Effects of a Very Low Fat, High Fiber Diet on Serum Hormones and Menstrual Function

Implications for Breast Cancer Prevention

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Background. **Low fat, high fiber dietary interventions that decrease blood estrogen levels may reduce breast cancer risk. Asian women consuming their traditional low fat, high fiber diets have lower blood estrogen levels before and after menopause and lower rates of breast cancer compared with Western women. The current controlled feeding study of premenopausal women was designed to determine the effects of a very low fat (10% of calories) and high fiber (35–45g/day) diet on blood estrogen levels and menstrual function.**

Method. **Twelve healthy premenopausal women with regular ovulatory cycles were followed for 3 months. Subjects consumed a diet providing 30% of their energy from fat and 15–25 g of dietary fiber per day for 1 month, and they consumed a very low fat, high fiber ad libitum diet providing 10% of their energy from fat and 25–35 g of dietary fiber per day for 2 months.**

Results. **At the end of the second month of the very low fat, high fiber diet, there was a significant reduction in serum estrone and estradiol levels during the early follicular and late luteal phases. There were no significant changes observed in serum estrone sulfate, sex hormone binding globulin, or progesterone. Despite a significant decrease in serum estradiol and estrone levels after 2 months of a very low fat, high fiber diet, there was no interference with ovulation or the magnitude of the midcycle leuteinizing hormone surge. Small changes in menstrual cycle length of up to 3 days were not ruled out due to the small sample size of the study.**

Conclusions. **A very low fat, high fiber diet in healthy premenopausal women can reduce estradiol and estrone levels without affecting ovulation, thereby providing a rationale for the prevention of breast cancer through a very low fat, high fiber diet. *Cancer* 1995;76:2491–6.**

Key words: breast cancer, menstrual cycle, fat, premenopausal, diet, hormones, ovulation.

Breast cancer is the second leading cause of mortality among women,[1] and the lifetime risk for developing breast cancer in the United States is one in eight.[2] International comparisons of age-adjusted breast cancer incidence reveal marked differences, with a much lower rate in Asia and in developing countries than in North America, Europe, and the developed countries of the Western world.[3,4] Substantial changes in incidence occur when populations move from low-incidence to high-incidence countries, indicating that these differences are not simply genetic.[5,6] The recent increase in breast cancer incidence rates reported in Japan suggest that environmental, dietary, and life-style changes within a country may also be important determinants of risk.[7]

Studies of dietary effects on reproductive hormone levels in premenopausal women are relevant to breast cancer prevention, because many of the hypotheses related to breast cancer prevention center on estrogens. There is substantial evidence that exposure to ovarian hormones, including estrogen and progesterone, may increase breast cancer risk. It has been established that age at menarche, age at menopause, parity, and age at first full-term pregnancy are important determinants of risk.[8] Although breast cancer occurs more commonly

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after menopause and occurs only rarely in women between the ages of 20 and 30, the rate of increase with age is greater before menopause. Antiestrogens, such as tamoxifen, and ovariectomy can be used to inhibit breast cancer growth.[9] Asian women eating a low fat, high fiber diet and living a traditional life-style have lower estrogen levels before and after menopause,[10,11] and these lower levels of estrogen have been considered important markers of the differences in breast cancer incidence observed in international epidemiologic studies.

Conversely, in several within-country case–control studies, investigators have not found an association between dietary fat intake and the age-adjusted incidence of breast cancer,[12–16] which has been observed in international correlations and migration studies. For example, analysis of the data of Willett et al. from the Nurses Health Study[17] did not reveal a relationship between dietary fat intake and breast cancer risk. A combined analysis of 12 case–control studies performed by Howe et al.[18] did show a positive association between fat intake and breast cancer for postmenopausal women but not for premenopausal women. These epidemiologic data rely on recalled food intake within countries where the range of dietary habits are more uniform than the are those found in international studies. Studies of mechanism by which diet can affect breast cancer risk such as the current experiment, complement these other studies.

