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ORIGINAL CONTRIBUTION

An incident case-referent study on plasma enterolactone and breast cancer risk

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Summary *Objective* Using a nested case-referent design, we evaluated the relationship between plasma levels of the lignan enterolactone and the risk of developing breast cancer. *Methods* 248 cases and 492 referents were selected from three population-based cohorts in northern Sweden. Blood samples were donated at enrolment. All blood samples were stored at –80 °C. Cases and referents were matched for age, date of blood sample and sampling centre. Breast cancer cases were identified through the regional and national cancer registries. *Results* Plasma enterolactone was lower among smokers in all cohorts and in subjects with BMI < 23 and BMI > 28 in one of the cohorts. Low plasma concentrations of enterolactone, below the 12.5th percentile (mean plasma enterolactone 2.9 nmol/l), were associated with an increased risk of breast cancer. Also, high values of plasma enterolactone, above the 87.5th percentile (mean plasma enterolactone 58.2 nmol/l) were significantly associated with an increased breast cancer risk among women from two cohorts with only incident cases and a higher number of pre-menopausal women. High plasma enterolactone concentrations among older women from a mammary screening project with mostly prevalent cases were associated with a non-significant slightly reduced breast cancer risk. *Conclusion* Very low plasma concentrations of enterolactone were associated with an increased breast cancer risk in all three cohorts. In two of the cohorts, with only incident cases, very high plasma concentrations were also associated with an increased breast cancer risk. In the third cohort with mainly screen-detected cases from a mammary screening program, high plasma enterolactone concentrations were associated with a weak decreased breast cancer risk.

Key words plasma – enterolactone – phyto-oestrogens – breast cancer

Introduction

A consensus meeting on dietary intake of cereals and fibre, and risk for cancer concluded that suggestive evidence exists that cereal fibre may protect against breast cancer [1]. Some studies have shown associations between higher cereal fibre intake and decreased breast cancer risk, although other studies have not demonstrated such associations, particularly not with total fibre intake [2]. The nature of the fibre is different in different countries, which may be one of several explanations for the diverging results. Therefore, insufficient evidence exists for firm conclusion with regard to fibre intake and breast cancer risk. Various mechanisms for an increased risk of breast cancer with lower fibre in-

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take have been discussed, e. g., increased concentrations of oestrogens, insulin, and insulin-like growth factor 1 (IGF-1) as well as low concentrations of phyto-oestrogens including lignans [3–6].

The detection [7, 8] and simultaneous identification of the mammalian lignans, later called enterolactone and enterodiol, by two groups [9, 10] initiated research on phyto-oestrogens in man. The urinary lignan excretion was found to correlate positively with fibre intake and plasma sex hormone binding globulin (SHBG) and negatively with plasma levels of free estradiol and testosterone [11, 12]. In case-referent studies the lowest urinary enterolactone excretion was found in breast cancer patients [13, 14, 15] and the highest in vegetarians, particularly macrobiotics [16].

Lignans may influence steroid production, metabolism and biological activity as well as intracellular enzymes, SHBG production, and malignant cell proliferation. These properties make them potential natural cancer protective compounds [5, 17].

Recently, we developed a time-resolved fluoroimmunoassay for the determination of enterolactone in plasma extracts corresponding to 20 µl of plasma [18, 19]. This technique was applied to plasma samples from the Northern Sweden Health and Disease Cohort Study, analyzed within a nested case-referent study on breast cancer risk related to earlier plasma enterolactone values. This report represents the first study with prospective data on exposure measured as enterolactone in plasma and breast cancer risk. Our hypothesis was that low levels of plasma enterolactone are associated with an increased risk of developing breast cancer.

Subjects and methods

Study sample

Data were collected within three ongoing cohort studies in northern Sweden. The Västerbotten Intervention Project (VIP) started in 1985 and comprised 30,000 men and 29,000 women by the end of year 2000. The northern Sweden component of the WHO multinational study for Monitoring of Trends and Cardiovascular Disease study (MONICA) included 2,700 men and 28,000 women recruited in 1986, 1990 and 1994. The Mammary Screening Project (MSP) started in 1995 and by the end of year 2000 39,000 women were involved. Two referents for each case were randomly selected from the corresponding cohort, with a few exceptions. In some rare cases, for the MONICA and MSP projects referents were selected from the VIP project. Referents were matched for age (± 6 months), date of blood sample (± 2 months), and sampling centre. Because of lack of blood samples, 28 subjects were excluded from the study. The final study population with blood samples was thus 740 women (248 cases and 492 referents). The VIP contributed 140 cases and 282 referents, the MONICA 15 cases and 25 referents and the MSP 93 cases and 185 referents. The number of cases with samples collected less than 3 months before diagnosis were 11 in the VIP, none in the MONICA and 61 in the MSP.

