

Serum Enterolactone and Risk of Breast Cancer: A Case-Control Study in Eastern Finland

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Abstract

Phytoestrogens have been linked to a risk of breast cancer. The main phytoestrogens in the Finnish diet are lignans, and enterolactone is quantitatively the most important circulating lignan. The purpose of this study was to examine the association between serum enterolactone and risk of breast cancer in Finnish women. The subjects were participants of the Kuopio Breast Cancer Study. This analysis concerns 194 breast cancer cases (68 premenopausal and 126 postmenopausal) who entered the study before diagnosis and 208 community-based controls. They completed a validated food frequency questionnaire referring to the previous 12 months and gave serum samples before the examinations. The measurement of serum enterolactone was performed by time-resolved fluoroimmunoassay. The statistical analyses were done by the logistic regression method. The mean serum enterolactone concentration was 20 nmol/l for the cases and 26 nmol/l for the controls (P 0.003). The mean serum enterolactone concentration in the lowest quintile was 3.0 nmol/l and 54.0 nmol/l in the highest. The odds ratio in the highest quintile of enterolactone values adjusted for all of the known risk factors for breast cancer was 0.38 (95% confidence interval, 0.18–0.77; P for trend, 0.03). The inverse association between serum enterolactone and risk of breast cancer was seen both among premenopausal and postmenopausal women. High enterolactone level was associated with higher consumption of rye products and tea and higher intake of dietary fiber and vitamin E compared with those with low serum enterolactone values. Serum enterolactone level was significantly inversely associated with risk of breast cancer.

Introduction

Phytoestrogens are a group of biologically active compounds that have been shown to influence not only hormone metabolism but also intracellular enzymes, protein synthesis, growth factors, malignant cell proliferation, and angiogenesis (1). Originally, the definition of phytoestrogens included all estrogenic compounds in plants including also estrogens produced by fungi living on the plants as well as steroidal estrogens. Later, phytoestrogens were mainly restricted to the isoflavones found in soybeans and some other legumes, to coumestans, which seldom occur in human food, and to resocyclic acid lactones, which should be defined as fungal estrogens and not phytoestrogens (2). The three main isoflavones in soy are genistein, daidzein, and glycitein, occurring as various types of glycosides. The mammalian lignans enterolactone and enterodiol were found much later to have very weak estrogenic activity (3) and to have similar biphenolic structure as the isoflavones (4). These mammalian lignans are formed from precursors in plants by intestinal bacterial action (5, 6). Two main precursors of enterolactone are known, secoisolariciresinol and matairesinol, both occurring as glycosides in the plant. A few flavones, excluding the isoflavones, have also been found to have estrogenic activity (7), as well as some other compounds such as β -sisterol, isocoumarin, anethole, and some compounds in hops (2). The isoflavones are metabolized to numerous compounds (8–10), the most well-known metabolite being the daidzein metabolite equol, because it causes the “clover disease” in sheep grazing clover (11, 12) and is produced only by ~36% of human subjects (13).

The low incidence of breast cancer in Japanese as well as other Asian women has been suggested to be at least partly explained by their high intake of phytoestrogens (14, 15). Soy-consuming Asian populations have typically high intakes of isoflavones, whereas lignans are more important sources of phytoestrogens in the diet of many other populations (16).

In earlier studies, it was shown that postmenopausal breast cancer patients excrete very low amounts of enterolactone in urine, and equol excretion also tended to be lower than in controls (17, 18). A case-control study showing a protective effect of both equol and enterolactone on breast cancer was reported recently from Australia by Ingram *et al.* (19). Another case-control study among Chinese women in Shanghai showed that the overnight urinary excretion of isoflavonoids, particularly daidzein, glycitein, and total isoflavonoids, was strongly inversely related to risk of breast cancer (20). In other epidemiological studies using dietary questionnaires, the intake of soy products has been associated with lower breast cancer risk (21, 22). In one study in Shanghai and Tianjin, China, no protection by soy intake was found, but high intake of fiber decreased breast cancer risk (23).

Because it is difficult to collect 24-h urines in epidemiological studies, methodology to analyze phytoestrogens from very small serum or plasma samples was developed recently

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(24). Using this method, we investigated the association between serum enterolactone level and risk of breast cancer in a case-control study in Eastern Finland.