Studies have shown that white women who have a higher risk of breast cancer have higher circulating estrogens, urinary excretion of total estrogens, estrone, estradiol and 2–4 hydroxy catechol estrogens than Asian women with lower breast cancer risk.[19,20] Between Asian and American women, there are differences in endogenous estrogen metabolism and excretion patterns.[21,22] The absolute levels and relative ratios of 16-alpha hydroxylated estrogens and 2-hydroxylated estrogens (catechol estrogens) in the body may play a role in the cause of breast cancer. The catechol estrogens can form quinones and free radicals, which can bind covalently to protein and form mutagenic DNA adducts.[23]

Epidemiologic, experimental, and clinical data have revealed a protective role of dietary fiber.[11,24,25] Mechanisms by which fiber could exert its protective effects include changes in the metabolism of estrogens, resulting in less estrogen bioavailability, and the introduction of phytoestrogens, antioxidants, and protease inhibitors. Dietary fiber could also act by removing fat or bile acids in the feces, thereby reducing fat absorption. The current controlled feeding study of premenopausal women eating a 10%-fat diet with 25–35 g of fiber per day was designed to confirm the combined effects of lowered dietary fat intake and increased fiber intake on circulating levels of estrogens in healthy premenopausal women. Moreover, ovulation and luteinizing hormone levels were also examined under conditions of controlled feeding and dietary change.

Methods

Twelve healthy white women age 25–45 years (mean age, 33.67 ± 6.48 years) with normal menstrual function were studied. The mean weight of the patients was 62.75 ± 6.72 kg, and the mean height was 1.66 ± 0.09 m (mean body mass index = 22.3 ± 1.7 kg/m^2). Seven of the 12 women were nulliparous, and the 5 parous women had breast-fed their infants at least 2 years before entering this study. Three of the 12 women were at slightly increased risk of breast cancer, with a history of breast cancer in one first-degree relative. All women selected for the study had discontinued using oral contraceptives 6 months before starting the study. Twelve of 14 women completed the study, and only their results are included in this report. The study was approved by the UCLA Institutional Review Board, and written informed consent was obtained.

Subjects first participated in a month of screening, during which they were asked to monitor their basal body temperature to determine the approximate duration of their menstrual cycle and to confirm normal ovulation. Basal body temperature was measured orally with a mercury thermometer and was recorded on a chart that was submitted monthly to the investigator. Basal body temperatures were monitored throughout the study. During the second month of the study, subjects consumed a prepared meal distributed by the UCLA General Clinical Research Center (GCRC) and followed a diet that provided 30% of calories from fat and 15–20 g fiber per day. During months 3 and 4, the subjects followed a very low fat, high fiber diet that provided 10% of calories from fat and 25 to 35 g fiber per day. The increased fiber intake was achieved by increasing the consumption of whole-grain cereals and legumes. During these 2 months, all of the major meals consumed by the subjects were prepared and distributed by the GCRC. The subjects were also instructed to carefully record any other food that they ate. The major meals were prepackaged frozen entrees. In addition, breads, fresh and frozen vegetables, fresh and canned fruits, and dairy foods were supplied according to the protocol requirements. The dietitian developed a 14-day menu cycle and reviewed it with each subject. An ad libitum controlled feeding study design was used, so that subjects were provided with more-than-adequate amounts food and were asked to return unused food, which was weighed to assess the amount of food eaten.

Table 1. Nutrient Intake Based on Four-Day Food Records

	30% AHA (month 1)	VLF/HFi (month 2)	VLF/HFi (month 3)
Energy (kcal)	1524 ± 282	1462 ± 159	1520 ± 201
Protein (g)	73.4 ± 17.4	66.6 ± 9.4	68.8 ± 14.4
Fat (g)	49.1 ± 16.4	17.2 ± 4.8*	20.2 ± 4.6*
Carbohydrate (g)	205.09 ± 27.2	272.5 ± 32.6*	280.0 ± 30.4*
Protein (%)	19.7 ± 2.85	18.38 ± 2.14	17.9 ± 1.6
Fat (%)	28.5 ± 4.8	10.5 ± 2.7*	11.9 ± 1.7*
Carbohydrate (%)	54.0 ± 3.9	74.5 ± 3.4*	74.0 ± 2.1*
Cholesterol (mg)	164.4 ± 48.6	59.5 ± 25.2*	73.8 ± 32.9*
P:S ratio	0.84	0.93	0.93
Fiber (g)	18.9 ± 5.5	28.9 ± 3.4*	27.9 ± 4.9*
Soluble fiber (g)	6.2 ± 2.0	9.3 ± 1.1*	8.7 ± 1.8*
Insoluble fiber (g)	12.6 ± 3.4	19.5 ± 2.5*	19.0 ± 3.2*

AHA: American Heart Association Diet; VLF/HFi: very low fat/high fiber diet. Values are mean ± SD.

* Significantly different from 30% fat diet phase, $P < 0.05$.

