Plasma Levels of Enterolactone and Percentage Mammographic Density among Postmenopausal Women

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Abstract

Aims: **Certain phytoestrogens, such as lignans, may protect against developing breast cancer. Enterolactone is a lignan metabolite produced by the intestinal flora from dietary precursors such as whole grains, vegetables, and fruits. Enterolactone has been shown to have weak estrogenic and antiestrogenic properties. We decided to examine the association between plasma levels of enterolactone and mammographic density, a biomarker for breast cancer risk.**

Methods: **We included data from postmenopausal women ages 55 and older who participated in a cross-sectional mammogram study in Tromsø, Norway. Mammograms, plasma enterolactone measurements, as well as information on anthropometric and hormonal/reproduction factors were available on 616 women. We assessed mammographic density using a previously validated computer-assisted method. We estimated correlation coefficients and conducted multiple regression analyses.**

Results: **Mean mammographic density increased slightly across quartiles of enterolactone; the women in the highest quartile had, on average, 3.1% (absolute difference) higher percentage mammographic density compared with the lowest quartile (P_{trend} < 0.01). After adjustment for age, body mass index, number of full-term pregnancies, age at first birth, and use of postmenopausal hormone therapy, the mean difference in density was reduced to 2.0% (P_{trend} = 0.05). Results were similar when restricted to the 454 current hormone nonusers. The fully adjusted statistical model explained 28.3% of the total variability in mammographic percentage density, with body mass index contributing 18.2% and enterolactone only 0.9%.**

Conclusion: **In our study, higher levels of enterolactone were associated with slightly higher percentage mammographic density. Our results suggest that if higher enterolactone levels reduce the risk of developing breast cancer in postmenopausal women, then this effect is not through lowering mammographic density.** (Cancer Epidemiol Biomarkers Prev 2005;14(9):2154–9)

Introduction

The radiographic appearance of a mammogram is determined by the relative amounts of translucent fat tissue to the denser epithelial and/or stromal tissues (1). The percentage of total breast area that appears radiologically dense has been shown to be associated with breast cancer risk, with women having the densest mammograms being four to six times at higher risk of developing breast cancer compared with women with no densities (2-4).

Mammographic density has also been shown to change with regimens known to increase or decrease breast cancer risk, such as decreased density with the antiestrogen tamoxifen (5, 6), menopause (7), and increased density with postmenopausal hormone therapy use (8, 9).

Phytoestrogens, such as isoflavones and lignans, could protect against developing breast cancer (10). In Western diets, lignans are probably the most important source of phytoestrogens and enterolactone, formed by oxidation of plant precursors by the intestinal microflora (11), the most abundant lignan metabolite (12). Lignan precursors are found in a wide variety of plant foods, such as seeds, especially flaxseeds, whole grains, vegetables, fruits, and berries (13, 14).

The association between lignans and the risk of developing breast cancer has been investigated by dietary assessments of lignans (15-19) by measuring the main mammalian metabolite, enterolactone, in urine (20-22) and blood (23-27). Some investigators (15, 19) have suggested that to elucidate the association between lignans and chronic diseases, such as cancer, long-time dietary assessment of lignans by food frequency methods would be of more relevance than measuring human lignan metabolites, which are influenced by recent lignan precursor intake (28). However, food frequency methods suffer from imprecision as well, and the ability to correctly quantify lignan intake may be variable. Further, more recently detected lignan precursors (29) are not included in the food composition databases of phytoestrogens, which limits the ability to estimate total lignan intake. Also, the low correlation found between lignan-containing food and the main biological active metabolite, enterolactone (30, 31), limits the interpretation of dietary assessment of lignans. With respect to mammographic density, which has been shown to be modified by short-time changes in hormone levels (32, 33), assessing the human active metabolite, enterolactone, would be a feasible approach for assessing lignan exposure.

The biological properties of lignans (10) could, separately or combined, possibly affect the radiographic appearance of a mammogram. Whereas several recent studies have provided increased evidence for the reduced breast cancer risk with soy or isoflavone consumption (34-36), results from the few studies conducted on isoflavone consumption and mammographic density have been contradictory (37-40).

