Published in final edited form as:
J Steroid Biochem Mol Biol. 2008 November ; 112(1-3): 32–39. doi:10.1016/j.jsbmb.2008.08.002.

Fat/Fiber intakes and sex hormones in healthy premenopausal women in USA

Mylène Aubertin-Leheudre[1], **Sherwood Gorbach**[2], **Margo Woods**[2], **Johanna T Dwyer**[3], **Barry Goldin**[2], and **Herman Adlercreutz**[1,*]

[1]*Folkhälsan Research Center, Institute of Preventive Medicine, Nutrition and Cancer, Division of Clinical Chemistry, University of Helsinki, Haartmaninkatu 8 (P.O.Box 63), 00014 University of Helsinki, Finland.* [2]*Department of Public Health and Family Medicine, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA.* [3]*Jean Mayer USDA Human Nutrition Research Center on Aging and Friedman School of Nutrition Science and Policy, Tufts University, and Frances Stern Nutrition Center, Tufts-New England Medical Center, 750 washington street, 02111 Boston, MA, USA*

Abstract

The mechanisms by which diet affects breast cancer (BC) risk are poorly understood but a positive relationship between fat and a negative association with fiber intake and BC risk have been demonstrated. Here we study the association between dietary fat/fiber ratio and estrogen metabolism. Fifty women were recruited, 22 were included in the low fat/high fiber and 22 were in the high fat/low fiber and 6 did not meet our criteria. Estrogens (determined in plasma, urine and feces) and dietary records were collected during 3 following days. All data were collected in winter and in summer. The high fat/low fiber group had significantly higher urinary total estrogens, estriol-3-glucuronide, 2-hydroxyestradiol, 16α-hydroxyestrone, and a higher 2-hydroxyestrone/4-hydroxyestrone ratio. Total fat intake correlated significantly with plasma estrone, estradiol, urinary 2-hydroxyestrone, 2-hydroxyestradiol, 2-hydroxyestrone/4-hydroxyestrone ratio, and total urinary estrogens, even after adjustment for total fiber intake. The high fat/low fiber diet was associated with high values both for catechol and 16α-hydroxylated estrogens and a high 2-hydroxyestrone/4-hydroxyestrone ratio, but 2-hydroxyestrone/16α-hydroxyestrone ratio was not different between the groups. Our results suggest that fat affects estrogen metabolism more than does fiber and that one mechanism resulting in high estrogen values is an increased reabsorption of biliary estrogens.

Keywords

Diet; Estrogens; Breast cancer risk; Women

1. Introduction

Breast cancer (BC) incidence is significantly lower in Asian than in Western women [1]. One explanation is that traditional Asian diets may be protective because they are often rich in soy

*__Corresponding author:__ Herman Adlercreutz, MD; PhD., Folkhälsan Research Center, Biomedicum Helsinki, Haartmaninkatu 8 (P.O.Box 63), FIN-00014 University of Helsinki, Tel: +358-9-191 25380, Fax:+358-9-191 25452, Email: herman.adlercreutz@helsinki.fi.

products and lower in fat [2]. The mechanism by which diet influences BC risk is unclear although it has been shown that changes in dietary habits may increase or decrease BC risk [3]. A meta-analyses and a pooled study [4,5] demonstrated positive relationship between fat and BC risk, but negative results have also been reported [6,7].

More recently, the Women's Health Initiative Dietary Modification Trial (WHI-DMT, [8]) found a statistically non significant 9% reduction of BC incidence in the low-fat intervention group compared to a control group. That study also reported significant differences between the intervention and control groups for body weight, total energy, and vegetable fiber intake; factors which are also thought to influence BC risk. Alcohol intake was similar and unchanged in both groups.

It is believed that fiber consumption protects against BC by binding enteral carcinogens, and estrogens during their enterohepatic circulation thus facilitating their fecal elimination [9,10]. Reduction of intestinal β-glucuronidase activity, necessary for hydrolysis of the estrogen conjugates before absorption, is another mechanism diminishing estrogen levels [11]. In premenopausal Finnish women total fiber or grain fiber intake/kg body weight showed a statistically significant negative association with the excretion of 10 of 13 estrogens measured in urine [12]. An inverse relationship between high fiber intake and BC risks was noted in a Swedish prospective study [13]. However, in most other prospective studies the associations between dietary fiber and BC were null [14]. In a meta-analysis, Wu et al. demonstrated that 4/14 interventional studies showed that a high level of dietary fiber combined with a low level of fat had an effect on BC risk [15].

Evidence from both epidemiologic and biomolecular studies indicate that estrogens are implicated in human BC [16–18]. Some cohort studies have revealed a strong relationship between endogenous estrogen levels and BC risk [19,20]. In one study [21] but not in another [22], women who were switched from a high-fat (40% of calories) to a low fat (25%) showed a significant decrease of the level of 16α-OHE1 which is recognized to be a potent estrogen receptor agonist [23]. It has been demonstrated that the level of 16α-OHE1 is elevated in women with BC [24]. In addition it was suggested that 2-OHE1 is only weakly estrogenic and possibly antiestrogenic [24] and it has been assumed that a high level could reduce BC risk. However, it has also been shown that in postmenopausal women a high catechol estrogen concentration is associated with increased BC risk [25]. Catechol estrogens are thought to be carcinogenic because of their capability to form DNA adducts [17]. Furthermore, it has been suggested that the ratio 2-OHE1/16α-OHE1 may serve as a marker of BC risk [26]. Early evidence indicated that postmenopausal vegetarians, known to consume twice the fiber than omnivores, had significantly lower blood estrogen and androgen levels [27–29]. Cross-sectional studies among postmenopausal women with low fiber intakes reported no association between fiber and endogenous estrogen levels [30–32]. Recently, Monroe et al. demonstrated that fiber intake was significantly negatively associated with a decrease of serum E1 and E2 in postmenopausal Mexican-American women [33].

The aim of this study was to examine the association between, on the one hand, a high fat/low fiber Western-type diet, and on the other hand, an ideal low fat/high fiber diet with the sex hormone pattern in plasma and urine, including some catechol estrogens, in premenopausal healthy women living in USA. To our knowledge no such study on subjects consuming their habitual diets has been carried out. To obtain information about the enterohepatic circulation of estrogens we also included assays of estriol-3-glucuronide in urine, which is a marker of conjugation in the mucosal cells of the gastrointestinal tract.

2. Materials and methods

2.1. Subjects

The participants were premenopausal healthy volunteer women and were recruited in the Boston area (USA). Women who had maintained their habitual diet, including omnivores, lacto-ovo-vegetarians and vegans, for at least 2 years were recruited by advertisements in local newspapers, bulletins and magazines. Subjects were excluded if they had: a BMI over 120 percent or under 90 percent of ideal body weight, history of heart disease, history of cancer, arthritis, hypertension, diabetes mellitus, thyroid problems, renal or liver disease, irregular menstrual cycle, regular consumption of alcohol (≥ 15 g/d) or using prescribed medications (oral contraceptives, corticosteroids or antibiotics).

For the purpose of this study, subjects were divided by the percent of fat of energy intake and the amount of fiber consumed per day in two groups. Based on their dietary intakes, the two groups included: a low fat/high fiber group consisting of women who consumed less than 21% of energy/day as fat and more than 21g/day of fiber, and a high fat/low fiber group made up of women with >21% calories from fat and less than 21 g fiber per day. Among the 50 premenopausal subjects studied, 22 women were classified in low fat/high fiber group and 22 women in high fat/low fiber group (6 women could not be included in our analysis because their food intakes did not match the inclusion criteria used).

