Enterolactone Is Differently Associated with Estrogen Receptor β–Negative and –Positive Breast Cancer in a Swedish Nested Case-Control Study

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Abstract

Background: **Differences in the estrogen receptor (ER) status of tumors may explain ambiguities in epidemiologic studies between the blood concentrations of enterolactone and breast cancer. To our knowledge, the association between enterolactone and ERβ-defined breast cancer has previously not been examined.**
Methods: **A nested case-control study within the Malmö Diet and Cancer cohort used 366 cases and 733 matched controls to identify the major determinants of plasma enterolactone and to examine the association between enterolactone concentration and breast cancer risk and if this association differs depending on the ERα and ERβ status of tumors. A modified diet history method assessed dietary habits. Time-resolved fluoroimmunoassay determined enterolactone concentrations and immunohistochemistry using tissue microarray determined ER status.**
Results: **Dietary fiber, as well as fruits and berries, and high-fiber bread showed statistically significant correlations with enterolactone (*r*, 0.13-0.22). Smoking and obesity were associated with lower enterolactone concentrations. Enterolactone concentrations above the median (16 nmol/L) were associated with reduced breast cancer risk when compared with those below [odds ratio, 0.75; 95% confidence interval (95% CI), 0.58-0.98]. The reduced risk was only observed for ERα [positive (+); odds ratio, 0.73; 95% CI, 0.55-0.97] and ERβ [negative (−)] tumors (odds ratio, 0.60; 95% CI, 0.42-0.84), with significantly different risks for ERβ (−) and ERβ (+) tumors (*P* for heterogeneity = 0.04).**
Conclusions: **This study supports the suggestion that enterolactone is a biomarker of a healthy lifestyle. The protective association between enterolactone and breast cancer was significantly different between ERβ (−) and ERβ (+) tumors and most evident in tumors that express ERα but not ERβ.** (Cancer Epidemiol Biomarkers Prev 2008;17(11):3241–51)

Introduction

We have previously observed a breast cancer–protective association with high-fiber, low-fat diets (1) in the Malmö Diet and Cancer (MDC) cohort. Fiber-rich foods like whole grains, seeds, legumes, berries, and vegetables contain lignans (2, 3), which are converted to enterolignans, mainly enterolactone, by the mammalian intestinal microflora (4). Enterolignans are estrogen-like compounds that have been hypothesized to protect against breast cancer (5). A high circulating concentration of estrogens is an established risk factor for breast cancer, and enterolignans may decrease the estrogen exposure through influences on the sex hormone–binding globulin concentration (6) and aromatase activity (7). They may also modulate the activity of estrogen receptors (ER; refs. 8-10). However, other nonhormonal mechanisms for the breast cancer–protective effects of enterolactone have been suggested, including antioxidative effects (11). Case-control studies have shown decreased breast cancer risks at high circulating enterolignan concentrations (12, 13), but cohort studies have, however, been less clear (14-20), which may be related to too low concentrations in several of these populations. The conflicting results seen in prospective epidemiologic studies may also depend on the dietary sources of enterolactone in the population. Apart from its own biological effect, enterolactone has been suggested to be a marker of a high-fiber diet (21), and it seems to be the dietary fiber, including the associated lignans that protects against diseases (5). Coffee, tea, fruit juice, and wine increase plasma enterolactone without adding any fiber to the diet (22), and a protective effect of enterolactone may not be observed in populations wherein these food groups are major contributors to enterolactone concentrations. In addition to lignan-containing foods, lifestyle factors, including smoking and obesity, may influence the enterolactone concentrations (23-25), and recent antibiotic use has been shown to decrease enterolactone concentrations (26). The MDC study has a great opportunity to estimate the major determinants of the

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enterolactone concentrations with its extensive database and dietary data of high relative validity (27, 28).

