Low-fat, high-fiber diet and serum estrone sulfate in premenopausal women\(^{1-3}\)

Margo N Woods, Sherwood L Gorbach, Christopher Longcope, Barry R Goldin, Johanna T Dwyer, and Ann Morrill-LaBrode

**ABSTRACT** The effect of diet on serum estrogen levels was investigated in 17 healthy premenopausal women consuming defined diets prepared in a metabolic unit. During an initial 4-wk control period all women consumed a typical Western diet (40% of total calories from fat, 400 mg cholesterol/d, 12 g dietary fiber/d, and a ratio of polyunsaturated to saturated fatty acids [P:S] of 0.5). After this control period they were switched to a low-fat, high-fiber diet for 8–10 wk, which consisted of 25% of calories from fat, P:S of 1.0, cholesterol of 200 mg cholesterol/d, and 40 g dietary fiber/d. Compared with the control period 16 of 17 women had lower serum estrone sulfate levels on the low-fat, high-fiber diet. There was an average decrease of 36% with mean levels decreasing from 2.11 ± 0.25 nmol/L (\(\bar{x} \pm \text{SEM}\)) on the control diet to 1.29 ± 0.19 nmol/L on the experimental diet \((p < 0.001)\). We conclude that a low-fat, high-fiber diet can significantly reduce serum estrone sulfate levels.

**KEY WORDS** Estrone sulfate, estrogens, low-fat diets

**Introduction**

Diet appears to influence the production, metabolism, and excretion of endogenous estrogens. Armstrong et al \((1)\) in their study of American vegetarians and omnivores found lower levels of urinary estrogen and serum: prolactin and higher sex-hormone–binding globulin in the vegetarians. In another report of premenopausal Seventh-day Adventists (SDA) and premenopausal omnivores, the SDA vegetarian women consumed significantly less fat, especially saturated fatty acids and their plasma levels of estrone and estradiol were lower than those of the omnivores \((2)\). A study from our laboratory showed that omnivores eating a high-fat, Western diet had higher plasma and lower fecal estrogen levels than vegetarians and there was an inverse correlation between plasma estrogen levels and fecal estrogen excretion \((3)\). We also observed a positive correlation between dietary fat intake and plasma estrogen levels \((4)\). Rose et al \((5)\) reduced the fat intake by dietary counseling from 35% to 21% in 16 premenopausal women with cystic breast disease. After 3 mo on the low-fat diet, significant reductions were noted in serum estrone, estradiol, and total estrogen.

Several epidemiologic studies as well as animal experiments have shown a relationship between dietary fat intake and breast cancer \((6–13)\). Dietary fat may influence the initiation and promotion of breast tumors through several mechanisms including the endocrine system. The effects of diet on estrogen metabolism are especially interesting because of the possible role of endogenous estrogen in human breast cancer: for example, the estrogen dependence of many human breast tumors, the effectiveness of anti-estrogen therapy in treating the disease, the positive association between breast cancer incidence and reproductive events (early menarche, late menopause, late first pregnancy, and nulliparity), and the dramatic influence of early oophorectomy in lowering the incidence of breast cancer \((14–16)\). High levels of plasma and urinary estrogens have been correlated with human breast cancer, as shown in studies using improved analytical techniques for estrogen determination. Morreal et al \((17)\) found elevated urinary estrogens in postmenopausal women with breast cancer. Higher levels of total serum estradiol and nonprotein-

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1 From the Departments of Community Health and Medicine, Tufts University School of Medicine, Boston, MA; the Frances Stern Nutrition Center, New England Medical Center Hospitals, Boston, MA; the USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA; and the Departments of Obstetrics and Gynecology and Medicine, University of Massachusetts School of Medicine Worcester, MA.

2 Supported by NIH grant R37 CA-45128, ACS grant PDT 254, and NIH grant M01-RR-0054.

3 Address reprint requests to SL Gorbach, Department of Community Health, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111.