Four-day food records were kept each month by all subjects and were analyzed by means of the University of Minnesota Nutrient Data Base System, which was established in the UCLA Clinical Nutrition Research Unit. Body weights were determined with a calibrated physician's scale, and heights were measured with a stadiometer. Percentage of body fat was measured with a bioelectrical impedance meter (RJL Systems, Detroit, MI).

To determine the effects of the very low fat, high fiber diet on menstrual function and the secretion of reproductive hormones during the cycle, we collected blood samples every other day during the diet intervention period for measurement of estradiol, estrone, estrone sulfate, progesterone, luteinizing hormone (LH), and sex hormone binding globulin (SHBG). Blood samples were allowed to clot for 30 minutes at room temperature before they were processed, and the serum was stored at −70°C until the time of analysis.

Serum LH level was measured in duplicate with use of a commercially available kit (Diagnostic Products, Torrance, CA). Serum estradiol level was measured with an ^{125}I-estradiol tracer at a specific activity of 0.14 μCi/ml and a first antibody produced in rabbits with estradiol-6-carboxymethyl–bovine serum albumin (6-CMO-BSA) and a goat anti-rabbit second antibody separation. Serum estrone level was measured with ^{125}I-estrone, a first antibody developed against estrone-6-CMO-BSA in sheep and a donkey anti-sheep second antibody. For both assays, 500 μl of serum was extracted with hexane:ethyl acetate (3:2) for 5 minutes and separated by centrifugation at 1000 rpm for 5 minutes. The bottom aqueous phase was frozen with liquid nitrogen, and the upper phase was decanted and evaporated by heating the sample to 40°C and exposing it to a stream of nitrogen gas. Sample aliquots, standards, and serum pools, were incubated at 37°C for 30 minutes (60 minutes for estrone) before a second antibody was added. Estrone sulfate was measured after thermal solvolysis at 100°C for 18 hours after reconstitution of ethanol extracts of serum in sodium acetate buffer. After thermal treatment, samples were extracted again with 2.5 ml diethyl ether to remove unconjugated steroids. Total estrone levels were measured after reconstitution of extracts as described above, and the serum estrone concentration was subtracted to determine the estrone sulfate concentration. A statistical analysis was performed by the Statistical Coordinating Unit of the UCLA Clinical Nutrition Research Unit with the use of the nonparametric sign rank test and paired *t* test to assess the differences between the diet phases.

Results

The ad libitum feeding methods resulted in similar total energy intakes during the periods of study when dietary fat intake was varied, as determined by analysis of 4-day food records (see Table 1). We found a decrease in fat intake (49.1 ± 16.4 to 17.2 ± 4.8 [month 1] and 20.2 ± 4.6 [month 2]) and an increase in fiber intake (18.9 ± 5.5 to 28.9 ± 3.4 [month 1] and 27.9 ± 4.9 [month 2]) and carbohydrate intake (205.09 ± 27.2 to 272.5 ± 32.6 [month 1] and 280.0 ± 30.4 [month 2]).

Subjects were weighed weekly, and body composition determined in the GCRC. There was a small but statistically significant overall loss of body fat and body weight among the 12 women studied, as shown in Table 2.

We determined cycle length by examining the records of basal body temperature recorded daily by each subject. Serum LH levels measured on alternate-day samples were used to estimate the day of the LH peak and the day of ovulation (LH surge, Day 0). Follicular

Table 2. Effects of VLF/HFi Diet on Body Weight

	30% fat diet (month 1)	VLF/HFi (month 2)	VLF/HFi (month 3)
Weight (kg)	61.7 ± 8.6	60.7 ± 8.7*	59.7 ± 9.0*
Weight change (kg)		−0.99 ± 1.1†	−2.0 ± 1.4
Height (m)	1.66 ± .09	1.66 ± .09	1.66 ± .09
Body mass index (kg/m^2)	22.3 ± 1.7	21.9 ± 1.9*	21.5 ± 1.8*
% body fat	23.5 ± 4.9	4.0 ± 6.6	21.6 ± 5.5*

AHA: American Heart Association Diet; VLF/HFi; very low fat/high fiber diet. Values are mean ± SD.

* Significantly different from 30% fat diet phase, $P < 0.05$.

