoli was shown to induce the cytochrome P-450 enzyme CYP1A2, which is involved in estrone 2-hydroxylation (28). Consumption of indole-3-carbinol by mice resulted in a dose-dependent increase in the cytochrome P-450 content of hepatic microsomes (77), suggesting that indole-3-carbinol is one of the compound(s) in broccoli capable of inducing cytochrome P-450 enzymes. In some animals, testosterone and other steroids can induce certain cytochrome P-450 enzymes (76). Given these findings and the structural similarity of enterodiol and enterolactone to endogenous steroids, we propose that these compounds may induce cytochrome P-450 enzymes involved in the estrogen 2-hydroxylation pathway in humans. This is one possible explanation for the observed effect of F on the urinary 2:16α-OHE1 ratio.

Because diets high in phytoestrogens also tend to be high in dietary fiber, it can be difficult to distinguish between these components when investigating biomarkers for cancer risk. We attempted to analyze these separately by feeding 10 g of F as a source of lignans with little dietary fiber (2 g), alone and in combination with 28 g of WB as a high source of dietary fiber (10 g). Our finding that F significantly increased the urinary 2:16α-OHE1 ratio, whereas WB had no effect, suggests that components in F other than dietary fiber are responsible for the effect.

We also found that WB did not interact with the effect of F on the 2:16α-OHE1 ratio. Tew *et al.* (78) found that a wheat fiber-supplemented diet containing 40 g of dietary fiber reduced dietary absorption of the phytoestrogen genistein from tofu and textured vegetable protein in premenopausal women. Other investigators have shown that water-insoluble dietary fiber binds steroid hormones *in vitro* (79). Based on these findings, we anticipated that WB might interfere with lignan absorption

Table 4   Urinary 2-OHEstrogen, 16α-OHE1, and 2:16α-OHE1 ratio during the luteal phase of each feeding treatment[a]

	Ctrl ($n = 15$)	F ($n = 16$)	WB ($n = 15$)	WBF ($n = 16$)
2-OHEstrogen (μg/24 h)	34.65 ± 3.78	45.29 ± 3.78[b]	39.55 ± 4.23	42.16 ± 3.84
16α-OHE1 (μg/24 h)	15.64 ± 1.42	17.59 ± 1.42	17.49 ± 1.59	18.88 ± 1.45
2:16α-OHE1 ratio	2.26 ± 0.19	2.73 ± 0.19[c]	2.18 ± 0.21	2.55 ± 0.19

[a] LS mean ± SE, adjusted for energy (kcal) and fat intake.
[b] Significantly different from ctrl, $P = 0.048$.
[c] Significantly different from WB, $P = 0.045$.

Table 5   Main effects of E and WB on urinary 2-OHEstrogen, 16α-OHE1, and 2:16α-OHE1 ratio during the luteal phase of each feeding treatment[a]

	Main effect of F		Main effect of WB	
	No F	10 g of F	No WB	28 g of WB
2-OHEstrogen (μg/24 h)	37.10 ± 2.89	43.73 ± 2.60	39.97 ± 2.76	40.86 ± 2.73
16α-OHE1 (μg/24 h)	16.56 ± 1.09	18.23 ± 0.98	16.61 ± 1.04	18.18 ± 1.03
2:16α-OHE1 ratio	2.22 ± 0.14	2.64 ± 0.13[b]	2.49 ± 0.14	2.36 ± 0.13

[a] LS mean ± SE adjusted for energy (kcal) and fat intake. Combines data from the F feeding treatments and the WB feeding treatments to analyze the main effects from F and WB.
[b] Significantly different from "No F"; $P = 0.034$.

from F, which in turn would decrease any lignan-induced physiological effect. However, our results do not support this hypothesis. It is possible that 28 g of WB is insufficient to affect lignan absorption. It is also possible that another component in F was responsible for the increased 2:16α-OHE1 ratio.

Although lignans are present in other plant foods, such as legumes, whole cereals, fruits, and vegetables, F is by far the most significant source. By weight, mammalian lignan production from F meal is over 120 times higher than most legumes, over 180 times higher than most whole cereals, and over 260 times higher than most fruits and vegetables (35). Therefore, it is very difficult for an individual to obtain the quantity of lignans supplied by 10 g of F by consuming other food sources.