2.2. Collection of samples

Subjects were studied for 3 consecutive days on 2 occasions (one visit in the summer and one in the winter season). An average of the winter and summer values was used in the statistical analyses to counteract the seasonal effect. In fact, it is recognized that diet during the seasons are different [34]. Each of these visits included a 3 times 24-h collection of urine and feces (totally 6 days of collection), 3 different blood samples in heparinized tubes during the morning after urine collection and 3-day food records. The 3-day blood samples were pooled to reduce the number of analyses. All samples were collected during the mid-follicular phase of the menstrual cycle [days (3)5–7(9)]. To prevent oxidation of the hormones and stop bacterial growth, we added as preservative 0.1 % of ascorbic acid and 0.1 % of sodium azide in heparinized tubes. One g of ascorbic acid powder per liter of volume was added to the plastic bottle before urine collection. Feces were collected in plastic containers and after the first defecation 10 ml of 1 % ascorbic acid was poured on the sample. The urine and feces samples were stored in a cold place (in the winter outside) and brought to the hospital every day and then 0.1 % sodium azide added. The samples for 3 days were pooled and feces homogenized. Suitable volumes of the urine and feces samples for analysis were transferred to new tubes. The storage was at −20 °C. The samples were sent to Helsinki frozen in dry ice.

2.3. Hormones

The plasma samples were collected from fasting subjects. All analyses in duplicate were carried out within a year of sample collection. If the results of the duplicates deviated more than 10 % from the mean value the analysis was repeated. Quality control samples based on pooled samples that had been analyzed ten times, were included in each batch.

Plasma estrogens, estrone (E1) and estradiol (E2), were determined by radioimmunoassay (RIA) after chromatographic separation on an LH-20 column as described in detail [29,35]. After addition of tritiated internal standards, the plasma was extracted with 5 volumes of 20 per cent ethyl acetate in petroleum ether (vol/vol) in one step by freezing. After chromatography on Sephadex LH-20 with methanol-toluene 9:1 (vol/vol), the separated estrogens were determined by radioimmunoassay, with free and bound ligand separated by dextran-coated charcoal [35]. The coefficient of variation (CV%), which was calculated from the differences

between duplicate analyses of a control serum pool, was 5.1 % for E1 and 3.9 % for E2. The corresponding inter-assay CV% calculated from the mean values of these duplicates during a 3-year period (n = 15) was 2.0 % for E1 and 1.4 % for E2 [29].

Plasma testosterone (T) and androstenedione (A) were determined by radioimmunoassay as described in detail by Kuoppasalmi et al. [36]. Free testosterone (Free T) was measured according to Bergink et al. [37] and sex hormone binding globulin (SHBG) as described by Rosner [38] with slight modifications [39]. Dehydroepiandrosterone-sulfate (DHEAS) was measured with a commercial kit (Wien Laboratories,inc. Succansunna, NJ,USA). The intra-assay CV% were for T 8.3 %, for A 5.7 %, for DHEAS 6.2 % for SHBG 8.8 %, and for free T 7.1 %. The inter-assay CV% were for T 12.7 %, for A 9.2 %, for DHEAS 7.5 %, for SHBG 11.1 %, and for free T 8.5 %.

Urinary estrogen metabolites were measured by GC/MS in the selected ion monitoring mode as described by Fotsis et al. [40]. All details including validation of the procedure were published [40]. Urinary “immunoreactive” estriol-3-glucuronide (E3-3G), a biomarker of enterohepatic circulation of estrogens, was determined by radioimmunoassay as described [41] using reagents kindly provided by Drs A. Kellie and P. Samarajeewa. This estriol conjugate is formed exclusively in the mucosal cells of the intestine and thereafter excreted without further metabolism in the urine and may, therefore, serve as a biomarker of the reabsorption of biliary estrogens in the intestine.

Fecal analyses of unconjugated E1, E2 and E3 were carried out by RIA using a method previously described in detail [42]. The inter-assay CV% for E1 is 5.8 %, for E2 5.9 % and for E3 1.7 %.

All the methods have been validated in regard to the accuracy, sensitivity and reproducibility. The E3-3G method probably also measures small amounts of other 16-hydroxylated estrogen-3-glucuronides, but this does not invalidate its use as a biomarker of the extent of enterohepatic circulation of estrogens, because the intestinal mucosa is the only organ where 3-glucuronidation of polar estrogens occur. Plasma or serum for SHBG analyses cannot be stored for long periods of time because this leads to the combination of two SHBG molecules (dimerization) and these dimers cannot be detected in the immunoassay resulting in low values. This problem was avoided in the present study. Our own studies and discussion on the methodological issues have been published [43].

2.4. Dietary intake

Subjects were instructed to maintain their usual diets throughout the three days of the dietary recordings in each season [44]. Each subject was provided with a postal scale balance and was instructed on how to complete the dietary records. It has been demonstrated that a 3-day dietary record is valid to estimate dietary intakes in adults without cognitive impairments [44]. Dietary analyses were completed by a nutritionist using the Nutrient Data Base at the University of Massachusetts at Amherst. Dietary fiber intake was calculated individually by nutritionist using the values of Paul and Southgate [45].

2.5. Statistical analysis

Results are presented as means ± SD. Normality of distribution was determined using the Kurtosis test. Data were log-transformed if abnormally distributed. Homogeneity of variances was tested using the Levene test. We used an independent sample t-test to compare groups for all variables and a General Linear Model (GLM) multivariate analysis with total Kcal intake as the covariable. Finally, we used Pearson and partial correlation tests to examine the relation

between dietary components and sex hormone levels. $P \leq 0.05$ was considered significant (SPSS 15.00).

3. Results

We observed no significant differences in the anthropometric data between the 2 groups (Table 1). For dietary intake, we found significant differences between the groups for all the dietary variables except for protein intake and PUFA (Table 2). The high fat/low fiber group consumed more fat, SFA and MUFA and had higher energy intake. The fat/fiber ratio was, related to the grouping of the subjects, significantly higher in the high fat/low fiber group compared to the low fat/high fiber group.

Plasma E1 (p = 0.069), E1+E2 (p = 0.067) and SHBG (p = 0.051) were lower in the low fat/high fiber group compared to the high fat/low fiber group (Table 3).

We found significantly higher values in the high fat/low fiber group for the urinary E3 (p = 0.025), total estrogens (p = 0.013), E3-3G (p = 0.002), 2-OHE2 (p = 0.004), 16α-hydroxylated-estrogens (16α-OHE1+E3; p = 0.032), 2-OHE1/4-OHE1 ratio (p = 0.045), 16β-hydroxyestrone (p = 0.023), 16-*epi*estriol (p = 0.033), and a tendency for the urinary 16α-OHE1 (p = 0.055), 2-OH-estrogens (p = 0.078) and 16-keto-estradiol (p = 0.067) (Table 4).

We carried out a GLM multivariate analysis for all the plasma sex hormones and urinary estrogen metabolites using total energy intake as a covariable (Table 5). We observed significantly higher values in the high fat/low fiber group for the plasma E1+E2 (p = 0.046), plasma SHBG (p = 0.045), urinary E3-3G (p = 0.024), 16α-OHE1 (p = 0.024) and 16β-hydroxyestrone (p = 0.024). We found a trend toward higher values in the high fat/low fiber group for the plasma E1 (p = 0.052), plasma E2 (p = 0.078), urinary E2 (p = 0.068), 2-OHE2 (p = 0.054), 16-keto-estradiol (p = 0.061) and 16α-OH-estrogens (p = 0.072).

Total energy intake correlated significantly and positively with the plasma E2, E1+E2, and urinary 2-OHE2, 2-OHE1/4-OHE1 ratio, 2-OHE and total urinary estrogens (Table 6).