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To our knowledge, the association between enterolactone levels and mammographic density has not been assessed before.

Materials and Methods

Study Population. The present study used data from the Tromsø Mammography and Breast Cancer Study, the main objectives of which are to identify genetic, hormonal, reproductive, and lifestyle characteristics associated with mammographic patterns/densities that may place women at higher risk of developing breast cancer. Details of this cross-sectional study are described elsewhere (41). Briefly, women ages 55 and older who resided in the municipality of Tromsø, in the northern part of Norway, attending the National Breast Cancer Screening Program during 2001 and 2002, were invited to participate in the study.

Study Sample. Our study of enterolactone and mammographic density was done among women recruited during the spring of 2002 as these women had completed an extensive dietary questionnaire. Altogether, 1,212 women received an invitation to the screening program, and 1,209 of these women were simultaneously invited to participate in the scientific study. In addition, 12 women were invited to the scientific study by a letter from the University of Tromsø. Nine hundred seventy-five women (80%) attended the screening. The 653 women included in our study constituted 53% of the women invited to the screening. Finally, two women who self-referred subsequently to the screening were included in the scientific study.

At the day of the mammographic screening, a blood sample was drawn and anthropometric measures were obtained from the participants. The study subjects were interviewed by a trained nurse about their current and previous postmenopausal hormone therapy use, reproductive and menstrual factors, previous history of cancer, and smoking status. The participants were asked to complete an eight-page questionnaire at home. The questionnaires contained items on demographic, menstrual, and reproductive factors, as well as lifestyle and dietary factors. The study was approved by the Regional Medical Ethical Committee and written informed consents were obtained from all participants.

Among the 655 women who agreed to participate, 14 women with new or previous breast cancer diagnosis were excluded, leaving 641 women in the study. Among these, 619 had enterolactone measured. Three women were equivocal for menopausal status and were not included in the analysis on enterolactone and mammographic density. For the multivariate analysis on enterolactone and mammographic density, complete information was available for 616 women.

Assay of Plasma Samples. Nonfasting venous samples were obtained from study participants at the day of mammographic screening. Samples were stored at −20°C or colder until analysis in December 2002. Samples had been thawed once during storage time. The enterolactone analysis was performed by time-resolved fluoroimmunoassay (42) with slight modifications (43). Briefly, the method used was as follows: 200 μL of hydrolysis reagent containing 0.1 mol/L acetate buffer (pH 5), 2 units/mL sulfatase, and 0.2 units/mL β-glucuronidase was added to the plasma samples (200 μL). After overnight incubation at 37°C, free enterolactone and hydrolyzed conjugates were extracted with 1.5 mL diethylether. The ether phase was evaporated to dryness in water bath and the dry residue then dissolved in 200 μL assay buffer. Twenty microliters of the solution, corresponding to 20 μL of the original sample, was then taken for time-resolved fluoroimmunoassay, using DELFIA Research Reagents (Wallac Oy, Turku, Finland) for measurement of enterolactone. By this method, europium-labeled and sample enterolactone compete for a limited amount of highly specific antibody. Enhancement solution dissociates the europium ions from the labeled enterolactone into solution and they form fluorescent chelates with components in the enhancement solution. Fluorescence was measured in a VICTOR 1420 (Wallac Oy) multilabel counter. Fluorescence from each sample is inversely proportional to the concentration of enterolactone in the sample. All of the samples were analyzed in duplicate and values were averaged. The mean (between batches) coefficient of variation of our duplicates was 5.4.

Processing of Mammograms. Percentage mammographic density was determined using the University of Southern California Madena computer-based threshold method of assessing density; this method has been described and validated elsewhere (44). Briefly, the craniocaudal mammographic images are digitized using a high-resolution Cobrascan CX-612T scanner (Radiographic Digital Imaging, Torrance, CA) and then viewed on a computer screen. A reader first defines the total breast area using a special outlining tool. Next, the region of interest, excluding the pectoralis muscle, prominent veins, and fibrous strands, is defined. The reader then uses a tinting tool to apply a yellow tint to dense pixels with gray levels at or above some threshold X and 255. The reader searches for the best threshold where all pixels X within the region of interest are considered to represent mammographic densities. The software estimates the total number of pixels and the number of tinted pixels within the region of interest. Absolute density represents the count of the tinted pixels within the region of interest. Percentage density, or the fraction (%) of the breast with densities, is the ratio of absolute density to the total breast area.