Baseline questionnaire information

In the VIP and MONICA cohorts, information on dietary habits, working conditions and social factors were collected at baseline by questionnaires. For the MSP, questionnaire information was obtained only for reproductive factors. In the VIP and MONICA cohorts, weight was measured with light indoor clothing without shoes, and height was measured by a graded scale fixed to the wall. In the MSP height and weight were self-reported. (Where participation in the MSP cohort overlapped with that of the VIP and the MONICA cohorts, the self reported anthropometric information from the MSP have shown fair correspondence, $r = 0.97$ for height and 0.75 for weight).

Blood sampling

In all the three studies 20 ml of blood was collected at baseline from every subject. 10 ml was collected with heparin and 10 ml with EDTA, as anticoagulants. The blood was thereafter aliquoted into 10 tubes; 6 tubes with plasma, 2 with buffy coat and 2 with erythrocytes. The aliquots were stored at –80 °C. For the VIP and MONICA cohorts 95 % (147 cases, 293 referents) of the subjects had fasted at least 4 hours and 57 % (84 cases, 175 referents) had fasted more than 8 hours before donating the blood sample. For the MSP cohort it was not required to come in fasting state and only a very small proportion had actually fasted. The study was approved by the Ethical Committee of UmeÜ University and all study participants have given their informed consent for future use of blood samples for research purposes.

Follow-up

Incident cases of breast cancer from baseline up to year 2000 were identified through linkage with the regional cancer registry covering the northern region of Sweden, complemented by linkage with the national cancer registry covering the whole of Sweden. The Swedish, unique personal identification number was used for linkage. Follow-up for vital status (death), or losses to follow-up due to migration from the country was also carried out for the whole study population through local and national population registries.

Reproductive history data

Data about reproductive history were not systematically collected at baseline. Therefore a questionnaire was sent out retrospectively to all cases with breast cancer within the three cohorts and to the selected referents. An 85 % response rate was obtained (211 cases and 418 referents). The questionnaire data included age at menarche (years), number of full-term pregnancies, age at first full-term pregnancy (years), menopausal status, age at menopause (years), use of oral contraception, use of hormone replacement therapy and family history of breast cancer. The same information was collected at baseline in the Mammary Screening Project.

Determination of enterolactone

The detailed synthesis of 5-O-carboxymethoxyenterolactone and the immunogen as well as the labelling of 5-O-carboxymethoxyenterolactone with europium and the methodology used and its validation have been described [18, 19]. The method is in brief as follows: After addition of (6,7–3H) estradiol-17β-glucuronide as a check of the hydrolysis and extraction steps (conjugated enterolactone standards are not available) the plasma sample (200 µl) (on heparin) is diluted with buffer and the enterolactone conjugates are hydrolysed with β-glucuronidase and sulfatase. The hydrolysed enterolactone is extracted twice with diethyl ether. After evaporation of the ether the dry residue was concentrated to the bottom of the tube by dissolving in 0.5 ml of methanol followed by mixing and evaporation. Assay buffer (200 µl) is then added to the tubes, and after careful mixing 20 µl of the solution corresponding to 20 µl of the original plasma sample is taken in duplicate for time-resolved fluorescence immunoassay (TR-FIA). Another 20 µl of the solution is taken for liquid scintillation counting for determination of recovery.

The fluoroimmunoassay was carried out by adding 20 µl of standard or hydrolyzed extract in buffer to prewashed goat anti-rabbit IgG coated micro-titration wells (Wallac Ltd, Turku, Finland), 100 µl of diluted antiserum (dilution 1:250,000) in 0.5 % BSA Tris-HCl buffer and 100 µl of the tracer (dilution 1:400,000). After incubation and shaking the strips at room temperature for 90 min, the strips were washed using DELFIA plate washer (using the no. 29 T3 programme) (Wallac). Enhancement solution (Wallac) (200 µl) was added to each well and the strips were shaken slowly for an additional 5 min. Fluorescence was measured in a VICTOR 1420 multi-label counter (Wallac). Calculation of the final result was done according to the formula:

$$\text{Final result} = \text{Concentration (read)} \times 1/\text{recovery (\%)} \times \text{dilution factor} \quad (1)$$