Conflicting results among studies may also be explained by differences in tumor biology. It has been suggested that the hormone receptor status may indicate etiologically distinct breast cancer types (29), and studies have shown that the association between breast cancer and reproductive factors, obesity, and alcohol consumption vary by hormone receptor status (30-32). Two subtypes of ERs have been identified: ERα and ERβ. ERα expression in breast tumors is used to guide treatment and provide prognostic information. ERβ was recently identified (33) and is still not used in the clinical setting, although a treatment predictive value of ERβ has been reported (34, 35). Although the biological function of ERβ is not yet fully understood, it is suggested that ERα and ERβ differ in their interaction with other proteins (36, 37) and that ERβ may have a negative modulatory effect on ERα (36). Although it is not clear whether enterolactone have preference for ERα or ERβ, a recent study showed that enterolactone had higher affinity toward ERα than ERβ (10). A Danish study has examined the association between plasma enterolactone and breast cancer defined by ERα status (14). We are, however, not aware of any study that has examined the association between enterolactone and ERβ-defined breast cancers. The aims of this project were therefore to identify the major determinants of enterolactone in this population and to examine if enterolactone concentrations are associated with the risk for breast cancer and if the association with enterolactone differs depending on the ERα and ERβ status of the tumors.

Materials and Methods

Study Population. The MDC study is a population-based prospective cohort study in the third largest city of Sweden. During the baseline examinations from March 1991 to October 1996, all men born between 1923 and 1945 and all women born between 1923 and 1950 and living in Malmö were invited to participate in the study. Limited Swedish language skills and mental incapacity were the only exclusion criteria. Participants came to the study center on two occasions. At the first visit, trained project staff provided groups of participants with information on the background and aims of the project, gave detailed instructions about the dietary data collection procedure, and distributed the dietary questionnaire and menu book and the extensive lifestyle and socioeconomic questionnaire. Nurses conducted direct anthropometric measurements and collected blood samples. At the second visit, individual interviews were conducted by trained dietary interviewers to complete the diet history and to check the correctness of completed questionnaires. With a participation rate of ~40%, 28,098 individuals (11,063 men and 17,035 women) with complete data represent the cohort. A more detailed description of the cohort has been given elsewhere (38, 39). Ethical permission for the study was obtained.

Ascertainment of Cases and Selection of Controls. The Swedish Cancer Registry and the Southern Swedish Regional Cancer Registry provided information on breast cancer cases. Participants with prevalent cancers were excluded, except cervix cancer *in situ*. A total of 544 invasive breast cancer cases occurred until the end of follow-up, December 31, 2004. The breast cancer diagnoses occurred 0 to 13 years (mean, 6 years) after baseline examinations. We included only cases with data on ERβ status in this project (n = 370). For the remaining cases, adequate tumor samples were not available because of either surgery done at other hospitals or insufficient amount of tumor material left for histopathologic evaluation. Age and anthropometric measures did not differ between cases with determined ER status and cases with unknown ER status. The cases were 45 to 73 years at baseline and the mean age at diagnosis was 63 years (range, 46-81 years). The National Tax Board provided information on vital status. For each case, two controls were selected matched by age (±3 months) and date of blood collection (±1 month) from the cohort members at risk at the time of diagnosis of the case. Blood samples were not available for 11 subjects, leaving 366 cases and 733 controls for the analyses.

ER Status Assessment. ER status assessments were done at the Center for Molecular Pathology in Malmö. For the construction of tissue microarrays, two 0.6-mm tissue cores were collected from each tumor block and arranged in a recipient block using a manual tissue arrayer (Beecher, Inc.). Slides were then processed in an automatic immunohistochemistry staining machine. The antibodies used were ERα (prediluted anti-ER 6F11, Ventana) and ERβ (1:25 EMR02; ref. 40). Tumors were grouped into categories according to the expression of ERα and ERβ [0-1%, 2-10%, 11-50%, and 51-100% positive (+) nuclei]. One person evaluated the hormone receptor status of all breast tumors in a standardized way, thereby eliminating interobserver variation. All arrays were evaluated independently twice, and in the case of discrepancy, a third examination was done followed by a final decision, thereby reducing the potential intra-observer bias. We classified the tumors as (+) and (−) using the clinically established cutoff value of 10% (+) nuclei.