Bradlow *et al.* (8, 19) have proposed that altered estrogen metabolism exists before the onset of cancer and is not a byproduct of it. This suggests that influencing the hormonal environment in a protective fashion may help prevent cancer initiation. The findings from our study suggest that daily consumption of 10 g of ground F may offer some protection against breast cancer in some premenopausal women by significantly increasing the urinary 2:16α-OHE1 ratio. Further research is required to understand the biological mechanisms and the active compound(s) in F.

References

1. Bernstein, L., and Ross, R. K. Endogenous hormones and breast cancer risk. Epidemiol. Rev., *15:* 48–65, 1993.

2. Toniolo, P. G. Endogenous estrogens and breast cancer risk: the case for prospective cohort studies. Environ. Health Perspect., *105* (Suppl. 3)*:* 587–592, 1997.

3. Madigan, M. P., Troisi, R., Potischman, N., Dorgan, J. F., Brinton, L. A., and Hoover, R. N. Serum hormone levels in relation to reproductive and lifestyle factors in postmenopausal women (United States). Cancer Causes Control, *9:* 199–207, 1998.

4. Cauley, J. A., Lucas, F. L., Kuller, L. H., Stone, K., Browner, W., and Cummings, S. R. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Study of Osteoporotic Fractures Research Group (see comments). Ann. Intern. Med., *130:* 270–277, 1999.

5. Hankinson, S. E., Willett, W. C., Manson, J. E., Colditz, G. A., Hunter, D. J., Spiegelman, D., Barbieri, R. L., and Speizer, F. E. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women (see comments). J. Natl. Cancer Inst., *90:* 1292–1299, 1998.

6. Thomas, H. V., Reeves, G. K., and Key, T. J. Endogenous estrogen and postmenopausal breast cancer: a quantitative review. Cancer Causes Control, *8:* 922–928, 1997.

7. Bradlow, H. L., Telang, N. T., Sepkovic, D. W., and Osborne, M. P. 2-hydroxyestrone: the "good" estrogen. J. Endocrinol., *150* (Suppl.)*:* S259–S265, 1996.

8. Bradlow, H. L., Hershcopf, R. E., and Fishman, J. F. Oestradiol 16α-hydroxylase: a risk marker for breast cancer. Cancer Surv., *5:* 573–583, 1986.

9. Fishman, J., Osborne, M. P., and Telang, N. T. The role of estrogen in mammary carcinogenesis. Ann. NY Acad. Sci., *768:* 91–100, 1995.

10. Fishman, J., Bradlow, H. L., and Gallagher, T. F. Oxidative Metabolism of Estradiol. J. Biol. Chem., *235:* 3104–3107, 1960.

11. Schneider, J., Huh, M. M., Bradlow, H. L., and Fishman, J. Antiestrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells. J. Biol. Chem., *259:* 4840–4845, 1984.

12. Martucci, C., and Fishman, J. Direction of estradiol metabolism as a control of its hormonal action—uterotropic activity of estradiol metabolites. Endocrinology, *101:* 1709–1715, 1977.

13. Martucci, C. P., and Fishman, J. Impact of continuously administered catechol estrogens on uterine growth and luteinizing hormone secretion. Endocrinology, *105:* 1288–1292, 1979.

14. Loukovaara, M., Carson, M., and Adlercreutz, H. Regulation of sex-hormone-binding globulin production by endogenous estrogens *in vitro*. Biochem. Biophys. Res. Commun., *206:* 895–901, 1995.

15. Fishman, J., and Martucci, C. Biological properties of 16 α-hydroxyestrone: implications in estrogen physiology and pathophysiology. J. Clin. Endocrinol. Metab., *51:* 611–615, 1980.

16. Fishman, J., Schneider, J., Hershcope, R. J., and Bradlow, H. L. Increased estrogen-16 α-hydroxylase activity in women with breast and endometrial cancer. J. Steroid Biochem., *20:* 1077–1081, 1984.

17. Schneider, J., Kinne, D., Fracchia, A., Pierce, V., Anderson, K. E., Bradlow, H. L., and Fishman, J. Abnormal oxidative metabolism of estradiol in women with breast cancer. Proc. Natl. Acad. Sci. USA, *79:* 3047–3051, 1982.