Received March 28, 1988.
Accepted for publication July 12, 1988.
bound serum estradiol were found in postmenopausal women with breast cancer and nonprotein-bound estradiol was elevated in premenopausal breast cancer patients (18). Drafla et al (19) reported elevated plasma estradiol concentrations in postmenopausal women with breast cancer. MacMahon et al (20) found relative risks between 2.5 and 3.5 associated with the highest quartile of urinary estrogen values in premenopausal women. Elevated urinary estrogens were also observed in the healthy daughters of women who had breast cancer (21). In addition, Henderson et al (22) found elevated serum estrogens and Pike et al (23) found elevated plasma estrogens in women with a family history of the disease. Bernstein et al (24) demonstrated that nulliparous women, presumably at higher risk for breast cancer, had higher plasma estradiol and estrone levels and lower sex-hormone–binding globulin levels than parous women.

However, there have been other studies that have not agreed with these findings. England et al (25) found no difference in total serum estradiol between premenopausal breast-cancer patients and control subjects. Two other studies (26, 27) failed to find differences in total plasma estradiol between postmenopausal cases and control subjects.

Thus, it is unclear whether the level of estradiol in blood and urine is associated with breast cancer risk. It may be that other estrogen fractions would be more sensitive indicators. In this regard the free fraction of serum estradiol has associated positively with breast cancer risk in six other studies (26–31).

Diet may play a pivotal role in estrogen metabolism and may thereby influence breast cancer risk. The dietary studies cited above, however, were based on self-reported food recalls collected from women eating their usual diets. These diets were highly variable not only in fat content but in cholesterol, fiber, and polyunsaturated to saturated fat (P:S) ratio. We conducted this study to determine more precisely the role of dietary intake in estrogen metabolism.

Materials and methods

Subjects

Seventeen premenopausal women were recruited. Mean values (±SD) for age, height, and weight of the subjects were 25.4 ± 3.1 y, 161.5 ± 8.2 cm, and 60.4 ± 6.5 kg, respectively, with no significant weight changes occurring during the study. All were healthy and taking no drugs as determined by a medical history and blood studies. All women reported normal menses, were nulliparous, and had not used oral contraceptives within the last year. Before entering the study all subjects were interviewed by a nutritionist to ensure that they consumed a diet similar to the typical Western diet and did not go on frequent diets or food binges; women whose eating pattern could be classified as typically Western were selected for the study. The study was conducted according to the ethical standards of the Institutional Review Board of Tufts—New England Medical Center.

Diets

The participants were randomly assigned to a cohort of four to five women. They committed themselves to eating only food prepared by the Metabolic Research Unit at the USDA Human Nutrition Research Center on Aging at Tufts University. All meals, Monday through Friday and breakfast on Saturday morning were consumed in the unit; the remaining meals were packaged for the subjects to consume at home. The food was weighed to 0.1 g and trays were checked after each meal for complete consumption of food items. Women began the study 1 wk before the expected onset of menses. Daily for the first 4 wk of the study each member of the four cohorts, which consisted of a total of 17 subjects, consumed a control diet similar to the typical Western diet (40% of calories from fat, P:S of 0.5, 400 mg cholesterol, 17% protein, 43% carbohydrate, 12 g dietary fiber). Subjects were then placed on the experimental diet (25% of calories from fat, P:S of 1.0, 200 mg cholesterol, 18% protein, 57% carbohydrate, 40 g dietary fiber). The increase in the P:S was achieved by using safflower-oil salad dressing, reducing the amount of red meat, and replacing butter with margarine. These changes resulted in relatively high amounts of linoleic and linolenic acid. The control and experimental diets were isocaloric. Women consumed the experimental diet for a minimum of 8 wk and a maximum of 10 wk (two menstrual cycles) before hormone levels were remeasured.

Hormone determinations

Fasting blood samples were collected on three consecutive days during the early follicular phase of the menstrual cycle (days 4–7) while women were on the control diet and then again after 8–10 wk on the experimental diet. Samples were centrifuged at 1400 × g rpm for 22 min at 4 °C and the serum was stored at −70 °C. Pooled serum samples from the 3 d were used for hormone analysis. Serum hormone measurements were carried out for estrone, estradiol, free estradiol, estrone sulfate, androstenedione, and testosterone by radioimmunnoassays involving solvent extraction and celite chromatography as previously described (32, 33). The percent free testosterone was measured using an ultrafiltration technique (34–36). Sex-hormone–binding globulin was measured by the DEAE cellulose filter technique (35, 37). All samples were coded by using a random number system and blinded duplicates were sent as a quality control measure.