Entry and statistical analysis of data

Data collected from the questionnaire were coded and edited with SPSS (Release 7.0, 1995), and descriptive statistics were also obtained with SPSS. Conditional logistic regression analyses were performed using stratified Cox proportional Hazards procedure in SPSS (Release 9.0, 1998) [20]. Odds ratios and 95 % confidence intervals (CI) were calculated to estimate the relative risk for the exposure variable as well as the potential confounding variables. Our odds ratio closely approximates a relative risk because our sample is derived from population-based cohorts. Cut points for enterolactone were determined based on all referents from the three cohorts pooled. To investigate if very low levels were associated with risk, the lowest quartile was halved and thus cut off points were set at 12.5 % and 25 %. Unexpectedly, the highest quartile showed an overrepresentation of cases in comparison to the quartiles close to the median (Fig. 1). Subjects between the quartiles 25 % and 75 % were thus chosen as the reference group. Also the highest quartile was halved and cut-off points were set at 75 % and 87.5 % for further investigations. As this is an unconventional approach of presenting data we have also included calculations of relative risks and 95 % confidence intervals based on quartile categories. Body mass index (BMI), age at menarche, parity, age at first full-term pregnancy, use of hormone replacement therapy, menopausal status, cotinine (a marker of recent exposure to tobacco smoke) and hours of fasting were all evaluated for potential confounding effects in the multivariate logistic regression models. All potential confounding variables were categorised, with one category for the missing data, in order to also enable inclusion of those subjects with missing information on some variables to the model. These subjects may thus have contributed with information on other variables where data were complete. Quartiles were set for variables with continuous data, i. e. BMI, age at menarche, age at first full-term pregnancy and number of full-term pregnancies. Menopausal status was dichotomised as pre- or post-menopausal. Use of oral contraceptive or hormone replacement therapy during or after menopause was classified as "yes" or "no". Hours of fasting were categorised as less than four hours, four to eight hours and more than eight hours. Finally, adjustments were made for BMI, smoking based on plasma cotinine levels and menopausal status. Where information on age at menopause was uncertain, the women were categorized as pre-menopausal when younger than 51 years and post-menopausal at 51 years and older. Women whose age for menopause occurred the same year as the blood samplings were classified as pre-menopausal.

Results

Background characteristics did not vary remarkably between cases and referents with a few exceptions. Parity was slightly higher among referents than among cases in the VIP and MONICA projects (Table 1). Subjects from the MSP were generally older than the VIP and MONICA subjects and therefore contributed with a higher number of post-menopausal women, 85 % in the MSP compared with 46 % in the VIP and MONICA cohorts. Mean enterolactone concentration was higher among cases than referents in the VIP and MONICA cohorts although the lowest quartile and the median were the same in cases and referents. Enterolactone levels were also lower among the MSP subjects than in the VIP and MONICA subjects. Time between blood collection and date of cancer diagnosis was evaluated as a separate variable in the study. This period however did not show any correlation with enterolactone levels within the projects (data not shown). Smokers, all through the cohorts, and subjects from the MSP with extreme BMI in either direction (Figs. 2, 3) had lower plasma enterolactone levels. These characteristics were all more common among the MSP cohort than in the VIP and MONICA cohorts. Fasting status did not influence plasma enterolactone in the VIP and MONICA cohort where these data were available.

Low enterolactone levels, below the 12.5th percentile (mean plasma enterolactone 2.9 nmol/l), were associated with an increased breast cancer risk. The adjusted relative risk and 95 % CI at the lowest percentile were 1.6 (95 % CI 1.0–2.6). Also, high plasma enterolactone levels were associated with an increased breast cancer risk with an adjusted relative risk and 95 % CI of 1.8 (1.4–4.3) (Table 2 and Fig. 4). When stratifying for cohort membership the association of low levels and increased risk