18. Telang, N. T., Suto, A., Wong, G. Y., Osborne, M. P., and Bradlow, H. L. Induction by estrogen metabolite 16 α-hydroxyestrone of genotoxic damage and aberrant proliferation in mouse mammary epithelial cells. J. Natl. Cancer Inst., *84:* 634–638, 1992.

19. Bradlow, H. L., Hershcopf, R. J., Martucci, C. P., and Fishman, J. Estradiol 16 α-hydroxylation in the mouse correlates with mammary tumor incidence and presence of murine mammary tumor virus: a possible model for the hormonal etiology of breast cancer in humans. Proc. Natl. Acad. Sci. USA, *82:* 6295–6299, 1985.

20. Leelawattana, R., Ziambaras, K., Lyss, C., Roodman-Weiss, J., Wagner, D., Klug, T., and Civitelli, R. Estrogen metabolites in urine of early postmenopausal women correlate with bone density (Abstract). J. Bone Miner. Res., *12* (Suppl. 1)*:* S132, 1997.

21. Lim, S. K., Won, Y. J., Lee, J. H., Kwon, S. H., Lee, E. J., Kim, K. R., Lee, H. C., Huh, K. B., and Chung, B. C. Altered hydroxylation of estrogen in patients with postmenopausal osteopenia. J. Clin. Endocrinol. Metab., *82:* 1001–1006, 1997.

22. Kabat, G. C., Chang, C. J., Sparano, J. A., Sepkovic, D. W., Hu, X. P., Khalil, A., Rosenblatt, R., and Bradlow, H. L. Urinary estrogen metabolites and breast cancer: a case-control study. Cancer Epidemiol. Biomark. Prev., *6:* 505–509, 1997.

23. Zheng, W., Dunning, L., Jin, F., and Holtzman, J. Correspondence re. G. C. Kabat *et al.*, Urinary estrogen metabolites and breast cancer: a case-control study. Cancer Epidemiol. Biomark. Prev., *6:* 505–509, 1997. Cancer Epidemiol. Biomark. Prev., *7:* 85–86, 1998.

24. Coker, A. L., Crane, M. M., Sticca, R. P., and Sepkovic, D. W. Re. Ethnic differences in estrogen metabolism in healthy women. J. Natl. Cancer Inst., *89:* 89–90, 1997.

25. Ho, G. H., Luo, X. W., Ji, C. Y., Foo, S. C., and Ng, E. H. Urinary 2/16α-hydroxyestrone ratio: correlation with serum insulin-like growth factor binding protein-3 and a potential biomarker of breast cancer risk. Ann. Acad. Med. Singapore, *27:* 294–299, 1998.

26. Anderson, K. E., Kappas, A., Conney, A. H., Bradlow, H. L., and Fishman, J. The influence of dietary protein and carbohydrate on the principal oxidative biotransformations of estradiol in normal subjects. J. Clin. Endocrinol. Metab., *59:* 103–107, 1984.

27. Longcope, C., Gorbach, S., Goldin, B., Woods, M., Dwyer, J., Morrill, A., and Warram, J. The effect of a low fat diet on estrogen metabolism. J. Clin. Endocrinol. Metab., *64:* 1246–1250, 1987.

28. Kall, M. A., Vang, O., and Clausen, J. Effects of dietary broccoli on human *in vivo* drug metabolizing enzymes: evaluation of caffeine, oestrone and chlorzoxazone metabolism. Carcinogenesis (Lond.), *17:* 793–799, 1996.

29. Bradlow, H. L., Michnovicz, J. J., Halper, M., Miller, D. G., Wong, G. Y., and Osborne, M. P. Long-term responses of women to indole-3-carbinol or a high fiber diet. Cancer Epidemiol. Biomark. Prev., *3:* 591–595, 1994.

30. Michnovicz, J. J., Adlercreutz, H., and Bradlow, H. L. Changes in levels of urinary estrogen metabolites after oral indole-3-carbinol treatment in humans. J. Natl. Cancer Inst., *89:* 718–723, 1997.

31. Michnovicz, J. J. Increased estrogen 2-hydroxylation in obese women using oral indole-3-carbinol. Int. J. Obes. Relat. Metab. Disord., *22:* 227–229, 1998.