Results

Estrone sulfate

After 8–10 wk on the experimental diet, plasma estrone sulfate levels decreased in 16 of 17 women. The average percent decrease was 36% (Table 1). The mean value of plasma estrone sulfate on the standard Western diet was 2.11 ± 0.25 nmol/L, which decreased to a mean of 1.29 ± 0.19 nmol/L on the experimental diet (p < .001, paired t test) (Table 1). Those women with initial estrone sulfate levels < 1.07 nmol/L (n = 4) showed a mean decrease of 15% whereas those women with initial levels > 1.07 nmol/L (n = 12) showed a mean decrease of 42%.

Other hormones

No statistically significant changes were seen in serum estrone, estradiol, free estradiol, androstenedione, testos-
TABLE 1
Serum hormone levels on the control and experimental diets*

<table>
<thead>
<tr>
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<th>Control diet</th>
<th>Experimental diet</th>
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<tbody>
<tr>
<td>Estrone sulfate (nmol/L)†</td>
<td>2.11 ± 0.25</td>
<td>1.29 ± 0.19</td>
</tr>
<tr>
<td>Estrone (pmol/L)</td>
<td>187.9 ± 27.3</td>
<td>200.1 ± 34.4</td>
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<tr>
<td>Estradiol (pmol/L)</td>
<td>223.1 ± 42.5</td>
<td>220.5 ± 43.6</td>
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<tr>
<td>Free estradiol (pmol/L)</td>
<td>2.89 ± 0.58</td>
<td>3.00 ± 0.36</td>
</tr>
<tr>
<td>Androstenedione (nmol/L)</td>
<td>3.97 ± 0.52</td>
<td>3.97 ± 0.41</td>
</tr>
<tr>
<td>Sex-hormone-binding globulin (nmol/L)</td>
<td>79.5 ± 9.3</td>
<td>60.5 ± 6.5</td>
</tr>
<tr>
<td>Testosterone (nmol/L)</td>
<td>1.41 ± 0.17</td>
<td>1.10 ± 0.13</td>
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<tr>
<td>Free testosterone (pmol/L)</td>
<td>17.6 ± 2.42</td>
<td>13.4 ± 1.73</td>
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* x ± SEM; n = 17.
† p < 0.001, paired t test.

Discussion

Our study showed that short-term dietary changes can reduce serum estrone sulfate levels. The experimental diet was meant to simulate the diet of Oriental countries. Several components are different in these diets, such as total fat, type of fat, cholesterol, and dietary fiber. It is therefore impossible to identify which specific dietary change or combination of changes was responsible for the lowering of serum estrone sulfate.

The importance of the estrogen conjugate, estrone sulfate, has been enhanced by recent observations that it accounts for 40–50% of the estrone present in the blood stream and has a concentration 10 times higher than that of estrone or estradiol (38). In addition, estrone sulfate has a longer half-life in serum than estrone or estradiol, 5–10 h for estrone sulfate (38, 39) compared with 1 and 2 h for estrone and estradiol, respectively (40). The decrease in estrone sulfate seen in our study could reflect reduced production of estrone and estradiol or a shift in the metabolism of estrone and estradiol away from sulfation toward other estrogen metabolites not measured in this study. In this regard, we reported recently that when omnivore women were shifted to this low-fat, high-fiber diet, there was a significant decrease in urinary excretion of the 16-α-hydroxylated estrogen metabolites, estriol and 16-α-hydroxyestrone (41).

A low-fat, high-fiber diet can also influence the enterohepatic circulation of estrogens. Studies conducted in our laboratory on vegetarians and recent Asian immigrants to Hawaii (3, 4) demonstrated that these women had increased fecal excretion of estrone, estradiol, and estriol. The fecal excretion was inversely associated with the plasma concentrations (3) and positively associated with fecal weight. It has also been shown that plasma estrone and estradiol are positively correlated with total and saturated fatty acid intake and negatively correlated with dietary fiber intake (4). These findings indicate that a low-fat, high-fiber diet may decrease the enterohepatic circulation of estrogens, resulting in increased fecal excretion. Reduced estrone sulfate may be an indicator of interrupted enterohepatic circulation because estrone sulfate like other estrogen conjugates is excreted in the bile (42). Our studies (3, 4) consistently found higher levels of free estrogen in the feces of women eating low-fat, high-fiber diets and it has been demonstrated that free estrogens in the feces are derived from estrogen conjugates that are cleaved by bacteria in the large intestine (43).