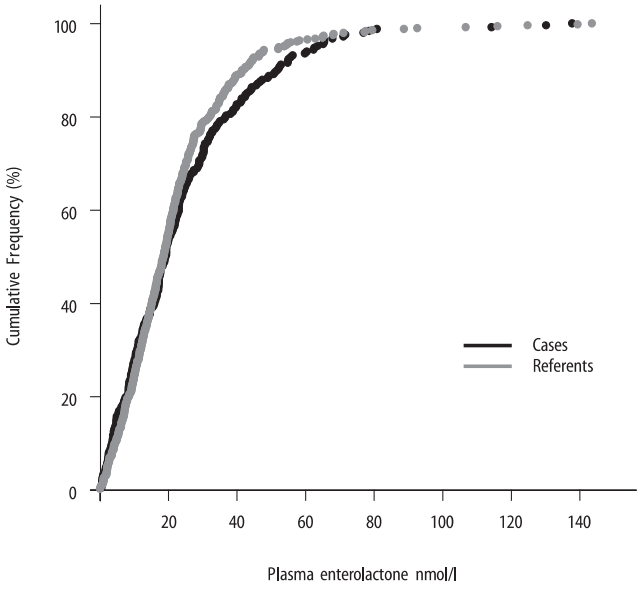

Fig. 1 Distribution of plasma enterolactone among cases and referents by the cumulative frequency.

Table 1 Descriptive characteristics of subjects stratified on cohort membership

Variables	Cases VIP & MONICA (n = 155) Cases MSP (n = 93)		Referents VIP & MONICA (n = 308) Referents MSP (n = 185)	
	Mean	Quartiles	Mean	Quartiles
Age (years)	51.3	49.0, 50.0, 59.0	51.2	49.0, 50.0, 59.0
	58.3	53.0, 59.0, 63.5	58.1	53.0, 58.0, 63.0
Reproductive variables[1]				
Age at menarche (years)	13.3	12.0, 13.0, 14.0	13.4	12.0, 13.0, 14.0
	13.2	12.0, 13.0, 14.0	13.5	13.0, 13.5, 14.0
Parity	2.1	1.0, 2.0, 3.0	2.6	2.0, 2.0, 3.0
	2.1	2.0, 2.0, 3.0	2.3	2.0, 2.0, 3.0
Age at first full-term birth	24.8	21.0, 24.0, 27.0	24.3	21.0, 24.0, 27.0
	24.1	21.0, 23.0, 26.0	24.3	21.0, 24.0, 27.0
Age at menopause[2]	49.5	48.0, 50.0, 52.0	49.2	47.0, 50.0, 52.0
	49.4	47.0, 50.0, 52.0	50.1	48.0, 50.0, 53.0
Anthropometry[3]				
Weight (kg)	67.5	61.0, 66.0, 73.0	68.5	61.0, 67.0, 75.0
	68.7	60.0, 67.0, 74.5	68.9	61.0, 67.0, 74.0
Height (m)	1.63	1.60, 1.64, 1.68	1.64	1.59, 1.64, 1.68
	1.65	1.61, 1.65, 1.69	1.64	1.60, 1.63, 1.68
BMI (kg/m^2)	25.2	22.3, 24.5, 27.3	25.6	22.9, 24.8, 27.7
	25.3	22.3, 24.6, 27.7	25.7	23.1, 25.0, 27.2
Blood measurement				
Enterolactone nmol/l	26.8	9.7, 19.9, 38.8	22.9	10.8, 19.6, 29.3
	19.3	8.6, 17.2, 24.7	20.4	9.1, 16.9 26.0

[1] Cases N = 142, Referents N = 281 (those covered by questionnaire on reproductive history)
[2] For sub sample of post-menopausal women, Cases N = 112, Referents N = 215
[3] Cases N = 155, Referents N = 298

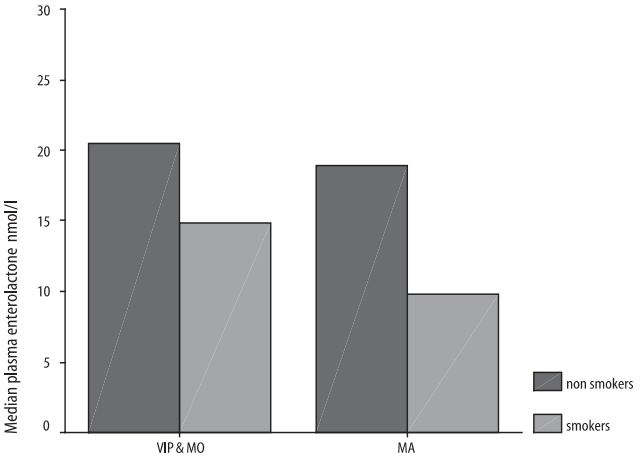

Fig. 2 Median plasma enterolactone levels among referents stratified by smoking and cohort membership.