Laboratory Analyses of Enterolactone. The nonfasting blood samples were processed and separated for plasma within 1 h of drawing as described previously (41). The samples were stored at -80°C until analysis. The quality control program of the biobank in the MDC study has been described previously (42). Enterolactone concentrations (nmol/L) were determined at Folkhälsan Research Center in Helsinki by time-resolved fluoro-immunoassay as previously reported (43, 44). In brief, plasma samples (200 μL) were hydrolyzed overnight with sulphatase and β-glucuronidase (pure enzyme), and unconjugated enterolactone was extracted with diethyl ether. Sample extracts were diluted in assay buffer and analyzed by AutoDELFIA 1235 Automatic Immunoassay System. ^{3}H-estradiol glucuronide was used as an internal standard in plasma controls. The mean values for recovery results were used for all samples for the correction of losses during hydrolysis and extraction. All analyses with the automatic instrument were carried out in duplicate. Three different control samples were used for each 96-well plate analyzed. The intra-assay coefficient of variation (CV) varied from 4.1% to 10.2%

(concentration, 12.0 nmol/L), from 2.3% to 8.5% (concentration, 31.6 nmol/L), and from 3.8% to 7.3% (concentration, 41.5 nmol/L). The interassay CV was 12.1% (concentration, 15.5 nmol/L), 10.0% (concentration, 37.7 nmol/L), and 11.5% (concentration, 60.7 nmol/L). The assay was repeated if the duplicates differed from their mean value by >15%. The laboratory analyses of plasma samples were approved by the Regional Ethical Review.

Reproducibility Study. During a 5-week period in 2005, 3 nonfasting and 2 overnight fasting samples were collected from 21 women born between 1940 and 1950 within the MDC cohort. Reliability was estimated by the intraclass correlation coefficient, which is an estimate of the proportion of the variation in the exposure explained by the variation between persons relative to the total variation. A reliable biomarker, useful for epidemiologic studies, is characterized by high intraclass correlation coefficient. Intraclass correlation coefficient was estimated to be 0.48 [95% confidence interval (95% CI), 0.22-0.72] for nonfasting samples in this reproducibility study. Details about this study have been published elsewhere (45).

Diet Assessment Methodology. The dietary assessment methodology of high relative validity has been described in detail elsewhere (27, 46). It combined (*a*) a 7-day menu book that collected information on lunch and dinner meals, cold beverages, medications, and dietary supplements, (*b*) a 168-items dietary questionnaire covering regularly consumed foods during the past year, including frequencies and portion sizes assessed using photographic aids, and (*c*) a 1-h interview. In the interview, participants were asked questions about portion sizes, food choices, and food preparation practices in the menu book, and the interviewer checked the menu book and dietary questionnaire for overlapping information. We calculated the average daily intake of foods from the information in the menu book (and interview) and in the questionnaire. Food intakes were converted to nutrient intake data using the MDC Food and Nutrient Database, specifically developed for the MDC study and originating from PC KOST2-93 of the Swedish National Food Administration. The relative validity of the dietary method has been examined among 105 women and 101 men, 50 to 69 years old, with 18 days of weighed food records (3 days every 2nd month) collected during 1 year as the reference. Energy-adjusted Pearson correlations in women were as follows: fat, 0.69; protein, 0.53; carbohydrates, 0.70; alcohol, 0.78; fiber, 0.69; fruit, 0.77; vegetables, 0.53; potatoes, 0.51; cereals, 0.73; bread, 0.58; rice and pasta, 0.24; wine, 0.63; beer, 0.74; and spirits, 0.67 (27, 28).