Total fat intake correlated significantly and positively with the plasma E1, E2, E1+E2, and the urinary E2, E3-3G, 2-OHE1, 2-OHE2, 2-OHE1/4-OHE1 ratio, 2-OHE, 16α-OH-estrogens, 16β-hydroxyestrone, 16-*epi*estriol, 17-*epi*estriol and total urinary estrogens (Table 6). Total carbohydrate intake correlated significantly and negatively with the urinary E3-3G, 16α-OHE1 and 16β-OHE1 and positively with fecal E1 and E2 (Table 6). Total SFA and MUFA correlated significantly and positively with the plasma E1, E2, E1+E2, and the urinary E2, E3, E3-3G, 2-OHE1, 4-OHE1, 2-OHE2, 2-OHE1/4-OHE1 ratio, 4-OHE1/E1 ratio, 2-OHE, 16α-OH-estrogens, 16β-OHE1, 16-keto-estradiol, 16-*epi*estriol and total urinary estrogens. Fat/fiber ratio correlated significantly and positively with the plasma E1, E1+E2 and urinary E2, E3, E3-3G and 2-OHE2. Total fiber intake correlated significantly but negatively with the urinary E3, total estrogens, E3-3G, 16α-OHE1, 16α-OH-estrogens and 16β-OHE1 (Table 6).

Urinary E3 (−0,360, p = 0.018) and E3-3G (−0.370, p = 0.015) still correlated negatively and significantly with the total fiber intake even after adjustment for the total fat intake. In addition, we found that the total fat correlated significantly with the plasma E1 (0.447, p = 0.003), E2 (0.358, p = 0.018), E1+E2 (0.432, p = 0.004), the urinary 2-OHE1 (0.499, p = 0.007), 2-OHE2 (0.646, p = 0.001), 2-OHE1/4-OHE1 ratio (0.540, p = 0.003), 2-OHE (0.552, p = 0.002) and all the urinary estrogens (0.563, p = 0.002) even after adjustment for total fiber intake. These results suggest that fat affects estrogen metabolism more than does fiber.

4. Discussion

The aims of this study were to investigate the hormonal pattern in healthy premenopausal women on a high fat/low fiber Western-type diet, which is associated with high BC risk, and to compare the results with women on a low fat/high fiber diet.

Our main results showed that women who consumed more fat and less fiber had significantly higher level of urinary E3-3G which is known as a reliable marker for the enterohepatic circulation [46,47], and higher catechol estrogens (particularly 2-OHE2), 2-OHE1/4-OHE1 ratio and 16α-hydroxylated-estrogens, than women who ingested less fat and more fiber. Our results confirm and extend the results obtained previously by Goldin et al. [29,48]. In another study vegetarians had lower plasma E2, and fiber intake correlated negatively with plasma E2 [28]. Furthermore, another study [49] and an intervention study [50] indicated that fiber reduces plasma estrogens. Fat seems to have the opposite effect [15].

The inhibitory effect of dietary fiber on breast cancer could differ according to the type of fiber [51]. Approximately 50% of the conjugated estrogens in the liver are excreted into the bile and reabsorbed in the intestine [47]. It was postulated that insoluble fiber from cereal might increase the fecal excretion of estrogens by several mechanisms (see below). Fruit and vegetable fibers that are mainly soluble may not affect the enterohepatic circulation of the estrogens to the same extent as insoluble fiber. It is important to note that the major source of dietary fiber differs between countries [52]. For instance, cereal fiber accounts for approximately 70% of total fiber in Sweden while vegetables are a major source of dietary fiber in USA [53]. These differences could explain the controversial results in the literature.

An important observation is the significant difference between the groups for E3-3G that is higher in the high fat/low fiber group. This finding in combination with the tendency to low fecal estrogens compared to the low fat/high fiber group indicates that the reabsorption of biliary estrogens is more extensive in the high fat/low fiber group compared to the low fat/high fiber group. Estriol-3-glucuronide is exclusively formed in the intestinal mucosal cells and excreted unchanged in urine without further metabolism [54]. The increased enterohepatic circulation of estrogens shown by the results for E3-3G explains the higher total plasma (E1 +E2) and urinary estrogen levels and lower fecal estrogen levels in this group. The 16α-hydroxylation takes place mainly in liver as demonstrated by the very low estriol formation in the cases of Gilbert's syndrome with poor liver uptake of bilirubin and probably also of estrogens [46]. The principal metabolites in bile are 16-oxygenated [55–57] being transported from the small intestine to the colon where they are hydrolyzed, reabsorbed and reconjugated in the mucosal cells [55,58]. The fecal E1, E2 and E3 values are rather similar suggesting that the 16α-hydroxylated estrogens are more readily reabsorbed. In the present study a significant ($p \leq 0.01$) negative correlation was found between plasma E1 and fecal E3, and plasma E1 + E2 and fecal E3 ($p \leq 0.05$), and fecal total estrogens and plasma E1 ($p \leq 0.05$) (data not shown). Several possible mechanisms have been suggested. All the biliary estrogens are conjugated [59] and have to be hydrolyzed before absorption. An increase of β-glucuronidase activity in feces associated with a high fat and low fiber diet [29] leads to increased reabsorption. The other mechanism proposed to explain this result, is that the estrogens are eliminated by the fecal route because fiber binds estrogens [9,33,51] and fiber speeds up intestinal transit [60]. Each of these mechanisms could account for the results obtained.

In a large number of studies a high androgen level has been found to be a risk factor for BC [20,61–65]. We found no differences between the groups in regard to plasma androgens (Table 3). In a previous study using identical hormone analyses in American postmenopausal women [27], we found significantly higher androstenedione and testosterone values in subjects with BC compared to vegetarians, but the omnivores had values in between. Our present results are

in accordance with some studies that showed no difference between dietary factors and serum androgens [30,66,67]. Androgens do not participate to any great extent in the enterohepatic circulation which could explain these results. The only significant result that we obtained was a negative correlation between the protein intake and plasma androstenedione (Table 6). The altered androgen levels in women with high BC risk may, therefore, not be due to dietary factors but to changes in endogenous hormone production caused by gene polymorphisms changing androgen production in the adrenals or by ovarian disease such as polycystic ovaries.

Catechol estrogens and 2-OHE1/4-OHE1 ratio were higher in the high fat/low fiber group. This ratio is in accordance with a study in Finnish women [43] who showed a high ratio in omnivores and BC women compared to vegetarians. The results are also in accordance with another previous study showing much higher catechol estrogen values and higher 2-OHE1/4-OHE1 ratio in Finnish premenopausal women consuming western diet compared to recent oriental immigrants to Hawaii [68] who had a very low fat diet. Because we observed the high levels of 2-OHE2 and 16α-OHE1 and no difference of the 2-OHE1/16α-OHE1 ratio in the high fat/low fiber group compared to the low fat/high fiber group, our results are not in concordance with those of Mutti et al. [69] who suggested that in women a high level of 2-OHE1 and a high 2-OHE1/16α-OHE1 ratio prevents BC, because a high fat/low fiber diet is associated with an increased BC risk.

The lower 16α-OHE1 in the low fat/high fiber group is in agreement with a study showing that a low fat diet in women decreases urinary 16α-OHE1 [21]. However, our results are not in agreement with the observation of an increase in catechol estrogens when women are on a low-fat diet [21].

16α-OHE1 binds covalently to primary amino groups in proteins and specifically to the estrogen receptors, and its effect is, therefore, prolonged [23]. The 2-hydroxylated estrogens have been shown to have a low estrogenic activity and this forms the basis for the hypothesis that a high 2-OHE1/16α-OHE1 ratio means diminished BC risk. However, the activation of the estrogen receptor by 4- and 2-hydroxyestradiol is qualitatively and quantitatively comparable to that of estradiol [70]. In the present study both the 2-hydroxylated, particularly 2-OHE2, and 16α-OHE1 were higher but the 4-OHE1 not in the high fat/low fiber group compared to the low fat/high fiber group. At the time of this study we were not able to measure 4-OHE2 and 2-MeOE2. Low values for the 2-hydroxylated estrogens in the low fat/high fiber group were not reflected in the values for 4-OHE1. This is the reason for the high 2-OHE1/4-OHE1 ratio in the high fat/low fiber group compared to the other group.