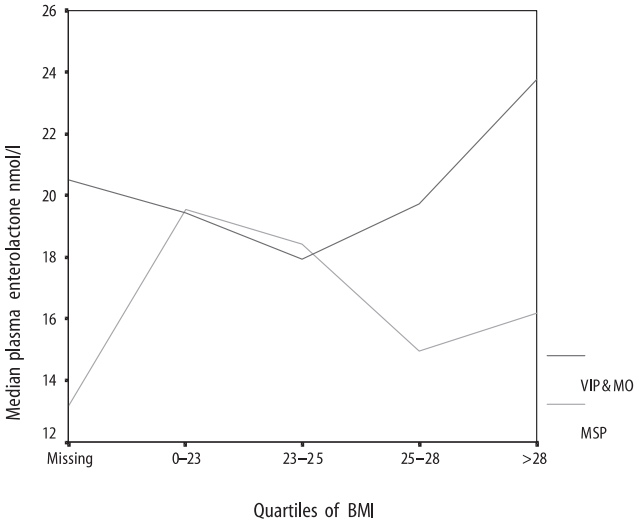

Fig. 3 Median plasma enterolactone levels in BMI quartiles among referents and stratified by cohort.

remained but was not significant. However, in the extremely high values (mean plasma enterolactone 58.2 nmol/l) there was a significant increased risk among the VIP and MONICA subjects 2.4 (95 % CI 1.4–4.3). In the MSP group there was a weak association of a decreased risk in the highest percentile 0.9 (95 % CI 0.4–2.3).

Relative risks and 95 % confidence intervals based on quartiles including women from all cohorts are presented in Table 3.

Discussion

Low levels of plasma enterolactone were associated with an increased breast cancer risk in this prospective study on women in northern Sweden. This finding was significant when all subjects were included in the analysis and a similar pattern was seen also in the cohorts separately. Surprisingly, we also found that high enterolactone levels were associated with a significantly increased breast cancer risk. The increase with high levels was only evident among women in the VIP and MONICA projects. In the MSP group, high levels were associated with a small non-significant decreased breast cancer risk. Our results should however be regarded with caution due to the relatively small sample size, especially after stratification by cohort membership. Ideally, they should be followed by larger prospective studies to further explore the role of enterolactone in relation to the development of breast cancer.

Table 2 Estimated crude and adjusted[1] relative risks of breast cancer at different levels of plasma enterolactone (VIP, MONICA and MSP)

Percentile cut-off points and plasma enterolactone (nmol/l)	Percentile mean and median plasma enterolactone (nmol/l)	Cases	Referents	RR	95 % CI
0–12.5	2.9[3]	41	61	1.6	1.0–2.6
(0.0–5.5)[2]	2.7[4]			1.6[1]	1.0–2.7
12.5–25	8.1	28	62	1.1	0.6–1.8
(5.5–10.2)	8.1			1.1	0.6–1.8
25–75*	18.4	101	247	1.0	
(10.2–27.4)	18.6			1.0	
75–87.5	32.8	32	60	1.3	0.8–2.1
(27.4–39.1)	32.6			1.2	0.7–2.0
87.5–100	58.2	46	62	1.8	1.1–2.8
(39.1–143.5)	51.4			1.8	1.4–4.3

[1] Adjusted for BMI, smoking and menopausal status
[2] Plasma enterolactone level at percentile cut-off point
[3] Mean plasma enterolactone level at percentile cut-off point
[4] Meadian plasma enterolactone level at percentile cut-off point
* Reference category

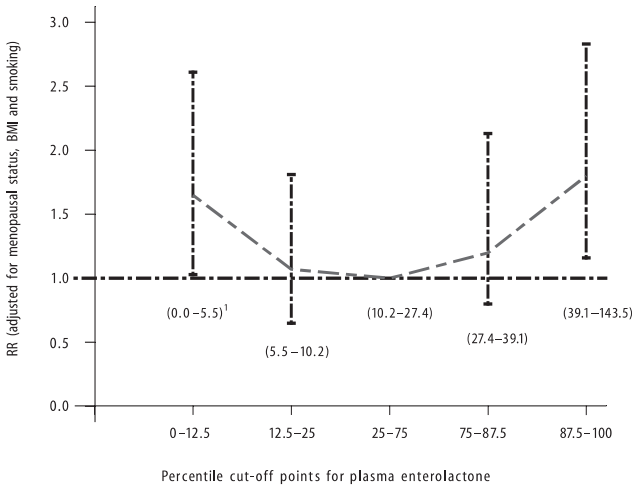

Fig. 4 Estimated adjusted relative risk of breast cancer and 95 % CI at different levels of plasma enterolactone. 1Plasma enterolactone level at percentile cut-off point.