Food Variables. The nutrient variables examined in this study were energy (kilocalories), fiber (grams per day), alcohol (grams per day), and percentage of nonalcohol energy contributed by protein, fat, and carbohydrates. The food groups (grams per day) examined were vegetables, fruits and berries, fruit juices, boiled potato, fried and deep-fried potato, cereals (grains, cereals, and flours), low-fiber bread (<6% of fiber for soft bread, <10% for crisp bread, <10% for biscuits and rusks), high-fiber bread (≥6% of fiber for soft bread, ≥10% for crisp bread, ≥10% for biscuits and rusks), rice and pasta, nuts, fermented milk, wine, beer, spirits, tea, and coffee. The selection of these variables was based on their known content of lignans (3) or their potential influence on the microflora (47).

Other Variables. Trained project staff measured weight (kilograms) using a balance-beam scale and height (centimeters) with a fixed stadiometer calibrated in centimeters, with subjects in light indoor clothing and without shoes. Body mass index (BMI, kilograms per square meter) was defined as weight (kilograms) divided by height in square meters. A three-category variable was created with subjects categorized as normal weight (<25), overweight (25-29]), or obese (≥30) according to the WHO classification (48). Bioelectric impedance analysis was used to estimate body composition (BIA 103, JRL Systems; single-frequency analyzer). The algorithm used to estimate body fat from impedance was supplied from the manufacturer. Body fat percentage was calculated from estimated body fat mass. Other lifestyle and socioeconomic variables were obtained through the standardized questionnaire. Educational status was categorized based on the type of education attained: elementary, primary and secondary, upper secondary, further education without a degree, and university degree. Smoking habits were categorized into current smokers (including irregular smoking), ex-smokers, and nonsmokers. Leisure-time physical activity was obtained from questions on different physical activities across the seasons, wherein minutes per week of each activity were multiplied with an intensity factor, creating a leisure-time physical activity score. The score was separated into quartiles. Household activities were divided into four categories: 0 to 9, 10 to 19, 20 to 29, and >29 hours per week. Alcohol consumption was divided into four categories. Individuals with no consumption of alcohol in the menu book and who indicated no consumption of alcohol during the previous year in the questionnaire were categorized as zero consumers. The other subjects were categorized into three groups according to their alcohol consumption: <15 g alcohol per day (low), 15 to 30 g (medium), and >30 g (high). Parity was aggregated into five categories: no children, one child, two children, three children, and four or more children. Age at birth of first child was constructed from the participant's year of birth and the year of birth of first child, and was divided into four categories: no children, <24, 24 to 30, and >30 years. Age at menopause was divided into five categories: <45, 45 to 49, 50 to 55, >55 years, and those who reported no cessation of menses at baseline. Information on the current use of menopausal hormone therapy (yes or no) and the current use of antibiotics (yes or no) were obtained from (*a*) the open-ended question ''Which medicines do you use on a regular basis?'' in the questionnaire and (*b*) the open-ended item for listing drug use in the 7-day menu book that was recorded during the consecutive days after blood collection (49). Dietary change in the past (yes or no) was derived from the questionnaire item ''Have you substantially changed your eating habits because of illness or some other reasons?'' A variable was created for the seasons of data collection: winter (December-February), spring (March-May), summer (June-August), and fall (September-November). In September 1994, the coding of dietary data was slightly altered (50).

Therefore, the analysis of dietary data was adjusted for method version to control for undue influences of dietary data processing.

