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 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 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, 2:16α-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

1 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-hydroxyestrone; LH, luteinizing 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. 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).

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
The approximate plant lignan content of the baked goods was determined by Kenneth D. R. Setchell, Ph.D., at the Children’s Hospitals and Clinics of Minnesota Medical Center (Cincinnati, OH) by measuring the estrogen metabolites in urine samples. These assays are competitive solid-phase enzyme immunoassays that use high affinity monoclonal antibodies. The monoclonal antibody to 2-OHEsterogen is specific for all 2,3-hydroxyalkylated estrogen metabolites, showing 100% reactivity with 2-OHE1 and 2-hydroxyestradiol, and 68% reactivity with 2-hydroxyestradiol. 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-OHEsterogen, 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-aminophenyl 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-OHEsterogen, 16α-OHE1, and the 2:16α-OHE1 ratio, respectively. Interassay coefficients of variation were 8.9%, 15.0%, and 14.5% for 2-OHEsterogen, 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-

### Table 1 Daily nutrient content of supplemental baked goods

<table>
<thead>
<tr>
<th></th>
<th>Ctrl</th>
<th>F (10g)</th>
<th>WB (28g)</th>
<th>WBF (28g of W + 10g of F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate (g)</td>
<td>67.8–95.2</td>
<td>41.6–62.5</td>
<td>50.8–116.8</td>
<td>51.5–112.1</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>4.5–5.7</td>
<td>5.3–10.0</td>
<td>6.0–10.2</td>
<td>7.6–11.5</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>2.0–6.1</td>
<td>7.2–9.7</td>
<td>2.0–7.5</td>
<td>5.3–10.4</td>
</tr>
<tr>
<td>Total dietary fiber (g)</td>
<td>4.8</td>
<td>5.5</td>
<td>14.8</td>
<td>20.7</td>
</tr>
<tr>
<td>Soluble fiber (g)</td>
<td>0.6</td>
<td>1.3</td>
<td>0.0</td>
<td>2.5</td>
</tr>
<tr>
<td>Insoluble fiber (g)</td>
<td>4.2</td>
<td>4.3</td>
<td>14.8</td>
<td>18.2</td>
</tr>
<tr>
<td>Lignans (mg)</td>
<td>79.6</td>
<td>70.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 settings. 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 (Estratet 2/16 Enzyme Immunoassay Kit, Immuna Care, Bethlehem, PA; Ref. 69) were used to measure 2-OHEsterogen 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-OHEsterogen is specific for all 2,3-hydroxyalkylated estrogen metabolites, showing 100% reactivity with 2-OHE1 and 2-hydroxyestradiol, and 68% reactivity with 2-hydroxyestradiol. 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-OHEsterogen, 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-aminophenyl phosphate was added as the enzyme substrate, and the plates were read kinetically at 405 nm.

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Effect of F and WB on Urinary Estrogen Metabolites

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 ($P = 0.034$; 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 ($P = 0.048$; 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 ($P = 0.048$; Table 4).

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-carbolin by mice resulted in a dose-dependent increase in the cytochrome P-450 content of hepatic microsomes (77), suggesting that indole-3-carbolin 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.
Acknowledgments

We gratefully thank the subjects who participated in this study for their dedication and support. We would also like to thank Susan Ratz, Ph.D., University of Minnesota Clinical Research Center, for developing the baked goods recipes, assisting with the study coordination, and analyzing the dietary intake data. In addition, we would like to thank United Grain Growers Ltd. for donating the Linola flaxseed, Amanda Walsh and Desiree Nichols for assisting with the study coordination, and Andy Evans and Rich Flores for entering the dietary intake data. Finally, we thank Kenneth D. R. Setchell, Ph.D., Children’s Hospital Medical Center (Cincinnati, OH) for conducting the lignan analysis, and Medallion Laboratories (Minneapolis, MN) for conducting the fiber analysis of the baked goods.

References


Table 4

<table>
<thead>
<tr>
<th></th>
<th>Ctrl (n = 15)</th>
<th>F (n = 16)</th>
<th>WB (n = 15)</th>
<th>WBF (n = 16)</th>
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<tbody>
<tr>
<td>2-OHEstrogen (μg/24 h)</td>
<td>34.65 ± 2.89</td>
<td>45.29 ± 3.78</td>
<td>39.55 ± 4.23</td>
<td>42.16 ± 3.84</td>
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<tr>
<td>16α-OHE1 (μg/24 h)</td>
<td>15.64 ± 1.42</td>
<td>17.59 ± 1.42</td>
<td>17.49 ± 1.59</td>
<td>18.88 ± 1.45</td>
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<tr>
<td>2:16α-OHE1 ratio</td>
<td>2.26 ± 0.19</td>
<td>2.73 ± 0.19</td>
<td>2.18 ± 0.21</td>
<td>2.55 ± 0.19</td>
</tr>
</tbody>
</table>

* LS mean ± SE adjusted for energy (kcal) and fat intake.
† Significantly different from WB, P = 0.045.
‡ Significantly different from “No F”; P = 0.034.

Table 5

<table>
<thead>
<tr>
<th></th>
<th>Main effect of F</th>
<th>Main effect of WB</th>
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<tbody>
<tr>
<td>2-OHEstrogen (μg/24 h)</td>
<td>37.10 ± 2.89</td>
<td>43.73 ± 2.60</td>
</tr>
<tr>
<td>16α-OHE1 (μg/24 h)</td>
<td>16.56 ± 1.09</td>
<td>18.23 ± 0.98</td>
</tr>
<tr>
<td>2:16α-OHE1 ratio</td>
<td>2.22 ± 0.14</td>
<td>2.64 ± 0.13</td>
</tr>
</tbody>
</table>

* 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.
† Significantly different from “No F”; P = 0.034.