In accordance with findings by Kilkkinen and colleagues, the plasma enterolactone levels were lower among smokers, underweight and overweight women [21]. As smoking and obesity were over represented in the Mammary Screening Project, this may explain the lower plasma enterolactone level in this cohort.

Subjects with breast cancer or those at high risk of breast cancer excrete low amounts of lignans in urine [13–15], whereas subjects living in areas with a traditional low risk of hormone-dependent cancers excrete high levels of phytoestrogens [5]. In Finland, higher urinary lignan excretion has been observed in women from rural north Karelia, in samples collected about 30 years ago, compared with women from urban Helsinki, collected about 20 years ago. This also correlated with breast cancer risk in these areas [16]. Today well-educated women in the Helsinki area have the highest enterolactone values. Its potential influence on future cancer risk can not be predicted at this point.

Our results are partly in agreement with those obtained by Pietinen and colleagues in the only other existing study to date including measurements of plasma enterolactone in relation to breast cancer [22]. In their case control study, which was part of the Finnish Kuopio Breast Cancer Study, low levels of plasma enterolactone were associated with an increased risk of breast cancer in both pre- and post-menopausal women. One prospective case control study of post-menopausal Dutch women reported a non-significant weak negative association between breast cancer risk and spot urine genistein/creatinine ratio, but reported a non-significant positive association with breast cancer risk of spot urine enterolactone/creatinine ratio [23].

One large American prospective cohort study did not show any protective effect of fibre against breast cancer risk [24]; however, in this study only the total amount of dietary fibre was estimated. Because of a lower intake of whole-grain cereals in the United States, total fibre-intake may be less related to the lignan content than is the case in northern Europe. In Scandinavia, whole grain cereals, especially rye bread, constitute the main source of fibre.

Some of the results of the present study are in agreement with the hypothesis that fibre-rich foods like whole-grain cereals, berries and some vegetables (all with a comparatively high content of lignans) may offer protection against breast cancer, at least in post-menopausal women. Plasma enterolactone concentration is determined by the amount of plant lignan in fibre-rich diet, combined with the intestinal capacity for bio-transformation of these plant lignans to mammalian lignans. The capacity of the intestinal microflora for the bio-transformation of lignans may be the most important determinant of the plasma enterolactone concentration [21, 25]. Urinary enterolactone excretion correlates significantly with fibre intake [11, 12], particularly if the fibre intake is adjusted for body weight. In northern Scandinavia the levels of enterolactone in the body are influenced by the intake of fibre in

Table 3 Estimated crude and adjusted[1] relative risks of breast cancer at quartile cut-points of plasma enterolactone (VIP, MONICA and MSP)

Quartiles of plasma enterolactone nmol/l	Quartile mean and median plasma enterolactone (nmol/l)	Cases	Referents	RR	95 % CI
0–10.2	5.3[2] 5.3[3]	69	122	1.0 1.0	
10.2–18.6	14.4 14.4	51	123	0.7 0.7	0.5–1.1 0.5–1.1
18.6–27.4	22.5 22.3	50	124	0.7 0.7	0.5–1.1 0.5–1.1
27.4–100	46.5 39.8	78	121	1.1 1.1	0.7–1.7 0.7–1.7

[1] Adjusted for BMI, smoking and menopausal status
[2] Quartile mean plasma enterolactone
[3] Quartile median plasma enterolactone

whole-grain products, berries, and some vegetables like carrots and other root vegetables [12, 26]. In an intervention study carried out in 1983 in north Karelia, Finland, a change in the diet from the traditional high-fat high-cereal diet (similar to the traditional diet in northern Sweden) to a low-fat, high-cereal, high vegetable and berry diet increased the concentration of enterolactone in plasma by almost 50 % (from 15.8 to 23.3 nmol/l) [27]. When the subjects switched back to the traditional diet, the plasma enterolactone levels decreased to almost baseline. The bio-transformation to mammalian lignans is thus dependent upon other components of the diet, such as amounts of fat and complex carbohydrates [28]. The composition of the gut flora also seems important and antibiotics seriously affect the bio-transformation of plant lignans to enterolactone [29]. In fact an association has been observed between consumption of antibiotics and increased breast cancer risk [30].