Statistical Methods. SPSS (version 14; SPSS, Inc.) and Stata (release 10; StataCorp LP) were used for the analyses. All statistical tests were done on $\log_e$-transformed enterolactone concentrations to normalize the distribution. The differences in enterolactone concentration and participant characteristics between cases and controls were tested using Mann-Whitney *U* test (enterolactone concentration), Student's *t* test (other continuous variables), and χ^2 test (categorical variables). Mean enterolactone concentration according to age, season, lifestyle factors (smoking status, educational status, alcohol consumption, BMI categories, leisure-time physical activity, and household activities), current use of menopausal hormone therapy, and antibiotics were estimated among the controls controlling for age and date of blood collection. Differences in means were tested using the general linear model, and multiple comparisons were examined using Tukey's test. Partial correlations between enterolactone and dietary variables ($\log_e$ transformed) and body composition measures were computed among the controls (n = 733), controlling for age, date of blood collection, diet method version, and total energy. We also formulated a minimally adjusted model, with all food variables and alcohol variables included simultaneously and adjusting for age, date of blood collection, method version, and total energy. Thereafter, in an exploratory analysis, all food group variables, alcohol variables (wine, beer, and spirits), tea, coffee, body fat percentage, smoking status, educational status, and leisure-time physical activity were included in a linear regression model followed by stepwise backward elimination of variables with $P > 0.10$. The model was adjusted for age, date of blood collection, method version, and total energy. The individuals were divided into quartiles based on the distributions of enterolactone among controls. Conditional logistic regression estimated odds ratios and 95% CI for enterolactone with the lowest quartile as the reference. The lowest quartile of enterolactone may include both women with long-term low plasma enterolactone concentrations as well as those with low concentrations due to the use of antibiotics. Because of the expected heterogeneity of the lowest quartile, additional analyses using the 2nd quartile as reference group were done. We evaluated the associations with and without adjustment for potential confounders (that is, weight, height, educational status, smoking habits, leisure-time physical activity, household activities, alcohol habits, age at menopause, parity, age at birth of first child, and current use of menopausal hormone therapy). These covariates were identified through the literature (that is, established risk factors) and from previous MDC projects and were graphically evaluated for being potential confounders (51). Missing values among these variables were recoded as separate categories to avoid the exclusion of individuals. We conducted a series of sensitivity analyses. The analyses were repeated with exclusion of individuals <50 years at baseline (in an attempt to exclude premenopausal women) and individuals diagnosed with breast cancer within 1 year after blood collection (to ensure that enterolactone concentrations was not influenced by preclinical cancer). We also repeated the analyses excluding individuals who reported dietary change in the past (to include only individuals more likely to have stable food habits and consequently more stable long-term enterolactone concentrations). Using the intraclass correlation coefficient of 0.48 from the reproducibility study (45), the risk estimates were corrected for within-person variation during 1 month in plasma enterolactone concentrations by the following equation $(\text{odds ratio})_{\text{observed}} = (\text{odds ratio})_{\text{true}}^{\text{intraclass correlation coefficient}}$ (52). Unconditional multinomial logistic regression estimated odds ratio and 95% CI of enterolactone separately for each type of ER-defined breast cancer [ERα (−), ERα (+), ERβ (−), ERβ (+), ERα (+)/ERβ (−), ERα (+)/ERβ (+)] adjusted for age, date of blood collection, weight, height, education, and current use of menopausal hormone therapy. Breast cancers that were ERα (−)/ERβ (−) (n = 31) and ERα (−)/ERβ (+) (n = 21) were not analyzed separately because of the small number of cases. A multivariate joint effect model simultaneously estimating the genuine impact of ERα and ERβ status was done. This model was checked for over- and underdispersion. Because of few cases, we divided the individuals into two categories (low or high) based on the median enterolactone concentration among controls. We used Wald's test to examine heterogeneity between different tumor subgroups about their association with enterolactone.

Results

Median concentration of enterolactone in plasma was 14.5 μmol/L among the cases and 16.1 μmol/L among the controls (Table 1). A higher frequency of nulliparous women and menopausal hormone therapy users and a lower frequency of individuals with 30 h/wk or more of household work were observed among cases compared with controls.