32. Osborne, M. P., Karmali, R. A., Hershcopf, R. J., Bradlow, H. L., Kourides, I. A., Williams, W. R., Rosen, P. P., and Fishman, J. Omega-3 fatty acids: modulation of estrogen metabolism and potential for breast cancer prevention. Cancer Invest., *8:* 629–631, 1988.

33. Axelson, M., Sjovall, J., Gustafsson, B. E., and Setchell, K. D. Origin of lignans in mammals and identification of a precursor from plants. Nature (Lond.), *298:* 659–660, 1982.

34. Nesbitt, P. D., and Thompson, L. U. Lignans in homemade and commercial products containing flaxseed. Nutr. Cancer, *29:* 222–227, 1997.

35. Thompson, L. U., Robb, P., Serraino, M., and Cheung, F. Mammalian lignan production from various foods. Nutr. Cancer, *16:* 43–52, 1991.

36. Borriello, S. P., Setchell, K. D., Axelson, M., and Lawson, A. M. Production and metabolism of lignans by the human faecal flora. J. Appl. Bacteriol., *58:* 37–43, 1985.

37. Adlercreutz, H., van der Wildt, J., Kinzel, J., Attalla, H., Wahala, K., Makela, T., Hase, T., and Fotsis, T. Lignan and isoflavonoid conjugates in human urine. J. Steroid Biochem. Mol. Biol., *52:* 97–103, 1995.

38. Setchell, K. D. R., and Adlercreutz, H. Mammalian lignans and phytooestrogens. Recent studies on their formation, metabolism and biological role in health and disease. *In:* I. R. Rowland (ed.), Role of the Gut Flora in Toxicity and Cancer, pp. 315–345. London: Academic Press Limited, 1988.

39. Adlercreutz, H., Mousavi, Y., Clark, J., Hockerstedt, K., Hamalainen, E., Wahala, K., Makela, T., and Hase, T. Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. J. Steroid Biochem. Mol. Biol., *41:* 331–337, 1992.

40. Hirano, T., Fukuoka, K., Oka, K., Naito, T., Hosaka, K., Mitsuhashi, H., and Matsumoto, Y. Antiproliferative activity of mammalian lignan derivatives against the human breast carcinoma cell line, ZR-75–1. Cancer Invest., *8:* 595–602, 1990.

41. Thompson, L. U., Seidl, M. M., Rickard, S. E., Orcheson, L. J., and Fong, H. H. Antitumorigenic effect of a mammalian lignan precursor from flaxseed. Nutr. Cancer, *26:* 159–165, 1996.

42. Wang, C., Makela, T., Hase, T., Adlercreutz, H., and Kurzer, M. S. Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. J Steroid Biochem. Mol. Biol., *50:* 205–212, 1994.

43. Adlercreutz, H., Bannwart, C., Wahala, K., Makela, T., Brunow, G., Hase, T., Arosemena, P. J., Kellis, J. T., Jr., and Vickery, L. E. Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. J. Steroid Biochem. Mol. Biol., *44:* 147–153, 1993.

44. Serraino, M., and Thompson, L. U. The effect of flaxseed supplementation on the initiation and promotional stages of mammary tumorigenesis. Nutr. Cancer, *17:* 153–159, 1992.

45. Serraino, M., and Thompson, L. U. The effect of flaxseed supplementation on early risk markers for mammary carcinogenesis. Cancer Lett., *60:* 135–142, 1991.

46. Serraino, M., and Thompson, L. U. Flaxseed supplementation and early markers of colon carcinogenesis. Cancer Lett., *63:* 159–165, 1992.

47. Jenab, M., and Thompson, L. U. The influence of flaxseed and lignans on colon carcinogenesis and β-glucuronidase activity. Carcinogenesis (Lond.), *17:* 1343–1348, 1996.

48. Johnston, P. V. Flaxseed oil and cancer: α-linolenic acid and carcinogenesis. *In:* S. C. Cunnane and L. U. Thompson (eds.), Flaxseed in Human Nutrition, pp. 207–218. Champaign, IL: American Oil Chemists Society, 1995.

49. Thompson, L. U., Rickard, S. E., Orcheson, L. J., and Seidl, M. M. Flaxseed and its lignan and oil components reduce mammary tumor growth at a late stage of carcinogenesis. Carcinogenesis (Lond.), *17:* 1373–1376, 1996.