Materials and Methods

Study Population. The subjects were participants in the Kuopio Breast Cancer Study. During the study period from April 1990 to December 1995, all women living in the catchment area of Kuopio University Hospital (the province of Kuopio) who had a suspected breast lump or breast symptom were referred for further examination and an interview. Each woman was interviewed by our study nurse, and a blood sample was drawn before entering the normal hospital examinations for diagnosis. The participation rate for cases was high; only 3% of the women entering the hospital for the breast examination refused to participate in this study. From all of them, 24% were later diagnosed with breast cancer. The age range of the study was limited to 25–75 years. A comparison of the confirmed breast cancer cases with the Finnish Cancer Registry showed that 96% of the women living in the province of Kuopio were referred to the Kuopio University Hospital, and only 4% were treated elsewhere.

A random sample of population controls was drawn from the National Population Register covering the same geographical area. The population controls were individually matched to the breast cancer cases by age (within ± 5 years) and area of residence (urban/rural). The participation rate of the population controls was 72%. The controls also completed the dietary questionnaires at home before coming to the hospital, where they were interviewed by the study nurse who also took a blood sample. The details of the recruitment of subjects and controls have been published (25, 26).

For the enterolactone analysis, a subset of cases and controls was selected, covering the time period from January 1992 to December 1995. During this time period, 237 women were diagnosed with breast cancer. After exclusions because of missing data, this report is based on 194 cases and 208 controls who all had given a blood sample, completed a dietary questionnaire, and provided anthropometric and other measurements. The study protocol was approved by the Research Ethics Committee of Kuopio University.

Data Collection. A semiquantitative FFQ² was mailed to the women with the invitation letter to Kuopio University Hospital. They completed it at home and returned it to the study nurse, who checked it during an interview. The FFQ assessed the diet over the preceding 12 months and consisted of 110 food items and mixed dishes. The reproducibility and validity of the FFQ were tested among 152 population controls, who kept a 7-day diet record immediately after the hospital visit and repeated both methods (the FFQ and the 7-day diet record) 3 months later. In comparison with other studies, the FFQ was reasonably accurate in measuring food consumption and nutrient intake (27). The FFQs were processed at the National Public Health Institute using the National Food Composition Database Fineli, where the nutrient values are mainly based on analyses of Finnish foods.

During the hospital visit, the study nurse interviewed the women with questionnaires on demographic, reproductive, and lifestyle characteristics. Weight, height, and waist and hip circumferences were measured to calculate body mass index and

waist:hip ratio. A blood sample was also drawn by venipuncture, and sera were divided into aliquots for storing. The women with suspected breast lumps entered the normal diagnostic procedures after the visit with the study nurse. The diagnostic procedures included clinical examination with inspection and palpation, radiological examinations (mammography or ultrasonography), and fine needle, core needle, or surgical biopsy, if necessary. In addition, the cytopathological or histopathological diagnoses were made according to the current practice at Kuopio University Hospital.

Assay of Serum Samples. The samples were stored deep-frozen and melted only once before the analyses. The measurement of serum enterolactone was performed by time-resolved fluoroimmunoassay (24) with slight modifications (28). The modified method is described briefly as follows. Two hundred μl of hydrolysis reagent containing 2 M sulfatase and 0.2 M β -glucuronidase were added to 200 μl of serum sample. After hydrolysis, the free enterolactone and hydrolyzed conjugates were extracted with 1.5 ml of diethyl ether. Diethyl ether was evaporated to dryness in a water bath, after which the dry residue is measured by time-resolved fluoroimmunoassay. All of the samples were analyzed in duplicate. The laboratory analyses were performed blind, and all of the batches were analyzed with two quality control samples going through the whole method and three samples controlling the immunoassay step only. The mean values and mean intra-assay coefficients of variation for the quality control samples, measured in duplicate in each batch, were as follows. Sixteen nmol/l (CV, 4.6%), 43 nmol/l (CV, 6.6%). The interassay CV varied between 6.3 and 10.5%. Isoflavonoids were not determined because of the very low serum concentrations in Finnish women.

Statistical Methods. Although individual matching was originally used, group matching was used in the analysis (29) because of two reasons: the matching was quite permissive (± 5 years, urban/rural), and the number of complete pairs would have been considerably smaller with individual matching. A logistic regression model was used in the analysis, and the quintiles were based on cases and controls combined. The regression models were first adjusted for only age and area and then also for age at menarche, age at first full-term pregnancy, use of oral contraceptives, use of estrogen replacement therapy, first-degree family history of breast cancer, history of benign breast disease, education, current alcohol intake, smoking, leisure time physical activity, waist:hip ratio, and body mass index in the same manner as in the larger study (25, 26). The data were analyzed separately for premenopausal and postmenopausal women. Women who were >50 and used postmenopausal estrogen replacement therapy were classified as postmenopausal; otherwise the self-reported menopausal status was used.