Determinants of Enterolactone Concentration. Individuals with a university degree had 55% higher enterolactone concentration than individuals with elementary degree as the highest educational level ($P < 0.001$). Nonsmokers had 38% higher enterolactone concentration than smokers ($P < 0.001$). Individuals consuming 15 to 30 g alcohol per day had 54% higher enterolactone concentration than zero consumers ($P = 0.012$). In contrast, a tendency toward lower enterolactone concentrations was observed among individuals consuming >30 g alcohol per day compared with those consuming 15 to 30 g/d ($P = 0.06$). Leisure-time physical activity was positively associated with enterolactone concentration (P for trend = 0.02). Current use of neither menopausal hormone therapy nor antibiotics was significantly associated with plasma concentrations of enterolactone (Table 2). The enterolactone concentration was positively correlated with fiber intake (r = 0.25; $P < 0.001$). Several food sources of fiber, vegetables, fruit and berries, high-fiber bread, and nuts (r = 0.10-0.17; $P < 0.01$), as well as alcohol intake (especially wine), were positively correlated to the enterolactone concentration when examined in separate models. BMI and body fat percentage were negatively correlated to the enterolactone concentration in plasma (Table 3). The r was similar when all food variables were simultaneously included in the model; however, vegetables were no

Table 1. Enterolactone concentration (nmol/L) and baseline characteristics of cases and controls from the MDC cohort, 1991 to 2004

Variables	Cases (n = 366)	Controls (n = 733)	P*	P †
ENL concentration (nmol/L), median (range)	14.5 (0.3-334)	16.1 (0.3-115)	0.45	
Fiber intake (g/d), mean (SD)	19.3 (6.9)	19.1 (6.1)	0.57	
Fat intake (E %), mean (SD)	38.4 (6.0)	38.7 (5.9)	0.53	
Age (y), mean (SD)	57.0 (7.1)	56.9 (7.1)	0.85	
Weight (kg), mean (SD)	69.1 (11.3)	69.0 (12.1)	0.84	
Height (cm), mean (SD)	164.4 (5.6)	163.8 (6.1)	0.12	
BMI (kg/m^2), mean (SD)	25.6 (4.2)	25.7 (4.5)	0.63	
Age at menarche (y), mean (SD)	13.6 (1.4)	13.6 (1.5)	0.85	
Age at menopause (y), mean (SD)	49.6 (4.8)	49.4 (4.3)	0.56	
Age at birth of first child			0.10	
≤24 y	161 (45 %)	326 (46 %)		0.91
25-29 y	103 (29 %)	244 (34 %)		0.09
≥30 y	46 (13 %)	82 (11 %)		0.49
No children	47 (13 %)	65 (9 %)		0.04
Educational status			0.59	
Elementary	136 (37 %)	305 (42 %)		0.15
Primary and secondary	121 (33 %)	226 (31 %)		0.47
Upper secondary	25 (7 %)	46 (6 %)		0.73
Further education without degree	21 (6 %)	46 (6 %)		0.72
University degree	62 (17 %)	106 (15 %)		0.29
Smoking status			0.21	
Smokers	99 (27 %)	200 (27 %)		0.92
Ex-smokers	116 (32 %)	197 (27 %)		0.10
Nonsmokers	151 (41 %)	335 (46 %)		0.16
Alcohol habits			0.36	
Zero consumers	23 (6 %)	55 (8 %)		0.46
<15 g alcohol per d	275 (75 %)	561 (77 %)		0.58
15-30 g alcohol per d	54 (15 %)	100 (14 %)		0.62
>30 g alcohol per d	14 (4 %)	16 (2 %)		0.12
Leisure-time physical activity			0.38	
Quartile 1	94 (26 %)	183 (25 %)		0.81
Quartile 2	100 (27 %)	182 (25 %)		0.38
Quartile 3	90 (25 %)	168 (23 %)		0.55
Quartile 4	81 (22 %)	197 (27 %)		0.09
Household activities (h/wk)			0.04	
0-9	67 (19 %)	101 (14 %)		0.06
10-19	142 (39 %)	279 (39 %)		0.88
20-29	112 (31 %)	219 (31 %)		0.86
≥30	39 (11 %)	117 (16 %)		0.02
Current menopausal hormone therapy use			<0.001	
Yes	109 (32 %)	133 (19 %)		
No	229 (68 %)	553 (81 %)		