Repeated measurements of plasma enterolactone one year apart showed an intra-class correlation of 0.6 in the New York Women Health Study suggesting that enterolactone is reasonably stable within individuals [31].

While low enterolactone is a marker of low dietary intake of certain fibre-rich foods [11, 12] and/or a disturbed intestinal microbial function, the results are not clearly causal. Latency time between blood sampling and diagnosis was evaluated although it is difficult to conclude that this fact did not influence the results as most MSP patients were indeed prevalent and mostly all VIP and MONICA patients were truly incident. Thus our results may be influenced by latency time between blood sampling and diagnosis but they may also be related to other factors such as hormonal status, e. g. sex hormones [3] and insulin [6], including insulin-like growth factor (IGF-1). In a recent prospective study, increased concentrations of IGF-1 has in fact been associated with an increased risk of both breast and prostate cancer [32, 33]. The unexpected tendency of an increased risk of breast cancer associated with a high concentration of enterolactone may have occurred by chance but other factors may be considered. High concentration of enterolactone in plasma is usually associated with an increased intake of lignan-rich foods related to an increased bio-transformation in the intestine. Plasma enterolactone levels above 100 nmol/l must be considered abnormal and may be due to an excessive intake of flaxseeds. Alternatively, the increased concentration of enterolactone is a marker of a disturbed hormonal metabolism. This may also be associated with an increased cancer risk. However, the physiological mechanisms of the mammalian lignans are yet complex and they may act differently depending on the hormonal status.

However, plasma enterolactone levels above about 80 nmol/l can hardly be obtained by consuming whole grain bread, fruits and vegetables because the amounts needed are very high. Some extreme vegetarians like macrobiotics may have values up to 1000 nmol/l but these subjects consume a lot of seeds like flax seed containing very high amounts of enterolactone precursors. Such high values, therefore, do not correlate well to the "healthy diet" which may reduce breast cancer risk. However, some animal experiments have shown that flax seed or purified secoisolariciresinol glucoside may protect against breast cancer [34.]

The high enterolactone levels in some subjects may also be due to regular intake of alcohol because 0.5–1 drink per day increases enterolactone levels in plasma by 131 % [35]. Alcohol intake is also a risk factor of breast cancer and alcohol increases estrogen levels in plasma which could explain the higher breast cancer risk in women with regular alcohol consumption [36]. The increase in enterolactone level due to alcohol consumption may have the same unknown mechanism as the increased estrogen level. On the other hand wine also contains a large amount of lignans [37, 38], but one drink per day would probably not be sufficient to increase the levels by 131 %, only in case of initially low levels. The connection between high risk and high enterolactone plasma levels was not observed in the Finnish study and there was no association between alcohol intake and serum enterolactone. The intake of alcohol was relatively low in the Finnish women because 51 % of the cases and 45 % of the controls were abstainers [39]. We suggest that the discrepancy between the results of the present study and the Finnish study with regard to the increased risk of breast cancer in the subjects with high plasma enterolactone could be due to higher intake of alcohol in Sweden, particularly in the cases. The mechanism by which alcohol increases plasma enterolactone (and estrogen) levels needs further study.

Very little is known about the mechanism of the possible protective effects of lignans with regard to breast cancer. We have suggested that enterolactone may affect the intracellular metabolism of estrogens in the breast cells by interfering with sulfatases and perhaps other steroid metabolizing enzymes [40]. We have some preliminary evidence that enterolactone monosulfate reduces the proliferative effect of estrone sulfate, or estrone sulfate + IGF-1 on MCF-7 breast cancer cell proliferation in culture.

In conclusion, in this study with prospective data of exposure and disease on plasma levels of the phytoestrogen enterolactone and breast cancer risk, a low plasma concentration of enterolactone was associated with an increased risk in all women as well as after stratification on cohort membership. Surprisingly, high plasma concentrations of enterolactone were also associated with an increased breast cancer risk among women from the population-based VIP and MONICA cohorts with only incident cases. In a mammary screening cohort with mainly post-menopausal women and

screen-detected cases, a weak but non-significant decreased risk was found among subjects in the highest percentile.

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