The catechol estrogens are easily auto-oxidized to semiquinones and further to quinones and these metabolites are both electrophiles capable of covalently binding to nucleophilic groups on DNA [16]. The quinones are initiators of breast and other cancers and it seems that catechol O-methyltranferase (COMT) inhibits this process by methylating the catechol estrogens [71, 72]. Both 16α-OHE1 and the catechol estrogens are carcinogenic.

The protection against formation of DNA adducts by COMT is interesting because 2-hydroxylation and methylation of the 2-hydroxy group seem to be a prerequisite for the stimulation of production of SHBG by estradiol [73] at least as judged from *in vitro* studies. A higher SHBG is also protective with regard to BC, because this reduces the concentration of free estradiol and particularly that of free testosterone. In the present study SHBG was significantly lower in the low fat/high fiber group compared to the high fat/low fiber group which is difficult to explain. In one study we found that plasma free fatty acids are positively associated with SHBG [74]. We did not measure free fatty acids, but it is likely that they are high in the high fat/low fiber group, which could explain the high SHBG. However, the

mechanism by which free fatty acids could influence SHBG values is not known, but it could also be a methodological problem.

Because 2-OHE1/16α-OHE1 ratio is not significantly different between the groups, this result does not fit with the hypothesis that a higher ratio is associated with lower BC risk [69]. The finding that urinary E1, E2 & E3, the catechol estrogens, 16α-OHE1 and plasma estrogen levels are higher in the high fat/low fiber group leads to the conclusion that a high fat/low fiber diet causes a general increase of all estrogens (Table 5). Because both the 16α-hydroxylated and the catechol estrogens are carcinogenic both of the groups of estrogens probably play a role in increasing BC risk. Because of the demonstrated carcinogenicity of the 4-hydroxylated estrogens [75,76], it is important to note that there was no difference for the values of 4-OHE1 between the groups and that Finnish women have 100% higher excretion of 4-OHE1 in urine compared to oriental women [68] with a low BC risk. Because 2-OHE1 is high during a high fat/low fiber diet and 4-OHE1 does not change and is low in a population with a low BC risk the 2-OHE1/4OHE1 ratio may be a good biomarker of BC risk.

We observed a significant difference in carbohydrate intake between the groups. A high carbohydrate intake is seen as necessary to maintain caloric intake in a low fat diet. A high carbohydrate diet seems to reduce sex hormone levels and to reduce BC risk [77,78]. However, one study did not find an association between carbohydrate intake and overall BC risk in pre- and postmenopausal women [79].

In conclusion, the high fat/low fiber diet reflects the Western diet compared to low fat/high fiber diet that is closer to the Asian diet. The results show that the Western diet is associated with high hormonal values and more specifically with the estrogens that might increase BC risk. Both catechol estrogen and 16α-hydroxylated estrogen levels are significantly higher but no difference for the 2-OHE1/16α-OHE1 ratio between the groups could be seen. The significantly higher 2-OHE1/4-OHE1 ratio in the high fat/low fiber group compared to the low fat/high fiber group that was seen previously in Finnish premenopausal women with BC [43], suggest that in premenopausal women this ratio is a better predictor of BC risk compared to the 2-OHE1/16α-OHE1 ratio. Our results also suggest that dietary fat has a greater influence on estrogen metabolism than does dietary fiber.

Acknowledgements

This work was supported by the NIH contract CB74104 and NIH grant CA-45128, the Sigrid Jusélius Foundation and the Folkhälsan Research Center, Helsinki, Finland. Mylène Aubertin-Leheudre is supported by the Canadian Institutes of Health Research. We wish to thanks Mrs Anja Koskela, Mrs Inga Wiik and Mrs Adile Samaletdin for their excellent technical assistances and the particpantes.

References

1. Parkin DM. Cancers of the breast, endometrium and ovary: geographic correlations. Eur. J. Cancer Clin. Oncol 1989;25:1917–1925. [PubMed: 2698807]
2. Barnes S. Evolution of the health benefits of soy isoflavones. Proc. Soc. Exp. Biol. Med 1998;217:386–392. [PubMed: 9492352]
3. Willett WC. Diet, nutrition, and avoidable cancer. Environ. Health Perspect 1995;103:165–170. [PubMed: 8741778]
4. Boyd NF, Martin LJ, Noffel M, Lockwood GA, Trichler DL. A meta-analysis of studies of dietary fat and breast cancer risk. Br. J. Cancer 1993;68:627–636. [PubMed: 8353053]
5. Armstrong B, Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int. J. Cancer 1975;15:617–631. [PubMed: 1140864]

6. Hunter DJ, Spiegelman D, Adami HO, Beeson L, van den Brandt PA, Folsom AR, Fraser GE, Goldbohm RA, Graham S, Howe GR, et al. Cohort studies of fat intake and the risk of breast cancer--a pooled analysis. N. Engl. J. Med 1996;334:356–361. [PubMed: 8538706]
7. Smith-Warner SA, Spiegelman D, Adami HO, Beeson WL, van den Brandt PA, Folsom AR, Fraser GE, Freudenheim JL, Goldbohm RA, Graham S, Kushi LH, Miller AB, Rohan TE, Speizer FE, Toniolo P, Willett WC, Wolk A, Zeleniuch-Jacquotte A, Hunter DJ. Types of dietary fat and breast cancer: a pooled analysis of cohort studies. Int. J. Cancer 2001;92:767–774. [PubMed: 11340585]
8. Prentice RL, Caan B, Chlebowski RT, Patterson R, Kuller LH, Ockene JK, Margolis KL, Limacher MC, Manson JE, Parker LM, Paskett E, Phillips L, Robbins J, Rossouw JE, Sarto GE, Shikany JM, Stefanick ML, Thomson CA, Van Horn L, Vitolins MZ, Wactawski-Wende J, Wallace RB, Wassertheil-Smoller S, Whitlock E, Yano K, Adams-Campbell L, Anderson GL, Assaf AR, Beresford SA, Black HR, Brunner RL, Brzyski RG, Ford L, Gass M, Hays J, Heber D, Heiss G, Hendrix SL, Hsia J, Hubbell FA, Jackson RD, Johnson KC, Kotchen JM, LaCroix AZ, Lane DS, Langer RD, Lasser NL, Henderson MM. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. J.A.M.A 2006;295:629–642.
9. Arts CJ, Govers CA, van den Berg H, Wolters MG, van Leeuwen P, Thijssen JH. In vitro binding of estrogens by dietary fibre and the in vivo apparent digestibility tested in pigs. J. Steroid Biochem. Mol. Biol 1991;38:621–628. [PubMed: 1645589]
10. Adlercreutz H. Western diet and Western diseases: some hormonal and biochemical mechanisms and associations. Scand. J. Clin. Lab. Invest. Suppl 1990;201:3–23. [PubMed: 2173856]
11. Goldin BR, Swenson L, Dwyer J, Sexton M, Gorbach SL. Effect of diet and Lactobacillus acidophilus supplements on human fecal bacterial enzymes. J. Natl. Cancer Inst 1980;64:255–261. [PubMed: 6766508]
12. Adlercreutz H, Fotsis T, Bannwart C, Hamalainen E, Bloigu S, Ollus A. Urinary estrogen profile determination in young Finnish vegetarian and omnivorous women. J. Steroid Biochem 1986;24:289–296. [PubMed: 3009980]
13. Mattisson I, Wirfalt E, Johansson U, Gullberg B, Olsson H, Berglund G. Intakes of plant foods, fibre and fat and risk of breast cancer--a prospective study in the Malmo Diet and Cancer cohort. Br. J. Cancer 2004;90:122–127. [PubMed: 14710218]
14. Holmes MD, Willett WC. Does diet affect breast cancer risk? Breast Cancer Res 2004;6:170–178. [PubMed: 15217490]
15. Wu AH, Pike MC, Stram DO. Meta-analysis: dietary fat intake, serum estrogen levels, and the risk of breast cancer. J. Natl. Cancer Inst 1999;91:529–534. [PubMed: 10088623]
16. Russo J, Russo IH. Genotoxicity of steroidal estrogens. Trends Endocrinol. Metab 2004;15:211–214. [PubMed: 15223050]
17. Yue W, Santen RJ, Wang JP, Li Y, Verderame MF, Bocchinfuso WP, Korach KS, Devanesan P, Todorovic R, Rogan EG, Cavalieri EL. Genotoxic metabolites of estradiol in breast: potential mechanism of estradiol induced carcinogenesis. J. Steroid Biochem. Mol. Biol 2003;86:477–486. [PubMed: 14623547]
18. Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. N. Engl. J. Med 2006;354:270–282. [PubMed: 16421368]
19. Toniolo PG, Levitz M, Zeleniuch-Jacquotte A, Banerjee S, Koenig KL, Shore RE, Strax P, Pasternack BS. A prospective study of endogenous estrogens and breast cancer in postmenopausal women. J. Natl. Cancer Inst 1995;87:190–197. [PubMed: 7707406]
20. Berrino F, Muti P, Micheli A, Bolelli G, Krogh V, Sciajno R, Pisani P, Panico S, Secreto G. Serum sex hormone levels after menopause and subsequent breast cancer. J. Natl. Cancer Inst 1996;88:291–296. [PubMed: 8614008]
21. Longcope C, Gorbach S, Goldin B, Woods M, Dwyer J, Morrill A, Warram J. The effect of a low fat diet on estrogen metabolism. J. Clin. Endocrinol. Metab 1987;64:1246–1250. [PubMed: 3571427]
22. Pasagian-Macaulay A, Meilahn EN, Bradlow HL, Sepkovic DW, Buhari AM, Simkin-Silverman L, Wing RR, Kuller LH. Urinary markers of estrogen metabolism 2- and 16 alpha-hydroxylation in premenopausal women. Steroids 1996;61:461–467. [PubMed: 8870165]