† Change in weight from month 1.

phase days were defined as those occurring before the surge, and luteal phase days were defined as those occurring after the ovulating LH surge. The effect of a very low fat, high fiber diet on menstrual function is shown in Table 3. Although average menstrual cycle duration at the end of the baseline and very low fat, high fiber diets was 28 days and 26 days, respectively, the dietary changes had no statistically significant effect on the duration of menstrual cycle, follicular phase, or luteal phase. The serum LH concentrations on the estimated day of ovulation are presented in Table 4. The very low fat, high fiber diet had no statistically significant effect on the LH concentrations on the estimated day of LH surge.

Serum hormone levels were determined during the early follicular phase (Days −10 and −12 from LH surge) and during the luteal phase (Days +8 and +10 from LH surge) of the menstrual cycle. Estrone, estradiol, estrone sulfate, progesterone, and SHBG concentrations were determined in samples pooled from the selected days for each subject. This was done to reduce variations due to pulsatility of hormone secretion within an individual subject. As shown in Table 5, at the end of the second month of the very low fat, high fiber diet, there was a significant reduction ($P < 0.05$) of 19% and 25% in serum estrone and estradiol concentrations, respectively, in the early follicular phase of the menstrual cycle. In the late luteal phase, serum estrone and estradiol concentrations decreased by 18% and 22%, respectively ($P < 0.05$). No significant changes were observed in serum estrone sulfate levels, luteal phase progesterone levels, or SHBG concentrations in either phase of the menstrual cycle after 2 months of the very low fat, high fiber diet.

Discussion

The current study revealed a significant effect of a very low fat, high fiber diet on serum estrogen levels without an adverse effect on ovulation. Although our study indicated a trend toward a shortening of the menstrual cycle duration when consuming a very low fat, high fiber diet, we recognize that the effects on menstrual cycle duration noted above were observed in only 12 women for only two menstrual cycles. Therefore, we could not detect potentially significant changes of 2–3 days in cycle length. Reichman et al.[26] demonstrated that a low fat diet (20% of total calories from fat) consumed for four menstrual cycles lengthened the follicular phase in 20 of 27 women compared with that of a higher fat diet (40% of calories from fat). In that study, four women had a shorter follicular phase and three had no change. However, the most important observation was that the changes resulting from the very low fat, high fiber diet were modest and did not interfere with ovulation in any subject in either the study by Reichman et al. or the present study.

We observed decreases ranging from 18% to 26% in the mean levels of estradiol and estrone in pooled samples collected during the follicular (Days −10 and −12 from LH surge) and luteal (Days +8 and +10 from LH surge) phases in women following the very low fat, high fiber diet compared with women following the 30%-fat diet. This change confirmed the findings of some earlier studies. Rose et al.[27] instructed 16 premenopausal females with benign breast disease to reduce fat consumption from 69 g to 32 g of fat per day and found a significant (30%) reduction in estradiol levels with no change in serum progesterone levels after 3 months of the diet. Rose et al.[28] also reported a decrease in luteal phase estradiol and estrone levels with a fiber intake that was increased from 15 to 30 g/ day. It has been shown that a vegetarian diet involving a significant reduction in fat consumption and a corresponding significant increase in fiber consumption can significantly increase SHBG concentration.[29] Although the results of the current investigation revealed no change in SHBG concentration in the follicular phase of the menstrual cycle, an increasing trend in SHBG concentration was observed in the luteal phase of the cycle. Additionally, a low protein-to-carbohydrate ratio of the diet has been reported to increase plasma levels of SHBG.[30] A decrease in protein-to-carbohydrate ratio (from 0.35 to

Table 3. Effects of VLF (10%)/HFi (35–45 g/day) Diet on Menstrual Cycle Length

	30% AHA (month 1)	VLF/HFi (month 2)	VLF/HFi (month 3)
Menstrual cycle length (days)	28.2 ± 3.4	28.2 ± 4.2	25.8 ± 5.2
Change from month 1		0 ± 3.46	−2.3 ± 6.7
Follicular phase (days)	15.8 ± 4.2	16.2 ± 3.7	13.4 ± 4.1
Change from month 1		0.3 ± 4.6	−2.4 ± 5.7
Luteal phase (days)	12.3 ± 3.0	12.0 ± 1.7	12.4 ± 3.0
Change from month 1		−0.3 ± 3.2	−0.1 ± 4.5

AHA: American Heart Association Diet; VLF/HFi; very low fat/high fiber diet. Values are mean ± SD.