In our study, the percentage of the breast with densities was estimated on the left breast. Density assessments were performed by Dr. G. Ursin, whereas breast area measurements were conducted by a research assistant trained by Dr. G. Ursin. The readers were blinded to all of the subject characteristics.

Data Analysis. We used ANOVA to study the association between enterolactone and selected variables. Enterolactone was $\log_{10}$ transformed in the analysis, and back transformed means and 95% confidence intervals are presented. Additionally, we conducted trend tests for enterolactone across categorized covariates using linear regression, with enterolactone ($\log_{10}$ transformed) entered as a continuous variable.

The association between percentage mammographic density and enterolactone was first estimated by Spearman's rank correlation and further studied by multiple linear regression analysis (analysis of covariance). We have previously shown that in this study population, percentage mammographic density decrease with higher body mass index (BMI), with increasing number of full-term pregnancies and with lower age at first birth (41). These variables, in addition to age and current use of postmenopausal hormone therapy, were adjusted for in the multivariate analysis. Enterolactone was categorized into quartiles, and the covariates were categorized as follows: BMI (tertiles), age (tertiles), number of full-term pregnancies (0, 1-2, 3, 4 and more), age at first birth (<20, 20-24, 25 and older), and current postmenopausal hormone therapy use (yes/no). Of the 616 participating women, 577 reported one or more full-term pregnancies. Of these, 59 did not provide information on age at first birth. To keep these women in the adjusted analyses, we replaced the missing values with the median age at first birth for women with the same age and same number of children. Trend tests across quartiles of enterolactone were conducted by treating the quartiles as a continuous variable in the regression analyses. Mammographic density was $\log_{10}$ transformed in the regression analyses. From the multivariate analyses, we report unadjusted and adjusted back-transformed marginal means with 95% confidence intervals. Further, from the adjusted model of enterolactone and percentage mammographic density, we report R^2 and partial

Table 1. Characteristics of women included in the study (n = 616)

Variable	Median (range)
Age (y)	60 (55-71)
BMI (kg/m^2)	27.1 (13.1-47.0)
No. full-term pregnancies	3 (0-11)
Age at first birth (n = 518)	22 (16-39)
Mammographic density (%)	8.5 (0-69.2)
Mammographic density (absolute, cm^2)	13.2 (0-155.2)
Enterolactone (nmol/L)	16.7 (0.3-176.9)
Postmenopausal hormone therapy use	**n (%)**
Current use	162 (26)
Current nonuse	454 (74)
Smoking (n = 616)	
Current daily smoking	170 (27)
Current nonsmoking	404 (66)
Smoking occasionally	42 (7)
Alcohol (n = 547)	
Drinks alcohol	455 (83)
Does not drink alcohol	92 (17)

η^2 values (analysis of covariance, SPSS for Windows, version 11.0; SPSS, Inc., Chicago, IL) for selected covariates.

Finally, the association between enterolactone and selected dietary items was estimated by Spearman's rank correlation.

The statistical analyses were done using SPSS for Windows (version 11.0). All P values are two sided.

Results

The characteristics of the study subjects are summarized in Table 1. Median percentage mammographic density was 8.5% (range: 0-69.2%) and 38% of the women had a percentage mammographic density lower than 5%. Median plasma enterolactone was 16.7 nmol/L (range: 0.3-176.9). Women using postmenopausal hormone therapy had, on average, 6.2% (absolute difference) higher median percentage mammographic density than did hormone nonusers (data not shown). Table 2 shows the association between plasma enterolactone levels and selected variables. Increasing age of first birth was positively associated with enterolactone levels. Enterolactone levels were lowest among women in the highest BMI category, and were lower for smokers than nonsmokers. Further, women who reported drinking alcohol had higher enterolactone levels compared with nondrinkers, although this was not statistically significant (P = 0.08).

Enterolactone and Mammographic Density. There was a weak positive correlation between plasma enterolactone levels and percentage mammographic density (Spearman's correlation coefficient, r = 0.13, P < 0.01).