23. Swaneck GE, Fishman J. Covalent binding of the endogenous estrogen 16 alpha-hydroxyestrone to estradiol receptor in human breast cancer cells: characterization and intranuclear localization. Proc. Natl. Acad. Sci. U S A 1988;85:7831–7835. [PubMed: 3186693]
24. Fishman J, Schneider J, Hershcope RJ, Bradlow HL. Increased estrogen-16 alpha-hydroxylase activity in women with breast and endometrial cancer. J. Steroid Biochem 1984;20:1077–1081. [PubMed: 6727352]
25. Wellejus A, Olsen A, Tjonneland A, Thomsen BL, Overvad K, Loft S. Urinary hydroxyestrogens and breast cancer risk among postmenopausal women: a prospective study. Cancer Epidemiol. Biomarkers Prev 2005;14:2137–2142. [PubMed: 16172222]
26. Bradlow HL, Davis DL, Lin G, Sepkovic D, Tiwari R. Effects of pesticides on the ratio of 16 alpha/2-hydroxyestrone: a biologic marker of breast cancer risk. Environ. Health Perspect 1995;103:147–150. [PubMed: 8593862]
27. Adlercreutz H, Hamalainen E, Gorbach SL, Goldin BR, Woods MN, Dwyer JT. Diet and plasma androgens in postmenopausal vegetarian and omnivorous women and postmenopausal women with breast cancer. Am. J. Clin. Nutr 1989;49:433–442. [PubMed: 2923075]
28. Barbosa JC, Shultz TD, Filley SJ, Nieman DC. The relationship among adiposity, diet, and hormone concentrations in vegetarian and non vegetarian postmenopausal women. Am. J. Clin. Nutr 1990;51:798–803. [PubMed: 2159209]
29. Goldin BR, Adlercreutz H, Gorbach SL, Warram JH, Dwyer JT, Swenson L, Woods MN. Estrogen excretion patterns and plasma levels in vegetarian and omnivorous women. N. Engl. J. Med 1982;307:1542–1547. [PubMed: 7144835]
30. Holmes MD, Spiegelman D, Willett WC, Manson JE, Hunter DJ, Barbieri RL, Colditz GA, Hankinson SE. Dietary fat intake and endogenous sex steroid hormone levels in postmenopausal women. J. Clin. Oncol 2000;18:3668–3676. [PubMed: 11054440]
31. Nagata C, Nagao Y, Shibuya C, Kashiki Y, Shimizu H. Fat intake is associated with serum estrogen and androgen concentrations in postmenopausal Japanese women. J. Nutr 2005;135:2862–2865. [PubMed: 16317133]
32. Wu AH, Stanczyk FZ, Seow A, Lee HP, Yu MC. Soy intake and other lifestyle determinants of serum estrogen levels among postmenopausal Chinese women in Singapore. Cancer Epidemiol. Biomarkers Prev 2002;11:844–851. [PubMed: 12223428]
33. Monroe KR, Murphy SP, Henderson BE, Kolonel LN, Stanczyk FZ, Adlercreutz H, Pike MC. Dietary fibre and endogenous serum hormones levels in naturally postmenopausal mexican-american women: the multiethnic cohort sutdy. Nutrition Cancer 2007;58:127–135.
34. Fahey MT, Sasaki S, Kobayashi M, Akabane M, Tsugane S. Seasonal misclassification error and magnitude of true between-person variation in dietary nutrient intake: a random coefficients analysis and implications for the Japan Public Health Center (JPHC) Cohort Study. Public Health Nutr 2003;6:385–391. [PubMed: 12795827]
35. Adlercreutz, H.; Fotsis, T.; Heikkinen, R. Advances in steroid analyses. Budapest: Ahadémia Kiado; 1982. Current state of the art in the analysis of estrogens; p. 3-33.
36. Kuoppasalmi K, Naveri H, Rehunen S, Harkonen M, Adlercreutz H. Effect of strenuous anaerobic running exercise on plasma growth hormone, cortisol, luteinizing hormone, testosterone, androstenedione, estrone and estradiol. J. Steroid Biochem 1976;7:823–829. [PubMed: 1011839]
37. Bergink EW, Holma P, Pyorala T. Effects of oral contraceptive combinations containing levonorgestrel or desogestrel on serum proteins and androgen binding. Scand. J. Clin. Lab. Invest 1981;41:663–668. [PubMed: 6461916]
38. Rosner W. A simplified method for the quantitative determination of testosterone-estradiol-binding globulin activity in human plasma. J. Clin. Endocrinol. Metab 1972;34:983–988. [PubMed: 4112000]
39. Kuoppasalmi K. Plasma testosterone and sex-hormone-binding globulin capacity in physical exercise. Scand. J. Clin. Lab. Invest 1980;40:411–418. [PubMed: 7192430]
40. Fotsis T, Adlercreutz H. The multicomponent analysis of estrogens in urine by ion exchange chromatography and GC-MS--I. Quantitation of estrogens after initial hydrolysis of conjugates. J. Steroid Biochem 1987;28:203–213. [PubMed: 3041111]
41. Baker TS, Jennison KM, Kellie AE. The direct radioimmunoassay of oestrogen glucuronides in human female urine. Biochem. J 1979;177:729–738. [PubMed: 435263]