Abbreviations: ENL, enterolactone; E %, percentage of nonalcohol energy.
*P values were computed using Mann-Whitney test for enterolactone concentration, Student t test for other continuous variables, and χ^2 test for categorical variables.
†P values were computed using χ^2 test (comparing the specific category with the rest of the variable categories).

longer significantly associated with enterolactone concentrations.

In the multivariate linear regression model, fruit and berries and high-fiber bread were significantly positively associated, and body fat percentage was significantly negatively associated with enterolactone concentrations (Table 3). University degree and the 3rd quartile of leisure-time physical activity were positively associated, whereas current smoking was negatively associated with enterolactone concentrations (data not shown). Fruit and berries, high-fiber bread, body fat percentage, university degree, current smoking, and 3rd quartile of physical activity explained 11.4% of the variation in enterolactone concentrations.

Enterolactone and Risk for Breast Cancer. A tendency toward a reduced breast cancer risk was observed for the highest compared with the lowest quartile of enterolactone concentration (odds ratio, 0.81; 95% CI, 0.55-1.20; P for trend = 0.14 in the multivariate model; Table 4). Among the potential confounding factors, educational status, age at birth of first child, and use of menopausal hormone therapy had greatest influence on the risk estimates. The heterogeneity of the lowest quartile (likely including individuals with low concentrations due to antibiotic use) seem obvious as individuals in the lowest quartile reported higher fiber intakes than individuals in the 2nd quartile. When the analyses only included women >50 years and cases with breast cancer diagnosis >1 year after the baseline examinations, a significant association was observed for the 4th quartile compared with the 2nd quartile (P = 0.04). Excluding individuals with reported dietary change in the past did not influence the risk estimates. When enterolactone concentrations were divided in two categories (low or high), high enterolactone concentrations were associated with decreased breast cancer risk compared with low enterolactone concentrations (odds ratio, 0.75; 95% CI,

0.58-0.98; Table 5). When we corrected the risk estimates for the within-person variation of enterolactone during 1 month (intraclass correlation coefficient, 0.48), the risk estimates were lower. The odds ratio of 0.75 for high enterolactone concentration compared with low enterolactone concentration was 0.55 after correction.

ER-Defined Breast Cancer. Most cases (84%) had ERα (+) tumors, and approximately half of the cases (49%) had ERβ (+) tumors (Table 5). There was no clear evidence of coexpression of ERα and ERβ (P = 0.16 for the χ^2 test). The reduced breast cancer risk with high enterolactone concentrations was only observed for ERα (+) (odds ratio, 0.75; 95% CI, 0.58-0.98) and ERβ (−) tumors (odds ratio, 0.60; 95% CI, 0.43-0.85). The risk was significantly different for ERβ (−) and ERβ (+) tumors (P for heterogeneity = 0.04; Table 6). The reduced risk for ERβ (−) tumors with high enterolactone concentration was also observed when analyzing odds ratios across quartiles of enterolactone [1.00 (reference), 1.13, 0.50, 0.78; P for trend = 0.045], whereas the risk for ERα (+) tumors across quartiles of enterolactone concentration did not reach significance [1.00 (reference), 1.06, 0.72, 0.78; P for trend = 0.07]. The risk for ERβ (+) tumors across quartile of enterolactone concentrations [1.00 (reference), 1.03, 0.99, 0.90; P for trend = 0.63] and ERα (−) tumors [1.00 (reference), 1.21, 0.71, 1.11; P for trend = 0.90] were not statistically significant. Because most tumors were ERα (+), high enterolactone concentrations were also associated with a decreased risk among ERα (+)/ERβ (−) tumors (odds ratio, 0.59; 95% CI, 0.41-0.86). There was a tendency toward heterogeneity between