Mean mammographic density increased slightly across quartiles of enterolactone levels; the women in the highest quartile had, on average, 3.1% (absolute difference) higher percentage mammographic density compared with women in the lowest quartile (P_{trend} < 0.01; Table 3). After adjustment for age, BMI, number of full-term pregnancies, age of first birth, and use of postmenopausal hormone therapy, the mean difference in density was reduced to 2.0% (P_{trend} = 0.05) .The results were essentially unchanged when the analysis was restricted to the women who did not have imputed values for age at first birth (n = 518). When the analysis was repeated among women with data available on alcohol consumption and smoking (n = 510), additionally controlling for these variables gave similar results (data not shown). Alcohol consumption (yes/no) or smoking (daily smoking/not smoking) did not modify the association between enterolactone and percentage mammographic density (test for interaction, P = 0.91 and 0.83, respectively).

When absolute mammographic density was used as dependent variable instead of percentage mammographic density in the multivariate analysis, the positive association with enterolactone was maintained (Table 3). In the adjusted analysis, women in the highest quartile of enterolactone had, on average, 2.5 cm^2 denser area than women in the lowest quartile of enterolactone (P_{trend} = 0.12). The weak positive association between percentage mammographic density and enterolactone levels was seen in both current hormone users (n = 162, adjusted difference = 2.7%, P_{trend} = 0.34) and hormone nonusers (n = 454, adjusted difference 1.7%, P_{trend} = 0.12). There was no statistically significant interaction between enterolactone and use of postmenopausal hormone therapy (current use/current nonuse) with respect to percentage mammographic density (test for interaction P = 0.70) or absolute mammographic density (test for interaction P = 0.80). In addition, if we replaced the current use/current nonuse hormone therapy variable with a hormone use variable categorized as current use/former use/never use in the analysis, this did not change the associations between enterolactone and mammographic density (results not shown). The use of this hormone therapy variable (current use/former use/never use) also did not modify the relationship between enterolactone and mammographic density with respect to percentage (test for interaction, P = 0.90) or absolute (test for interaction, P = 0.98) mammographic density.

Enterolactone plasma levels above 100 nmol/L are rare in humans if flaxseeds are not consumed. Therefore, we conducted an additional analysis where we only included the 602 women with enterolactone levels below 100 nmol/L and obtained essentially identical results.

Enterolactone and Diet. In this study, there were weak but statistically significant positive correlations between enterolactone levels and weekly servings of vegetables (Spearman's correlation coefficient, r = 0.13, P < 0.01), fruit (r = 0.20, P < 0.01), and grains (including whole meal bread, crisp bread, and

Table 2. Plasma enterolactone levels in relation with selected variables

	n (%)	Enterolactone (nmol/L)	P
Age in tertiles, n = 616			
<59	202 (33)	12.8 (10.8-15.2)	
59-63	223 (36)	14.5 (12.5-16.7)	
>63	191 (31)	12.7 (10.9-14.8)	0.41*
No. full-term pregnancies, n = 616			
0	39 (6)	17.7 (12.3-25.3)	
1-2	255 (41)	13.0 (11.3-15.0)	
3	181 (30)	13.9 (11.8-16.4)	
4 and more	141 (23)	12.3 (10.2-14.9)	0.29†
Age at first birth‡, n = 577			
<20	79 (14)	10.1 (7.9-13.1)	
20-25	343 (59)	13.3 (11.8-15.0)	
>25	155 (27)	14.5 (12.1-17.3)	0.05†
Postmenopausal hormone use, n = 616			
Current use	162 (26)	13.6 (11.2-16.6)	
Current nonuse	454 (74)	13.3 (12.0-14.7)	0.81*
BMI tertiles, n = 616			
BMI <25.2	184 (30)	14.0 (11.9-16.6)	
BMI 25.2-29.2	212 (34)	15.8 (13.5-18.4)	
BMI >29.2	220 (36)	10.9 (9.4-12.7)	0.02†
Smoking, n = 574			
Current nonsmoking	404 (70)	14.5 (12.9-16.2)	
Current daily smoking	170 (30)	10.0 (8.4-11.9)	<0.01*
Alcohol, n = 542			
Does not drink alcohol	92 (17)	11.2 (8.8-14.3)	
Drinks alcohol	455 (83)	14.1 (12.7-15.7)	0.08*

NOTE: Enterolactone was $\log_{10}$ transformed in the analyses; reported means are back transformed.