42. Adlercreutz H, Jarvenpaa P. Assay of estrogens in human faeces. J. Steroid Biochem 1982;17:639–645. [PubMed: 6757585]
43. Adlercreutz H, Fotsis T, Hockerstedt K, Hamalainen E, Bannwart C, Bloigu S, Valtonen A, Ollus A. Diet and urinary estrogen profile in premenopausal omnivorous and vegetarian women and in premenopausal women with breast cancer. J. Steroid Biochem 1989;34:527–530. [PubMed: 2626046]
44. Luhrmann PM, Herbert BM, Gaster C, Neuhauser-Berthold M. Validation of a self-administered 3-day estimated dietary record for use in the elderly. Eur. J. Nutr 1999;38:235–240. [PubMed: 10654160]
45. Paul AA, Southgate DA. McCance and Widdowson's 'The composition of foods': dietary fibre in egg, meat and fish dishes. J. Hum. Nutr 1979;33:335–336. [PubMed: 574147]
46. Adlercreutz H, Tikkanen MJ. Defects in hepatic uptake and transport and biliary excretion of estrogens. Some new concepts of liver metabolism. Med. Chir. Dig 1973;2:59–65. [PubMed: 4743208]
47. Adlercreutz H, Martin F. Biliary excretion and intestinal metabolism of progesterone and estrogens in man. J. Steroid Biochem 1980;13:231–244. [PubMed: 6991820]
48. Goldin BR, Adlercreutz H, Gorbach SL, Woods MN, Dwyer JT, Conlon T, Bohn E, Gershoff SN. The relationship between estrogen levels and diets of Caucasian American and Oriental immigrant women. Am. J. Clin. Nutr 1986;44:945–953. [PubMed: 3024478]
49. Kaneda N, Nagata C, Kabuto M, Shimizu H. Fat and fibre intakes in relation to serum estrogen concentration in premenopausal Japanese women. Nutr. Cancer 1997;27:279–283. [PubMed: 9101558]
50. Rose DP, Lubin M, Connolly JM. Effects of diet supplementation with wheat bran on serum estrogen levels in the follicular and luteal phases of the menstrual cycle. Nutrition 1997;13:535–539. [PubMed: 9263234]
51. Shultz TD, Howie BJ. In vitro binding of steroid hormones by natural and purified fibres. Nutr. Cancer 1986;8:141–147. [PubMed: 3010251]
52. Bingham SA, Day NE, Luben R, Ferrari P, Slimani N, Norat T, Clavel-Chapelon F, Kesse E, Nieters A, Boeing H, Tjonneland A, Overvad K, Martinez C, Dorronsoro M, Gonzalez CA, Key TJ, Trichopoulou A, Naska A, Vineis P, Tumino R, Krogh V, Bueno-de-Mesquita HB, Peeters PH, Berglund G, Hallmans G, Lund E, Skeie G, Kaaks R, Riboli E. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. Lancet 2003;361:1496–1501. [PubMed: 12737858]
53. Wolk A, Manson JE, Stampfer MJ, Colditz GA, Hu FB, Speizer FE, Hennekens CH, Willett WC. Long-term intake of dietary fibre and decreased risk of coronary heart disease among women. J.A.M.A 1999;281:1998–2004.
54. Adlercreutz H, Martin F, Pulkkinen M, Dencker H, Rimer U, Sjoberg NO, Tikkanen MJ. Intestinal metabolism of estrogens. J. Clin. Endocrinol. Metab 1976;43:497–505. [PubMed: 956337]
55. Emerman S, Twombly GH, Levitz M. Biliary and urinary metabolites of estriol-15-3H-3-sulfate-35S in women. J. Clin. Endocrinol. Metab 1967;27:539–548. [PubMed: 5336573]
56. Adlercreutz H, Ervast HS, Tenhunen A, Tikkanen MJ. Gas chromatographic and mass spectrometric studies on oestrogens in bile. I. Pregnant women. Acta Endocrinol. (Copenh) 1973;73:543–554. [PubMed: 4740847]
57. Adlercreutz H, Martin F, Lindstrom B. Gas chromatographic and mass spectrometric studies on oestrogens in bile--2. Men and non-pregnant women. J. Steroid Biochem 1978;9:1197–1205. [PubMed: 745416]
58. Adlercreutz H, Luukkainen T. Biochemical and clinical aspects of the enterohepatic circulation of oestrogens. Acta Endocrinol. (Copenh.) 1968;124:101. [PubMed: 5694253]
59. Adlercreutz H. Studies on oestrogen excretion in human bile. Acta Endocrinol. (Copenh.) 1962;42:1–220.
60. Lewis SJ, Heaton KW, Oakey RE, McGarrigle HH. Lower serum oestrogen concentrations associated with faster intestinal transit. Br. J. Cancer 1997;76:395–400. [PubMed: 9252210]

61. Secreto G, Recchione C, Cavalleri A, Miraglia M, Dati V. Circulating levels of testosterone, 17 beta-oestradiol, luteinising hormone and prolactin in postmenopausal breast cancer patients. Br. J. Cancer 1983;47:269–275. [PubMed: 6824571]
62. Dorgan JF, Longcope C, Stephenson HE Jr, Falk RT, Miller R, Franz C, Kahle L, Campbell WS, Tangrea JA, Schatzkin A. Relation of prediagnostic serum estrogen and androgen levels to breast cancer risk. Cancer Epidemiol. Biomarkers Prev 1996;5:533–539. [PubMed: 8827358]
63. Zeleniuch-Jacquotte A, Bruning PF, Bonfrer JM, Koenig KL, Shore RE, Kim MY, Pasternack BS, Toniolo P. Relation of serum levels of testosterone and dehydroepiandrosterone sulfate to risk of breast cancer in postmenopausal women. Am. J. Epidemiol 1997;145:1030–1038. [PubMed: 9169912]
64. Yu H, Shu XO, Shi R, Dai Q, Jin F, Gao YT, Li BD, Zheng W. Plasma sex steroid hormones and breast cancer risk in Chinese women. Int. J. Cancer 2003;105:92–97. [PubMed: 12672036]
65. Missmer SA, Eliassen AH, Barbieri RL, Hankinson SE. Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. J. Natl. Cancer Inst 2004;96:1856–1865. [PubMed: 15601642]
66. Dorgan JF, Reichman ME, Judd JT, Brown C, Longcope C, Schatzkin A, Forman M, Campbell WS, Franz C, Kahle L, Taylor PR. Relation of energy, fat, and fibre intakes to plasma concentrations of estrogens and androgens in premenopausal women. Am. J. Clin. Nutr 1996;64:25–31. [PubMed: 8669410]
67. Ingram DM, Bennett FC, Willcox D, de Klerk N. Effect of low-fat diet on female sex hormone levels. J. Natl. Cancer Inst 1987;79:1225–1229. [PubMed: 3480374]
68. Adlercreutz H, Gorbach SL, Goldin BR, Woods MN, Dwyer JT, Hamalainen E. Estrogen metabolism and excretion in Oriental and Caucasian women. J. Natl. Cancer Inst 1994;86:1076–1082. [PubMed: 8021957]
69. Muti P, Bradlow HL, Micheli A, Krogh V, Freudenheim JL, Schunemann HJ, Stanulla M, Yang J, Sepkovic DW, Trevisan M, Berrino F. Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16alpha-hydroxyestrone ratio in premenopausal and postmenopausal women. Epidemiology 2000;11:635–640. [PubMed: 11055622]
70. Schutze N, Vollmer G, Tiemann I, Geiger M, Knuppen R. Catecholestrogens are MCF-7 cell estrogen receptor agonists. J. Steroid Biochem. Mol. Biol 1993;46:781–789. [PubMed: 8274412]
71. Cavalieri E, Chakravarti D, Guttenplan J, Hart E, Ingle J, Jankowiak R, Muti P, Rogan E, Russo J, Santen R, Sutter T. Catechol estrogen quinones as initiators of breast and other human cancers: implications for biomarkers of susceptibility and cancer prevention. Biochim. Biophys. Acta 2006;1766:63–78. [PubMed: 16675129]
72. Lu F, Zahid M, Saeed M, Cavalieri EL, Rogan EG. Estrogen metabolism and formation of estrogen-DNA adducts in estradiol-treated MCF-10F cells. The effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin induction and catechol-O-methyltransferase inhibition. J. Steroid Biochem. Mol. Biol 2007;105:150–158. [PubMed: 17582757]
73. Loukovaara M, Carson M, Adlercreutz H. Regulation of sex-hormone-binding globulin production by endogenous estrogens in vitro. Biochem. Biophys. Res. Commun 1995;206:895–901. [PubMed: 7832802]
74. Adlercreutz, H.; Höckerstedt, K.; Bannwart, C.; Hämäläinen, E.; Fotsis, T.; Bloigu, S. The 3rd International Congress on Hormones and Cancer. Bresciani, FKR.; Lippman, ME.; Raynaud, J-P., editors. Hamburg: Progress in Cancer Research and Therapy; 1988 [September 1987]. p. 409-412.
75. Lottering ML, Haag M, Seegers JC. Effects of 17 beta-estradiol metabolites on cell cycle events in MCF-7 cells. Cancer Res 1992;52:5926–5932. [PubMed: 1327520]
76. Weisz J, Bui QD, Roy D, Liehr JG. Elevated 4-hydroxylation of estradiol by hamster kidney microsomes: a potential pathway of metabolic activation of estrogens. Endocrinology 1992;131:655–661. [PubMed: 1386303]
77. Boyd NF, Lockwood GA, Greenberg CV, Martin LJ, Tritchler DL. Effects of a low-fat high-carbohydrate diet on plasma sex hormones in premenopausal women: results from a randomized controlled trial. Canadian Diet and Breast Cancer Prevention Study Group. Br. J. Cancer 1997;76:127–135. [PubMed: 9218745]