Results

The mean age of the women was 55 years, and the majority of both the cases and the controls were postmenopausal urban dwellers (Table 1). The majority of all of the women were never-smokers, and about half were abstainers of alcohol. The mean serum enterolactone concentration was 20 nmol/l for all cases and 26 nmol/l for the controls (P for the difference, 0.003), 17 nmol/l for premenopausal cases and 21 nmol/l for controls (P for the difference, 0.10), and 21 nmol/l for the postmenopausal cases and 29 nmol/l for the controls (P for the difference, 0.01).

The mean serum enterolactone concentration in the lowest quintile was 3.0 and 54.0 nmol/l in the highest quintile

² The abbreviations used are: FFQ, food frequency questionnaire; CV, coefficient of variation; OR, odds ratio; CI, confidence interval.

Table 1 Descriptive characteristics of study

	Cases (n = 194)	Controls (n = 208)
Menopausal status		
Premenopausal, n	68	75
Postmenopausal, n	126	133
Age, yr (SD)	55 (11)	55 (11)
Urban residency, %	61	61
More education than comprehensive school, %	20	14
Body mass index, kg/m ² (SD)	26.9 (5.0)	26.2 (4.3)
Waist:hip ratio (SD)	0.83 (0.05)	0.83 (0.04)
Age at menarche, <12 yr, %	25	23
Age at first full-term pregnancy, %		
Nulliparous	19	13
<25 years	49	50
>30 years	12	10
First-degree family history, %	9	6
History of benign breast disease, %	45	42
Oral contraceptive use, %	42	49
Hormonal replacement therapy, %	13	16
Abstainers of alcohol, %	51	45
Current smokers, %	14	11
Leisure activity >3 times/week	42	48
Diet		
Energy intake, kcal (SD)	1888 (532)	1821 (551)
Fat intake, % of energy (SD)	33.1 (4.8)	34.0 (5.2)
Intake of dietary fiber, g (SD)	22.8 (8.1)	22.1 (7.9)
Mean serum enterolactone, nmol/l	19.6 (17.0)	25.9 (21.9)
Premenopausal women	16.6 (13.2)	20.7 (16.0)
Postmenopausal women	21.2 (18.6)	28.9 (24.2)

(Table 2). The OR adjusted for age and area was 0.38 (95% CI, 0.20–0.73) in the highest quintile compared with the lowest (*P* for trend, 0.01). The multivariate ORs were about the same after adjustment also for the known risk factors for breast cancer. The association between serum enterolactone and risk of breast cancer was seen both among the premenopausal and postmenopausal women (Table 2). However, the smaller number of cases in both groups widened the confidence intervals, and the risk reductions were not statistically significant.

Because the sources of plant lignans are whole grains, vegetables, and berries, we compared the dietary intakes against enterolactone values in the controls (Table 3). The consumption of rye products and tea increased significantly with increasing serum enterolactone levels. Intakes of total dietary fiber, water-insoluble fiber, and vitamin E also increased significantly with increasing enterolactone.

Discussion

Our study shows that serum enterolactone, the quantitatively most important circulating lignan, is associated with a substantial reduction in breast cancer risk. The results are very similar to those of the study by Ingram *et al.* (19), where the risk reduction between high and low quartile of urinary enterolactone excretion was on the order of >60%. Most of the earlier studies on phytoestrogens and breast cancer risk have been based on soy consumption (21–23, 30), and the results have been conflicting. There are very little epidemiological data on lignans and risk of breast cancer. In a small study of seven breast cancer cases studied during 1 year, significantly lower urinary excretion of enterolactone was found in postmenopausal cases compared with both premenopausal and postmenopausal omnivorous and vegetarian controls (17).

Our study was done in Eastern Finland, where the diet of the women is relatively high in fiber. Most people consume at least some rye bread, and berries grow locally. In addition, alcohol consumption is low, and the majority of women are nonsmokers. This is reflected in the generally high serum enterolactone levels found in our study compared with other population samples. In a population survey among adults in different parts of Finland, the mean serum enterolactone level was 16.6 nmol/l in women (31). The median serum enterolactone value in a subsample of the New York University Women's Health Study was 20.2 nmol/l (32). In the Australian breast cancer study, the average enterolactone excretion (nmol/24 h) was 1970 in cases in 3100 in controls (19). If those results are converted to the corresponding serum concentrations based on the correlation between urine and serum values ($r = 0.875^3$), the mean values would have been 12.0 nmol/l in cases and 15.8 nmol/l in controls.