*ANOVA.

†Test for trend.

‡Fifty-nine of the 577 women have imputed values for age at first birth.

Table 3. Unadjusted and adjusted means of mammographic density (95% confidence interval) in quartiles of enterolactone

	Quartiles of plasma enterolactone				P_{trend}
	1 (<7.19 nmol/L)	2 (7.20-16.59 nmol/L)	3 (16.60-28.29 nmol/L)	4 (>28.30 nmol/L)	
All women (n = 616)	153	155	154	154	
Percentage mammographic density					
Unadjusted	6.4 (5.4-7.8)	6.7 (5.6-8.1)	7.8 (6.5-9.4)	9.5 (7.9-11.5)	<0.01
Adjusted	8.2 (6.8-9.8)	8.1 (6.7-9.7)	8.5 (7.0-10.2)	10.2 (8.5-12.2)	0.05
Absolute mammographic density (cm^2)					
Unadjusted	9.1 (7.5-11.0)	9.6 (8.0-11.7)	10.7 (8.8-13.0)	12.9 (10.6-15.7)	<0.01
Adjusted	11.2 (9.1-13.7)	11.2 (9.1-13.7)	11.5 (9.3-14.1)	13.7 (11.2-16.8)	0.12
Current postmenopausal hormone therapy users (n = 162)	35	40	41	46	
Percentage mammographic density					
Unadjusted	10.4 (7.4-14.5)	12.6 (9.2-17.1)	11.3 (8.3-15.4)	13.4 (10.1-17.9)	0.34
Adjusted	9.1 (6.4-13.1)	11.2 (7.8-16.0)	10.3 (7.2-14.7)	11.8 (8.6-16.3)	0.34
Absolute mammographic density (cm^2)					
Unadjusted	14.2 (10.0-20.1)	17.2 (12.4-23.8)	16.3 (11.8-22.5)	18.2 (13.5-24.7)	0.35
Adjusted	13.0 (8.8-19.3)	15.7 (10.7-23.2)	15.1 (10.3-22.1)	16.5 (11.7-23.3)	0.40
Current postmenopausal hormone therapy nonusers (n = 454)	118	115	113	108	
Percentage mammographic density					
Unadjusted	5.6 (4.5-6.9)	5.4 (4.4-6.7)	6.8 (5.5-8.5)	8.2 (6.6-10.3)	<0.01
Adjusted	7.0 (5.7-8.6)	6.5 (5.3-8.1)	6.8 (5.5-8.4)	8.7 (7.0-10.8)	0.12
Absolute mammographic density (cm^2)					
Unadjusted	8.0 (6.4-10.0)	7.9 (6.3-9.9)	9.2 (7.3-11.5)	11.1 (8.8-14.1)	0.03
Adjusted	9.4 (7.5-11.9)	8.9 (7.1-11.3)	8.9 (7.0-11.3)	11.4 (9.0-14.6)	0.25

NOTE: Reported means are back transformed from estimated marginal means. Analyses are adjusted for age (tertiles), BMI (tertiles), full-term pregnancies (none, 1-2, 3, 4 or more), age at first birth (<20, 20-24, >24), and postmenopausal hormone therapy (current use/current nonuse).

cereals; r = 0.09, P = 0.03). Consumption of alcohol (gram alcohol per month) was positively associated with enterolactone (r = 0.10, P = 0.02).

There were no statistically significant associations with tea or coffee; however, if tea and coffee were categorized into groups of high and low consumption, median enterolactone tended to be higher with higher consumption of both beverages.

Using partial correlations (enterolactone $\log_{10}$ transformed) controlling for BMI did not substantially change the associations between these dietary variables and enterolactone.

In the final model (n = 616), R^2 was 28.3% and partial η^2 was highest for BMI 18.2% ($P < 0.01$); for number of full-term pregnancies, 4.0% ($P < 0.01$); for use of postmenopausal hormone therapy, 3.3% ($P < 0.01$), and for enterolactone levels, only 0.9% (P = 0.14).

Discussion

In this study, we found that higher levels of enterolactone were associated with a slightly increased mammographic density, which persisted after adjustment for BMI, number of full-term pregnancies, and use of postmenopausal hormone therapy. The difference of 2.0% in mammographic density between the lowest and the highest quartile of enterolactone is probably of limited clinical significance. In comparison, the average increase observed in women randomized to postmenopausal combined estrogen and progestin therapy is ~5% (8), and the decline when women undergo menopause is of similar magnitude (7).

We are not aware of any other studies on the association between plasma enterolactone levels and mammographic density.

Results from epidemiologic studies on the association between serum/plasma or urinary excretion levels of enterolactone and breast cancer risk have been inconclusive. A 1982 study of 28 American postmenopausal women (45), where diet and urinary lignans were assessed on four occasions over a year, showed significantly lower enterolactone levels for women with breast cancer than normal vegetarian and omnivorous women. Later, three retrospective studies, from Shanghai (34), Finland (24), and Australia (21), also reported an inverse relationship between enterolactone levels and breast cancer.

Results from prospective studies, however, have been more contradictory. In a nested case-control study from Sweden with 248 cases and 492 controls from three different sources (23), both very low and very high enterolactone levels were associated with increased breast cancer risk. A weak and nonsignificant increase in breast cancer risk was found with higher urinary enterolactone excretion in a nested case-control study of postmenopausal Dutch women (20). In an Italian study, conducted among 383 women (premenopausal and postmenopausal) with palpable cysts, enterolactone levels (measured at the occurrence of the cyst) were significantly lower among the 18 women who subsequently developed breast cancer (46). Also, in a recently done nested case-control study among Danish women (27), an inverse association between plasma enterolactone levels and breast cancer risk was found; however, this association was restricted almost entirely to estrogen receptor α–negative breast cancer.

In contrast, two recently conducted nested case-control studies, from United Kingdom (22) and New York (26), reported no association between breast cancer and serum enterolactone levels, and a Finnish study (25) reported an overall weak, nonsignificant, increased risk of postmenopausal breast cancer with increasing serum enterolactone levels.

Studies assessing the association between dietary intake of lignans and breast cancer risk have also given conflicting results. No protective effect of lignans was shown in a cohort study with both premenopausal and postmenopausal breast cancer included (17), whereas, although not statistically significant, a protective trend with higher calculated mammalian lignans was found in a Dutch prospective study, conducted among perimenopausal and postmenopausal women (19). In two case-control studies (15, 18), an inverse relationship between lignan consumption and premenopausal breast cancer risk was found.

Our findings of no or a slight positive association between mammographic density and enterolactone levels are consistent with the increasing number of epidemiologic studies that found no inverse association between enterolactone levels and breast cancer risk.

There are several ways that enterolactone could interfere with the effects of endogenous estrogen on breast tissue and, therefore, be expected to influence breast cancer risk.

It has been shown *in vitro* that enterolactone results in estrogen-like response in estrogen-responsive positive MCF-7 breast cancer cell lines (47); it has also been shown that enterolactone and estradiol inhibit the proliferative effects of each other on MCF-7 cells, the combination resulting in lower stimulation than any of them alone (48). *In vitro* enterolactone has been shown to reduce the activity of human estrogen synthetase (aromatase; refs. 50, 51) and, hence, potentially reduce the conversion of androgens to estrogens. Further, in cell cultures, it has been found that enterolactone stimulates the production of sex hormone–binding globulin (51), which could, in turn, reduce uptake of and the biological effect of estrogen on hormone-responsive cells. On the other hand, it has been shown that enterolactone inhibits the binding of estradiol to sex hormone–binding globulin in a dose-dependent manner (52), which could potentially increase levels of biologically active estradiol.

One explanation for the weak positive association of enterolactone and mammographic density found in our study could be a weak, net estrogenic biological effect of enterolactone. This could become visible on mammograms because of the lower endogenous hormonal level among postmenopausal women. If this is true, then one would expect that any potential risk lowering effect of enterolactone would be more pronounced among premenopausal women where hormone levels are higher.