78. Berrino F, Bellati C, Secreto G, Camerini E, Pala V, Panico S, Allegro G, Kaaks R. Reducing bioavailable sex hormones through a comprehensive change in diet: the diet and androgens (DIANA) randomized trial. Cancer Epidemiol. Biomarkers Prev 2001;10:25–33. [PubMed: 11205485]

79. Holmes MD, Liu S, Hankinson SE, Colditz GA, Hunter DJ, Willett WC. Dietary carbohydrates, fibre, and breast cancer risk. Am. J. Epidemiol 2004;159:732–739. [PubMed: 15051582]

Table 1

Anthropometric characteristics of the groups

Variables	high fat & low fiber group (n = 22)	low fat & high fiber group (n = 22)	p values
Age (yrs)	25 ± 3	26 ± 3	0.46*
Weight (kg)	57.1 ± 7.5	54.8 ± 6.6	0.28*
BMI (kg/m^2)	20.75 ± 1.94	20.46 ± 1.87	0.62*

Means ± SD.

Significant $p \leq 0.050$.

* p values obtained using log values.

Independent sample t-test.

BMI = body mass index.

Table 2

Dietary intake of the groups

Variables	high fat & low fiber group (n = 22)	low fat & high fiber group (n = 22)	p values
Total calorie intake (Kcal)	1813 ± 327	1599 ± 345	0.041
Fat intake (g/d)	84 ± 19	44 ± 19	0.001
Carbohydrate intake (g/d)	182 ± 36	250 ± 56	0.001
Protein intake (g/d)	65 ± 14	56 ± 15	0.080
SFA (g/d)	32 ± 10	12 ± 8	0.001
MUFA (g/d)	28 ± 6	13 ± 7	0.001
PUFA (gd)	14 ± 5	11 ± 6	0.164
Fat/Fiber ratio (g/d)	7.8 ± 2.9	1.4 ± 0.6	0.001
Fiber intake (g/d)	11 ± 3	31 ± 9	0.001

Means ± SD.

Significant $p \leq 0.050$.

Independent sample t-test.

SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids.

Table 3

Plasma and fecal sex hormone levels of the groups

Variables	high fat & low fiber group (n = 22)	low fat & high fiber group (n = 22)	p values
Estrone (pmol/l)	306 ± 60	264 ± 84	0.069
Estradiol (pmol/l)	249 ± 80	221 ± 84	0.148*
E1 + E2 (pmol/l)	556 ± 126	486 ± 159	0.067*
E1 / E2 ratio	1.27 ± 0.22	1.24 ± 0.30	0.618*
Androstenedione (nmol/l)	5.06 ± 1.20	4.94 ± 1.30	0.752
Testosterone (nmol/l)	1.55 ± 0.60	1.40 ± 0.44	0.375*
Free testosterone (pmol/l)	15.66 ± 7.19	15.64 ± 6.32	0.856*
SHBG (nmol/l)	54.47 ± 15.96	44.03 ± 18.47	0.051
DHEAS (µmol/l)	6.78 ± 3.13	6.96 ± 4.31	0.570*
F-E1 (nmol/24h)	2.30 ± 2.10	3.03 ± 2.72	0.226*
F-E2 (nmol/24h)	1.38 ± 1.32	1.77 ± 1.75	0.162*
F-E3 (nmol/24h)	2.15 ± 1.95	3.10 ± 2.96	0.177*
Total F-Estrogens (nmol/24h)	5.84 ± 5.23	7.90 ± 7.03	0.123*

Means ± SD.

Significant $p \leq 0.050$.

* p values obtained using log variables values.

Independent sample t-test.

E1 = estrone; E2 = estradiol; E3 = estriol; SHBG = sex hormone binding globulin, DHEAS = Dehydroepiandrosterone-sulfate; F = Fecal measures; Total F-estrogens = F-E1 +F-E2 +F-E3.

Table 4

Urinary estrogen metabolite levels of the groups

Variables	high fat & low fiber group (n = 22)	low fat & high fiber group (n = 22)	p values
E1 (nmol/24h)	22.70 ± 7.49	20.64 ± 8.11	0.266*
E2 (nmol/24h)	13.78 ± 6.87	10.85 ± 5.06	0.123*
E3 (nmol/24h)	26.74 ± 11.48	19.42 ± 10.38	0.025*
E3-3G (nmol/24h)	41.50 ± 17.98	27.34 ± 14.00	0.002*
2-OHE1(nmol/24h)	47.54 ± 19.45	37.16 ± 19.13	0.161
4-OHE1 (nmol/24h)	8.15 ± 2.02	8.75 ± 3.12	0.861*
2-OHE2 (nmol/24h)	12.61 ± 4.99	7.44 ± 3.93	0.004
16α-OHE1 (nmol/24h)	8.61 ± 4.47	5.58 ± 3.64	0.055
2-OHE1/4-OHE1 (nmol/24h)	5.87 ± 2.37	4.17 ± 1.78	0.045*
2-OHE1/16α-OHE1 (nmol/24h)	8.36 ± 8.68	10.16 ± 7.63	0.709*
2-OHE1/E1 (nmol/24h)	2.08 ± 1.12	1.69 ± 0.57	0.478*
4-OHE1/E1 (nmol/24h)	0.36 ± 0.17	0.45 ± 0.20	0.202*
16α-OHE1/E1 (nmol/24h)	0.37 ± 0.22	0.30 ± 0.24	0.295*
E3/16α-OHE (nmol/24h)1	4.78 ± 4.53	5.09 ± 3.17	0.663*
2-OHE (2-OHE1+ 2-OHE2; nmol/24h)	60.16 ± 23.63	44.60 ± 22.00	0.078
16α-OHE (16α-OHE1+E3; nmol/24h)	38.09 ± 13.34	27.07 ± 12.76	0.032
2-OHE/16-OHE (nmol/24h)	1.74 ± 0.85	2.14 ± 1.45	0.993*
2-MeO-E1 (nmol/24h)	7.90 ± 2.91	7.26 ± 3.25	0.590
15α-OHE1 (nmol/24h)	0.86 ± 0.59	0.70 ± 0.39	0.386
16β-OHE1 (nmol/24h)	3.02 ± 1.43	1.98 ± 1.90	0.023*
16-ketoE2 (nmol/24h)	5.76 ± 2.43	3.96 ± 2.58	0.067
16-epiE3 (nmol/24h)	6.40 ± 3.62	3.89 ± 2.18	0.033*
17-epiE3 (nmol/24h)	2.27 ± 1.53	1.48 ± 0.81	0.123*
Total urinary estrogens (nmol/24h)	174 ± 47	132 ± 38	0.013

Means ± SD.

Significant $p \leq 0.050$.

* p values obtained using log variables values.

Independent sample t-test.