50. Fritsche, K. L., and Johnston, P. V. Effect of dietary α-linolenic acid on growth, metastasis, fatty acid profile and prostaglandin production of two murine mammary adenocarcinomas. J. Nutr., *120:* 1601–1609, 1990.

51. Cameron, E., Bland, J., and Marcuson, R. Divergent effects of omega-6 and omega-3 fatty acids on mammary tumor development in C3H/Heston mice treated with DMBA. Nutr. Res., *9:* 383–393, 1989.

52. Adlercreutz, H. Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand. J. Clin. Lab. Investig. Suppl., *201:* 3–23, 1990.

53. Bagga, D., Ashley, J. M., Geffrey, S. P., Wang, H. J., Barnard, R. J., Korenman, S., and Heber, D. Effects of a very low fat, high fiber diet on serum hormones and menstrual function. Implications for breast cancer prevention. Cancer (Phila.), *76:* 2491–2496, 1995.

54. Adlercreutz, H., Fotsis, T., Bannwart, C., Hamalainen, E., Bloigu, S., and Ollus, A. Urinary estrogen profile determination in young Finnish vegetarian and omnivorous women. J. Steroid Biochem., *24:* 289–296, 1986.

55. Kaneda, N., Nagata, C., Kabuto, M., and Shimizu, H. Fat and fiber intakes in relation to serum estrogen concentration in premenopausal Japanese women. Nutr. Cancer, *27:* 279–283, 1997.

56. Adlercreutz, H., Hockerstedt, K., Bannwart, C., Bloigu, S., Hamalainen, E., Fotsis, T., and Ollus, A. Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). J. Steroid Biochem., *27:* 1135–1144, 1987.

57. Rose, D. P., Lubin, M., and Connolly, J. M. Effects of diet supplementation with wheat bran on serum estrogen levels in the follicular and luteal phases of the menstrual cycle. Nutrition, *13:* 535–539, 1997.

58. Arts, C. J., and Thijssen, J. H. Effects of wheat bran on blood and tissue hormone levels in adult female rats. Acta Endocrinol., *127:* 271–278, 1992.

59. Jenab, M., and Thompson, L. U. The influence of phytic acid in wheat bran on early biomarkers of colon carcinogenesis. Carcinogenesis (Lond.), *19:* 1087–1092, 1998.

60. Gamache, P. H., and Acworth, I. N. Analysis of phytoestrogens and polyphenols in plasma, tissue, and urine using HPLC with coulometric array detection. Proc. Soc. Exp. Biol. Med., *217:* 274–280, 1998.

61. Mazur, W., Fotsis, T., Wahala, K., Ojala, S., Salakka, A., and Adlercreutz, H. Isotope dilution gas chromatographic-mass spectrometric method for the determination of isoflavonoids, coumestrol, and lignans in food samples. Anal. Biochem., *233:* 169–180, 1996.

62. Adlercreutz, H., and Mazur, W. Phyto-oestrogens and Western diseases. Ann. Med., *29:* 95–120, 1997.

63. Schakel, S. F., Sievert, Y. A., and Buzzard, I. M. Sources of data for developing and maintaining a nutrient database. J. Am. Diet. Assoc., *88:* 1268–1271, 1988.

64. Berg, F. D., and Kuss, E. Urinary excretion of catecholestrogens, 2-methoxy-estrogens and "classical estrogens" throughout the normal menstrual cycle. Arch. Gynecol. Obstet., *249:* 201–207, 1991.

65. Chen, C., Malone, K. E., Prunty, J., and Daling, J. R. Measurement of urinary estrogen metabolites using a monoclonal enzyme-linked immunoassay kit: assay performance and feasibility for epidemiological studies. Cancer Epidemiol. Biomark. Prev., *5:* 727–732, 1996.

66. McGuinness, B. J., Power, M. J., and Fottrell, P. F. Radioimmunoassay of 2-hydroxyestrone in urine. Clin. Chem., *40:* 80–85, 1994.

67. Naganuma, H., Hershcopf, R. J., Michnovicz, J. J., Miyairi, S., Bradlow, H. L., and Fishman, J. Radioimmunoassay of 16 α-hydroxyestrone in human urine. Steroids, *53:* 37–48, 1989.

68. Sepkovic, D. W., Bradlow, H. L., Ho, G., Hankinson, S. E., Gong, L., Osborne, M. P., and Fishman, J. Estrogen metabolite ratios and risk assessment of hormone-related cancers. Assay validation and prediction of cervical cancer risk. Ann. NY Acad. Sci., *768:* 312–316, 1995.

69. Klug, T. L., Bradlow, H. L., and Sepkovic, D. W. Monoclonal antibody-based enzyme immunoassay for simultaneous quantitation of 2- and 16α-hydroxyestrone in urine. Steroids, *59:* 648–655, 1994.

70. Ziegler, R. G., Rossi, S. C., Fears, T. R., Bradlow, H. L., Adlercreutz, H., Sepkovic, D., Kiuru, P., Wahala, K., Vaught, J. B., Donaldson, J. L., Falk, R. T., Fillmore, C. M., Siiteri, P. K., Hoover, R. N., and Gail, M. H. Quantifying estrogen metabolism: an evaluation of the reproducibility and validity of enzyme immunoassays for 2-hydroxyestrone and 16α-hydroxyestrone in urine. Environ. Health Perspect., *105* (Suppl. 3)*:* 607–614, 1997.

71. Lampe, J. W., Martini, M. C., Kurzer, M. S., Adlercreutz, H., and Slavin, J. L. Urinary lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. Am. J. Clin. Nutr., *60:* 122–128, 1994.

72. Kurzer, M. S., Lampe, J. W., Martini, M. C., and Adlercreutz, H. Fecal lignan and isoflavonoid excretion in premenopausal women consuming flaxseed powder. Cancer Epidemiol. Biomark. Prev., *4:* 353–358, 1995.

73. Phipps, W. R., Martini, M. C., Lampe, J. W., Slavin, J. L., and Kurzer, M. S. Effect of flax seed ingestion on the menstrual cycle. J. Clin. Endocrinol. Metab., *77:* 1215–1219, 1993.

74. Haggans, C. J., Hutchins, A. M., Olson, B. A., Thomas, W., Martini, M. C., and Slavin, J. L. The effect of flaxseed consumption on urinary estrogen metabolites in postmenopausal women. Nutr. Cancer, *33:* 188–195, 1999.

75. Kolodziejczyk, P. P., and Fedec, P. Processing flaxseed for human consumption. *In:* S. C. Cunnane and L. U. Thompson (eds.), Flaxseed in Human Nutrition, pp. 261–280. Champaign, IL: American Oil Chemists Society, 1995.

76. Michnovicz, J. J., and Bradlow, H. L. Dietary and pharmacological control of estradiol metabolism in humans. Ann. NY Acad. Sci., *595:* 291–299, 1990.

77. Bradlow, H. L., Michnovicz, J., Telang, N. T., and Osborne, M. P. Effects of dietary indole-3-carbinol on estradiol metabolism and spontaneous mammary tumors in mice. Carcinogenesis (Lond.), *12:* 1571–1574, 1991.

78. Tew, B. Y., Xu, X., Wang, H. J., Murphy, P. A., and Hendrich, S. A diet high in wheat fiber decreases the bioavailability of soybean isoflavones in a single meal fed to women. J. Nutr., *126:* 871–877, 1996.

79. Whitten, C. G., and Shultz, T. D. Binding of steroid hormones *in vitro* by water-insoluble dietary fiber. Nutr. Res., *8:* 1223–1235, 1988.

80. Michnovicz, J. J., Hershcopf, R. J., Naganuma, H., Bradlow, H. L., and Fishman, J. Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. N. Engl. J. Med., *315:* 1305–1309, 1986.

The Effect of Flaxseed and Wheat Bran Consumption on Urinary Estrogen Metabolites in Premenopausal Women

Carol J. Haggans, Elsa J. Travelli, William Thomas, et al.

Updated version	Access the most recent version of this article at: http://cebp.aacrjournals.org/content/9/7/719

Cited articles	This article cites 70 articles, 11 of which you can access for free at: http://cebp.aacrjournals.org/content/9/7/719.full#ref-list-1
Citing articles	This article has been cited by 10 HighWire-hosted articles. Access the articles at: http://cebp.aacrjournals.org/content/9/7/719.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://cebp.aacrjournals.org/content/9/7/719. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.