For soy consumption, it has been suggested that a protective effect on breast cancer has been more pronounced for, or limited to, premenopausal women (53, 54).

For lignans, some studies have as well suggested that a potential breast cancer risk lowering effect could differ by menopausal status. An inverse relationship between lignans and breast cancer development has been found for premenopausal women only (15, 25) or more pronounced for premenopausal women (16, 34).

In other studies, however, differences by menopausal status for the association between lignans and risk of developing breast cancer have not been seen (21, 24). In general, the often low number of premenopausal breast cancer cases in the studies is a problem when assessing the potential differences in risk by menopausal status.

Randomized clinical trials of lignan intake, in the form of flaxseeds (containing large amounts of the enterolactone precursor secoisolariciresinol-diglycoside) and serum hormone levels, have yielded conflicting results and have not entirely been suggestive for a difference in risk for breast cancer by menopausal status. Among postmenopausal women, lower serum levels of 17β-estradiol and estrone sulfate, but higher prolactin concentrations, were shown in one study (55), whereas no significant changes in estradiol or estrone sulfate were found in another randomized, placebo-controlled study (56). In a randomized crossover trial among premenopausal women (57), consuming flaxseeds was associated with a longer luteal phase and a higher progesterone/estradiol ratio compared with control cycles. It is not clear, however, if such changes in the menstrual cycle would be associated with a lower risk for developing breast cancer. In another crossover designed study done among premenopausal women (58), consuming flaxseeds, wheat bran, or a combination of these were not associated with changes in serum hormones levels or levels of sex hormone–binding globulin in any of the treatments.

Limitations. In this study, we have focused on the specific biological properties of enterolactone when discussing its potential for influencing mammographic appearance. However, it is not known whether a possible protective effect of enterolactone on breast cancer risk would be related to the effects of enterolactone itself or if it is simply a biomarker of a healthy diet (10). Enterolactone was determined only once, and recent studies on short and long time variation of measurements of enterolactone have suggested that enterolactone in epidemiologic studies should be measured in several samples (59-61); moreover, samples should preferably be fasting (61).

In the study done by Hausner et al. (61) among Danish postmenopausal women, the overall intraindividual variation of samples collected at random times and on different days was estimated to be 64%. Among Finnish premenopausal women, Stumpf et al. (59) found within-week and within-month variation for serum enterolactone measurements to be 67% and 68%, respectively. In our study, having enterolactone measured only once could have resulted in nondifferential misclassification of enterolactone levels among the study subjects, and our reported differences in mammographic density then is likely to be biased against no association.

Antibiotics can interfere with enterolactone production (62). None of the women used antibiotics at the day of examination. However, because we only had information on current medication, women who had taken antibiotics the last months before screening could not be excluded. We think that it is unlikely that this is a major source of error in our study.

Both percentage mammographic density and plasma enterolactone levels were predominantly in the lower range. However, the median enterolactone value of 16.7 nmol/L and the range from 0.3 (lowest detectable concentration) to 176 nmol/L is comparable with values found in a Finnish cross-sectional study (31) and in accordance with results from Swedish, Italian, and some American studies as well (23, 30, 63).

Our correlations between enterolactone and the various food items are probably a substantial simplification of these associations; however, the significant weak positive correlations found between fruits, vegetables, and whole grain products and enterolactone are in accordance with the known contents of lignan precursors in these food groups (14) and with enterolactone determinants found by others (30, 31). Also, the negative associations of enterolactone with BMI and smoking are in accordance with findings from other studies (30, 31).

The distribution of mammographic density in this study from the northern part of Norway was skewed toward lower values compared with what has been found in other studies (4). The relatively high BMI among the women in this study (mean 27.7; SD, 4.5) may partly explain the high number of low-density mammograms.

In conclusion, in our analysis, mammographic density increased slightly across quartiles of enterolactone, but enterolactone accounted for a very small part of the variation in mammographic density. Thus, if higher levels of enterolactone contribute to reducing the risk of developing breast cancer in postmenopausal women, our results suggest that this is not through lowering mammographic density.

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