E1 = estrone; E2 = estradiol; E3 = estriol; Total estrogens = E1+ E2 +E3; E3-3G = estriol-3-glucuronide; OHE1 = hydroxyestrone; OHE2 = hydroxyestradiol: OHE = hydroxylated estrogens, all urinary estrogens = total estrogens + all estrogens metabolites (except E3-3G).

Table 5

Hormonal differences between groups with total kcal intake as covariable

Variables	high fat & low fiber group (n = 22)	low fat & high fiber group (n = 22)	p values
In plasma:			
Estrone (pmol/l)	294 ± 20	240 ± 19	0.052
Estradiol (pmol/l)	234 ± 15	200 ± 14	0.078*
E1+E2 (pmol/l)	529 ± 34	440 ± 31	0.046*
E1/E2 ratio	1.25 ± 0.06	1.22 ± 0.06	0.587*
Testosterone (nmol/l)	1.81 ± 0.16	1.53 ± 0.15	0.289*
SHBG (nmol/l)	62.11 ± 5.29	47.20 ± 4.89	0.045
In urine:			
E1 (nmol/24h)	23.56 ± 2.47	21.68 ± 2.28	0.562*
E2 (nmol/24h)	16.04 ± 1.68	11.96 ± 1.55	0.068*
E3 (nmol/24h)	28.6 ± 3.2	22.12 ± 2.96	0.127*
E3-3G (nmol/24h)	41.28 ± 4.32	29.97 ± 3.99	0.024*
2-OHE1(nmol/24h)	45.18 ± 5.66	38.24 ± 5.22	0.457
4-OHE1 (nmol/24h)	8.15 ± 0.81	8.66 ± 0.75	0.745*
2-OHE2 (nmol/24h)	11.61 ± 1.21	8.25 ± 1.12	0.054
16α-OHE1 (nmol/24h)	9.05 ± 1.19	5.36 ± 1.10	0.024
2-OHE1/4-OHE1 (nmol/24h)	5.47 ± 0.58	4.45 ± 0.53	0.271*
2-OHE (2-OHE1+ 2-OHE2; nmol/24h)	56.79 ± 6.59	46.50 ± 6.09	0.317
16α-OHE (16α-OHE1+E3; nmol/24h)	37.65 ± 3.97	27.48 ± 3.67	0.072
16β-OHE1 (nmol/24h)	3.01 ± 0.52	2.03 ± 0.48	0.024*
16-ketoE2 (nmol/24h)	5.81 ± 0.76	4.04 ± 0.70	0.061*
16-epiE3 (nmol/24h)	6.01 ± 0.85	4.30 ± 0.79	0.133
Total urinary estrogens (nmol/24h)	167 ± 12	138 ± 10	0.099

Means ± SE.

Significant $p \leq 0.050$.

* p values obtained using log variables values.

GLM multivariate with total Kcal as covariable.

Table 6

Pearson correlation between dietary components (g/d) and plasma, fecal or urinary sex hormones in premenopausal women

Variables	Total Kcal	Total Fat	Total CHO	Total Proteins	Total SFA	Total MUFA	Total PUFA	Fat/Fiber Ratio	Total Fibers
Plasma:									
E1	0.283	0.409**	−0.096	0.511	0.448**	0.462**	0.271	0.331**	−0.052
E2	0.302*	0.347*	0.027	0.099	0.367*	0.365*	0.150	0.220	0.000
E1+E2	0.313*	0.417**	−0.051	0.102	0.450**	0.456**	0.236	0.298*	−0.041
E1/E2 ratio	−0.061	0.067	−0.196	−0.031	0.093	0.116	0.165	0.130	−0.100
Androstenedione	−0.139	0.016	−0.180	−0.315*	0.073	0.072	−0.189	0.111	−0.108
Testosterone	0.017	0.074	−0.120	−0.132	0.018	0.088	−0.200	0.157	−0.178
Free Testosterone	−0.052	−0.062	−0.052	−0.126	−0.092	−0.052	−0.288	0.068	−0.085
SHBG	0.040	0.169	−0.233	0.006	0.100	0.174	0.074	0.218	−0.254
DHEAS	0.058	0.112	−0.077	−0.130	0.146	0.211	0.039	0.120	−0.055
Feces:									
E1	0.207	0.005	0.324*	0.151	−0.061	−0.008	−0.052	−0.139	0.228
E2	0.173	−0.021	0.321*	0.079	−0.089	−0.016	0.015	−0.193	0.226
E3	0.035	−0.075	0.157	0.007	−0.134	−0.068	−0.008	−0.205	0.087
Total estrogens	0.123	−0.070	0.293	0.072	−0.139	−0.071	−0.047	−0.207	0.204
Urine:									
E1	0.237	0.256	0.050	0.052	0.233	0.259	−0.008	0.236	−0.086
E2	0.294	0.314*	−0.063	0.261	0.341*	0.324*	−0.084	0.346*	−0.230
E3	0.142	0.266	−0.294	0.267	0.336*	0.273	−0.055	0.364*	−0.470**
E3-3G	0.167	0.382*	−0.407**	0.196	0.511**	0.431**	−0.007	0.445*	−0.548**
2-OHE1	0.336	0.401*	0.028	0.159	0.312	0.412*	0.145	0.161	−0.031
4-OHE1	0.057	0.033	0.117	0.021	−0.079	−0.034	0.024	−0.120	0.231
2-OHE2	0.561**	0.591**	0.039	0.420*	0.588**	0.597**	0.066	0.389*	−0.161
16α-OHE1	−0.038	0.237	−0.374*	−0.146	0.366	0.113	0.007	0.267	−0.397*
2-OHE1/4-OHE1	0.406*	0.478**	0.015	0.146	0.424*	0.498**	0.164	0.336	−0.171
2-OHE1/16α-OHE1	0.279	0.094	0.332	0.215	−0.054	0.039	0.027	−0.047	0.262
2-OHE1/E1	0.185	0.197	0.037	0.110	0.084	0.153	0.093	0.052	0.038
4-OHE1/E1	−0.251	−0.321	0.027	−0.038	−0.393*	−0.396*	−0.080	−0.329	0.245
16α-OHE1/E1	−0.247	0.017	−0.430*	−0.215	0.137	0.060	0.032	0.103	−0.333

J Steroid Biochem Mol Biol. Author manuscript; available in PMC 2009 November 1.

Variables	Total Kcal	Total Fat	Total CHO	Total Proteins	Total SFA	Total MUFA	Total PUFA	Fat/Fiber Ratio	Total Fibers
E3/16α-OHE1	0.339	0.058	0.296	0.554**	0.011	0.031	−0.031	−0.039	0.128
2-OHE	0.399*	0.460*	0.032	0.222	0.385*	0.470*	0.135	0.217	−0.060
16-OHE	0.224	0.370*	−0.294	0.308	0.514**	0.439*	0.005	0.301	−0.414*
2-OHE/16α-OHE	−0.062	−0.134	0.191	−0.143	−0.226	−0.160	−0.131	−0.194	0.355
2-MeO-E1	0.253	0.243	0.134	0.093	0.181	0.247	0.070	0.043	0.067
15α-OHE1	−0.017	0.062	−0.169	−0.028	0.068	0.072	0.125	0.070	−0.168
16β-OHE1	0.088	0.395*	−0.432*	0.027	0.514**	0.460*	0.025	0.358	−0.438*
16-ketoE2	0.102	0.302	−0.266	−0.027	0.389*	0.372*	0.081	0.306	−0.344
16-epiE3	0.185	0.385*	−0.329	0.151	0.507**	0.503**	0.116	0.324	−0.356
17-epiE3	0.250	0.374*	−0.151	0.184	0.325	0.373*	0.194	0.132	−0.153
Total urinary estrogens	0.469*	0.580**	−0.088	0.323	0.599**	0.634**	0.101	0.351	−0.254

* $p \leq 0.05$

** $p \leq 0.01$.

CHO = carbohydrates; SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids.