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

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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 (2:16α-OHE1), has been used as a biomarker for breast cancer risk. Women with an elevated 2:16α-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 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, 2:16α-OHE1, were measured by enzyme immunoassay. Flaxseed supplementation significantly increased the urinary 2:16α-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 (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 uterotrophic 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 uterotrophic 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, 2:16α-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 2:16α-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 2:16α-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

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3 The abbreviations used are: 2-OHEstrogen, 2-hydroxyestrogen; 2-OHE1, 2-hydroxyestrone; 16α-OHE1, 16α-hydroxyestrone; SHBG, sex hormone-binding globulin; 2:16α-OHE1, 2-hydroxyestrogen:16α-OHE1; LH, lutenizing hormone; BMI, body mass index; Ctrl, control; F, flaxseed; WB, wheat bran; WBF, WB F.
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 2:16α-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-OHE estrogen excretion and the urinary 2:16α-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 ± 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/m², 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
1 day after a LH surge. During the midluteal phase (days 6, 7, 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.

Commerically available enzyme immunoassay kits (Estramet 2/16 Enzyme Immunoassay Kit, Immuna Care, Bethlehem, PA; Ref. 69) were used to measure 2-OHE1strogen 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-OHE1strogen is specific for all 2,3-hydroxylated estrogen metabolites, showing 100% reactivity with 2-OHE1 and 2-hydroxyestradiol, and 68% reactivity with 2-hydroxyestril. 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-OHE1strogen, 16α-OHE1, and the 2:16α-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, para nitrophenyl 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-OHE1strogen, 16α-OHE1, and the 2:16α-OHE1 ratio, respectively. Interassay coefficients of variation were 8.9%, 15.0%, and 14.5% for 2-OHE1strogen, 16α-OHE1, and the 2:16α-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-
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 = 0.05); the groups with the highest intake of broccoli were shown to induce the cytochrome P-450 30.7% compared with the Ctrl treatment (P = 0.05). Analyses of the main effects of F and WB showed that the two treatments containing F significantly increased the urinary 2-OHEstrogen excretion by 25.2% compared with the Ctrl treatment (P = 0.05); the groups with the highest intake of broccoli were shown to induce 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...
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.

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