The extremely wide range of serum enterolactone concentrations, from 3 nmol/l in the lowest quintile to 54 nmol/l in the highest, deserves consideration. In the Australian study by Ingram *et al.* (19), where 72-h urines were collected, the difference between the lowest and highest quartile was only ~4-fold. Three 24-h urines are, of course, a much more reliable measure of a person's enterolactone status, and the main reason for our wide range is that we had only one blood sample. The attenuation caused by intra-individual variability probably explains our slightly more modest results. The reliability coefficient of a single serum enterolactone measurement has, however, been shown to be moderately high, 0.55 for three measurements repeated during 2 years (32). In fact, serum enterolactone measurement had the highest reliability coefficient of several phytoestrogen compounds measured in this methodological study.

One could argue that the cases might have had lower levels of serum enterolactone than the controls because they were worried before coming to the hospital for the examinations and diagnosis and, thus, could not eat normally. Feeding studies having a depletion baseline have shown that serum enterolactone levels do not decrease rapidly (33). It takes at least 3 days and in some subjects up to 8 days on a lignan-free diet to reduce the plasma level to a mean of ~10 nmol/l. It is unlikely that women who are worried about a possible disease change to a lignan-free diet, which would mean excluding bread, fruits, and vegetables. It is in fact more likely that women have tried to eat a healthy diet under the threat of possible breast cancer.

Because the risk of breast cancer started to decrease right after the lowest quintile of serum enterolactone and the diet of these women was not much different from the diet of the women in the higher quintiles, it raises a crucially important question: why are some women incapable of converting plant lignans to enterolactone? In previous studies, it was shown that antibiotics decrease urinary enterolactone excretion and that the pretreatment values were not obtained within 40 days (5). Thus, the reason for the large variation could be administration of antibiotics. These results provide evidence indicating that the condition of the intestinal microflora could have a large effect on enterolactone production and consequently on serum levels independent of the amount of fiber-rich food. A recent report on the increased risk of breast cancer among women with a history of chronic urinary tract infections suggests that chronically impaired function of the intestinal microflora by antibiotics

³ H. Adlercreutz, personal communication.

Table 2 Crude and adjusted ORs of breast cancer in quintiles of serum enterolactone concentration

	Quintiles of serum enterolactone concentration					P for trend
	1	2	3	4	5	
All women, cutoff values, nmol/l	<6.19	6.19–14.08	14.19–24.34	24.37–34.80	>34.80	
No. of cases	49	43	36	37	29	
OR ^a (95% CI)	1.00	0.68 (0.36–1.27)	0.57 (0.30–1.07)	0.57 (0.30–1.07)	0.38 (0.20–0.73)	0.01
OR ^b (95% CI)	1.00	0.60 (0.30–1.17)	0.57 (0.29–1.13)	0.53 (0.27–1.05)	0.38 (0.18–0.77)	0.03
Premenopausal women, cutoff values, nmol/l	<5.48	5.48–11.37	11.66–20.22	20.61–30.03	>30.03	
No. of cases	17	14	13	13	11	
OR ^a (95% CI)	1.00	0.61 (0.21–1.74)	0.65 (0.21–1.93)	0.57 (0.20–1.65)	0.46 (0.15–1.34)	0.26
OR ^b (95% CI)	1.00	0.82 (0.22–3.09)	0.39 (0.09–1.78)	0.52 (0.14–2.00)	0.42 (0.10–1.77)	0.18
Postmenopausal women, cutoff values, nmol/l	<6.30	6.33–14.90	15.07–26.01	26.11–37.65	>37.65	
No. of cases	32	29	23	24	18	
OR ^a (95% CI)	1.00	0.78 (0.36–1.71)	0.50 (0.23–1.10)	0.54 (0.25–1.19)	0.35 (0.16–0.78)	0.01
OR ^b (95% CI)	1.00	1.19 (0.46–3.07)	0.60 (0.24–1.49)	0.80 (0.32–2.02)	0.50 (0.19–1.28)	0.10

^a ORs adjusted for age and area.

^b ORs adjusted further for age at menarche, age at first full-term pregnancy, use of oral contraceptives, use of estrogen replacement therapy, first-degree family history of breast cancer, history of benign breast disease, level of education, current alcohol intake, smoking habits, physical activity, waist:hip ratio, and body mass index.