Table 4. Effects of VLF (10%)/HFi (35–45 g/day) Diet on Serum Luteinizing Hormone on the Day of Ovulation

	30% AHA (month 1)	VLF/HFi (month 2)	VLF/HFi (month 3)
LH (IU/l)	65.8 ± 42.9	89.9 ± 46.8	87.1 ± 42.3

AHA: American Heart Association Diet; VLF/HFi: very low fat/high fiber diet. Values are mean ± SD.

Table 5. Effects of VLF (10%)/HFi (35–45 g/day) Diet on Serum Hormones

	30% AHA (month 1)	VLF/HFi (month 2)	VLF/HFi (month 3)
Estradiol (follicular)* (pmol/l)	269.4 ± 61.3†	220.9 ± 70.1	201.1 ± 109.7§
Estradiol (luteal)‡ (pmol/l)	708 ± 315.7	774.5 ± 308.3	554.3 ± 256.9§
Estrone (follicular) (pmol/l)	271.5 ± 99.8	259.2 ± 149.8	219.7 ± 87.6§
Estrone (luteal) (pmol/l)	320.7 ± 129.4	268.5 ± 99.8	264.1 ± 121.6§
Estrone sulfate (follicular) (pmol/l)	831.5 ± 422.7	907.3 ± 460.8	920.6 ± 307.0
Estrone sulfate (luteal) (pmol/l)	1935.3 ± 752.7	2175.3 ± 849.6	2058 ± 949.5
Progesterone (nmol/l)	56.9 ± 21.6	49.2 ± 20.0	52.4 ± 16.8
SHBG (follicular) (nmol/l)	44.8 ± 21.3	639.7 ± 18.9	47.6 ± 29
SHBG (luteal) (nmol/l)	62.2 ± 36.3	73.8 ± 51	69 ± 49.2

AHA: American Heart Association Diet; VLF/HFi: very low fat/high fiber diet.

* Follicular: pooled sample of day −10, −12 from LH surge.

† Mean ± SD of pooled samples of the same subject in either the follicular or luteal phase.

‡ Luteal: pooled sample of day +8, +10 from LH surge.

§ Significantly different from 30% AHA phase, $P < 0.05$.

0.25) induced by a very low fat, high fiber diet could have been responsible for the increase in the SHBG concentrations observed in the current investigation.

There have been very few studies on the effects of a low fat, high fiber diet on serum estrone sulfate concentrations. A recent study conducted by Goldin et al.[25] indicated a significant decrease in serum estrone, estrone sulfate, and SHBG levels ($P < 0.05$) and a decrease in estradiol levels ($P < 0.07$) in premenopausal women consuming a low fat (20–25% of calories from fat), high fiber (40 g/day) diet. An examination of the independent effects revealed that high fiber caused a decrease in estradiol and SHBG levels, whereas both reduced fat and high fiber caused the decrease in estrone sulfate levels. However, we observed no significant changes in the follicular- and luteal-phase estrone sulfate levels.

Epidemiologic studies have revealed a decreased incidence of premenopausal breast cancer in obese women,[31–33] leading some investigators to suggest that obesity and high fat diets are protective for premenopausal women. However, anovulatory cycles, caused by an inadequate luteal phase, are common among markedly obese women. In such women, decreased progesterone levels during the luteal phase may counteract the promotional effects of increased estrogen levels produced by fat tissue through aromatization of adrenal androgens.[34] There are also changes in catechol estrogen metabolism among obese women, which may reduce breast cancer risk.[35] Although obesity and high fat diets are not recognized as risk factors for premenopausal women, we believe that the same risk factors pertain during the period when breast cancer develops before menopause. Because it has been estimated that breast cancer develops over a 10–15 year period, it seems prudent that for the longevity of women, prevention efforts should start in the premenopausal years.

Whether higher estradiol levels in the premenopausal period are associated with increased breast cancer risk is of fundamental importance to the understanding of breast cancer etiology. The current study shows that eating a very low fat, high fiber diet can lower circulating levels of estrone and estradiol. Biopsy studies have shown that although some cell division occurs in the follicular phase of the menstrual cycle, the mitotic rate of the epithelial cells is approximately fourfold higher during the luteal phase, when the amount of estradiol and progesterone are significantly greater.[36,37] Because estrogen is known to induce the progesterone receptor, reductions in estrogen levels may reduce breast cell proliferation rates and breast cancer risk by decreasing the induction of progesterone receptors. These questions can only be addressed in future studies at a cellular and molecular level. Nonetheless, the current studies demonstrated the significant effects of diet on reproductive hormone levels without interference with ovulation for premenopausal women.

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