Gurpide et al (44) showed in a continuous flow perfusion model that human endometrial slices from normal secretory tissue, postmenopausal tissue, and adenocarcinoma tissue, rapidly take up labeled estrone sulfate; the relative rates of entry of estrone sulfate compared with estrone were 0.49, 0.78, and 0.45, respectively. This is a high level of uptake considering the charged nature of estrone sulfate compared with the nonionic estrogen. It has also been shown that normal proliferative and secretory endometrial tissues as well as hyperplastic and adenocarcinoma endometrial tissues have aryl sulfatase activity in approximately equal amounts. This enzyme is capable of converting estrone sulfate to estrone (45). Vignon et al (46) injected radiolabeled estradiol into ovariec-tomized mice and found that 50–70% of the recovered radioactivity in mammary tumor cytosol was incorpo-rated into estrogen conjugates, the majority of which were sulfate conjugates. Similarly, estrone sulfate was shown to enter the MCF2 human breast cell line and was subsequently metabolized to free estrone and estradiol, which bound to the nuclear estrogen receptor.

The ability of homogenates of mammary carcinoma tissue to convert estrone sulfate into estrone and estradiol in vitro was also demonstrated in every such preparation from 23 patients (47). Santner et al (48) concluded that estrone sulfatase was the enzyme primarily responsible for intrauterine production of estrone in hormone-dependent breast carcinomas in postmenopausal women. Carlstrom (49) found that total hydrolysis of estrone sulfate was significantly higher in both hyperplastic endometrium and endometrial carcinoma than in atrophic endometrium and this difference was reflected in the formation of estradiol from estrone sulfate. This author concluded that estrone sulfate is an important prehormone for in-situ estradiol formation in target tissues such as breast or endometrium; this pathway is particularly pronounced in women with low peripheral estradiol levels.

There have also been studies comparing blood estrone sulfate levels in breast cancer cases and controls cases and in women at high and low risk for breast cancer. Prost et al (50) reported approximately twice the level of estrone sulfate in the plasma of women with breast cancer aged 56 and 80 y compared with matched control subjects. Fishman et al (51) found that women with a family history of breast cancer and thereby at higher risk for the disease had lower levels of glucuronide conjugates and a compensatory increase in sulfated estrogen conju-
gates when compared with matched control subjects. Studies from the same laboratory revealed that women with breast cancer had higher levels of 16-hydroxylated estrogen metabolites when compared with the matched control subjects (52, 53). These findings are similar to results from our laboratory, which showed that women on high-fat, low-fiber, diets have higher 16-hydroxylated products. 16-Hydroxyestrone is a highly estrogenic substance with a long half-life; it has been implicated as a factor in the etiology of breast cancer on the basis of these properties. There is also a link between the higher estrone sulfate levels and elevated 16-hydroxysterogens in breast cancer. Studies in the guinea pig (54) showed that estrogen sulfates are substrates for 16-hydroxylase. Therefore, this shift to higher estrone sulfate caused by either a high-fat, low-fiber diet or a family history of breast cancer can lead to higher levels of 16-hydroxyestrone.

Because of its higher concentration and longer half-life, plasma levels of estrone sulfate remain somewhat more constant during the menstrual cycle than those of estradiol or estrone (38–40). Thus, it may be easier to show a consistent change in the levels of estrone sulfate rather than estrone and estradiol when conducting short-term dietary studies. Rose et al (5) were unable to show a significant lowering in 2 mo but did achieve a reduction of these serum estrogens in a 3-mo, low-fat regimen in women with cystic breast disease. This suggests that a longer time is required than the 8–10 wk of dietary change used in our study to see lowering of serum estrone or estradiol.

We thank C. Franz and L. Gualtieri for their excellent assistance, the staff of the Metabolic Research Unit at the USDA Human Nutrition Research Center on Aging at Tufts University, and the Clinical Study Unit at New England Medical Center Hospital for their computer support.

References

DIET AND ESTRONE SULFATE