Table 3 Mean daily intakes of selected foods and nutrients across quintiles of serum enterolactone concentration among controls

	Quintiles of serum enterolactone, means (SD) ^a					P for trend ^b
	1	2	3	4	5	
Rye products, g	59.2 (44.1)	68.9 (41.9)	85.8 (47.9)	79.8 (54.4)	77.3 (43.5)	0.03
Vegetables, g	132.2 (73.2)	130.8 (60.9)	126.0 (62.4)	164.0 (108.1)	115.9 (58.6)	0.74
Fruits, g	110.9 (91.9)	96.3 (88.1)	96.2 (98.4)	124.8 (134.7)	102.1 (96.9)	0.62
Berries, g	30.1 (19.8)	35.6 (27.9)	35.5 (28.1)	36.4 (27.9)	39.7 (28.3)	0.21
Tea, g	54.7 (76.4)	33.6 (57.6)	94.9 (162.3)	105.9 (150.9)	84.1 (164.9)	0.04
Total fat, g	70.4 (19.9)	69.3 (26.3)	69.6 (21.1)	70.5 (25.9)	66.4 (25.3)	0.44
Dietary fiber, g	19.2 (7.9)	20.2 (7.41)	22.1 (6.9)	23.3 (9.5)	21.2 (7.3)	0.008
Water-insoluble fiber, g	7.9 (3.6)	8.4 (3.2)	9.4 (3.3)	9.6 (4.3)	8.9 (3.3)	0.02
Vitamin C, mg	126.4 (52.7)	132.0 (61.5)	118.8 (55.2)	139.0 (64.3)	125.6 (67.3)	0.74
Vitamin E, mg	8.4 (3.1)	8.4 (3.6)	8.9 (2.7)	10.0 (4.17)	8.9 (3.8)	0.008

^a Unadjusted values.

^b Adjusted for age, area, and energy intake.

could be important in the etiology of breast cancer (34). Unfortunately, we did not ask about the possible use of antibiotics in our study. There can be, of course, other reasons for impaired utilization of plant lignans. Feeding studies with large amounts of whole-grain rye bread have shown that although every person's enterolactone excretion increases, there is a 3–4-fold variation in the change (35).

The consumption of rye products, which means mainly rye bread, as well as intake of dietary fiber were significantly associated with serum enterolactone concentration. The differences were not, however, large in our study, because almost everybody consumes some rye bread daily, and based on the results obtained with a FFQ in this whole case-control study, the consumption of either rye bread or the intake of fiber was not associated with breast cancer risk (26). These results also provide evidence indicating that some other factor has a large effect on serum enterolactone level other than fiber or rye intake, and this factor could be the activity of the intestinal microflora, as suggested above, genetic background, medication, or alternative sources of lignans.

Tea is also a good source of lignans (36), and tea consumption was associated with serum enterolactone levels. However, the amounts of tea are small because Finland is typically a coffee-consuming country. The significant association of intake of vitamin E with serum enterolactone is interesting. This

could either be just a chance finding or a surrogate measure of an overall healthy diet. This latter interpretation is supported by a recent study among low- and high-vegetable consumers (37). In that study, urinary lignan excretion correlated with fruit and vegetable consumption as well as dietary fiber intake and was associated with an overall healthy diet. Unfortunately, we could not calculate the dietary intake of plant lignans because a sufficiently wide food composition database covering Finnish foods such as whole grain cereals and breads is not yet available.

In animal models, a diet very rich in lignans decreases both mammary tumorigenesis (38) and tumor growth (39), and there are several potential mechanisms that could explain why lignans may influence breast cancer development (1). Enterolactone may decrease the amount of active, circulating estrogens in many ways. Insoluble grain fiber rich in lignans binds steroid hormones and inhibits hydrolysis of estrogen conjugates, leading to a partial interruption of the enterohepatic circulation of estrogens and an increase in the fecal excretion of the hormone (40, 41). After absorption, enterolactone stimulates the synthesis of sex hormone binding globulin in the liver (42) and reduces estrogen synthesis by inhibiting human estrogen synthetase (aromatase; Ref. 43). It also affects the menstrual cycling by lengthening the luteal phase of the cycle (44). However, the results of the studies on the estrogenic effects of

enterolactone are conflicting. Both estrogenic (45, 46) and antiestrogenic (47, 48) effects of enterolactone on breast cancer cells in culture systems have been reported. What the net result will be in humans is an unsettled question. There is a hypothesis suggesting that enterolactone inhibits intracellular estrogen biosynthesis (49).

In conclusion, serum enterolactone concentration was inversely associated with risk of breast cancer. The range of its concentrations varies tremendously, and very low values are not explained simply by dietary patterns. We suggest that both diet and the intestinal flora and its activity play important roles, but we cannot exclude genetic influence as well as some yet unknown factors influencing lignan production and/or metabolism in the gut.

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BLOOD CANCER DISCOVERY

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