Table 2. Mean plasma concentrations of enterolactone (nmol/L) at baseline according to participant characteristics among the controls (n = 733) in a subsample from the MDC cohort

Participant characteristics	No. of subjects	Mean	95% CI	P*
Age (y)				0.23
44-50	162	22.2	19.6-24.8	
50-55	159	20.1	17.5-22.7	
55-60	170	19.4	16.8-21.9	
60-65	132	18.3	15.5-21.2	
65-70	65	16.1	12.1-20.2	
70-75	45	22.1	17.0-27.2	
Season of blood collection				0.19
Winter	154	20.6	18.0-23.3	
Spring	226	18.4	16.3-20.6	
Summer	101	19.2	15.9-22.4	
Fall	252	20.8	18.8-22.9	
Educational status				<0.001
Elementary	305	17.2 †	15.4-19.1	
Primary and secondary	226	20.0	17.8-22.2	
Upper secondary	46	19.9	15.1-24.7	
Further education without a degree	46	20.5	15.7-25.2	
University degree	106	26.6 †	23.4-29.8	
Smoking status				<0.001
Current smokers	200	16.2 †	13.9-18.5	
Ex-smokers	197	19.2	16.9-21.5	
Nonsmokers	335	22.4 †	20.6-24.1	
Alcohol consumption				0.007
Zero consumers	55	14.9 †	10.5-19.3	
<15 g/d	561	20.0	18.6-21.3	
15-30 g/d	100	22.9 †	19.7-26.2	
>30 g/d	16	13.5	5.4-21.6	
BMI (kg/m^2)				0.001
≤25	367	22.0 †	20.3-23.7	
25-30	245	18.7	16.7-20.8	
>30	121	15.4 †	12.5-18.3	
Leisure-time physical activity				0.005
Quartile 1	183	16.7 †	14.3-19.1	
Quartile 2	182	20.1	17.7-22.5	
Quartile 3	168	22.9 †	20.4-25.4	
Quartile 4	197	19.9	17.6-22.2	
Household activities (h/wk)				0.93
0-9	101	18.9	15.6-22.1	
10-19	279	20.6	18.7-22.5	
20-29	219	19.3	17.1-21.5	
≥30	117	19.5	16.5-22.6	
Current use of menopausal hormone therapy				0.91
No	553	19.6	18.3-21.0	
Yes	133	19.7	16.9-22.6	
Current use of antibiotics				0.79
No	718	19.7	18.5-20.9	
Yes	15	24.2	15.8-32.6	

*Test of differences in means ($\log_e$ transformed) using the general linear model, adjusted for age and date of blood collection.
† Indicates statistically significant differences in means at the 0.05 level using Tukey's multiple comparison test within each variable.

Table 3. Partial correlations between enterolactone concentrations, and dietary variables (grams per day) and body composition among the controls (n = 733) in a subsample from the MDC cohort

Variables	Separate models*		Minimal model*†		Multivariate model‡	
	Coefficient	P	Coefficient	P	Coefficient	P
Energy (kcal)	−0.006	0.88	−0.06	0.12	−0.07	0.06
Protein (E %)	0.05	0.21				
Fat (E %)	−0.06	0.12				
Carbohydrates (E %)	0.04	0.31				
Fiber	0.25	<0.001			0.22§	<0.001
Vegetables	0.10	0.008	0.006	0.87		N.S.
Fruit and berries	0.17	<0.001	0.14	<0.001	0.13	<0.001
Fruit juices	0.04	0.26	0.01	0.76		N.S.
Boiled potato	−0.003	0.95	−0.003	0.95		N.S.
Fried and deep-fried potato	−0.04	0.23	−0.04	0.24		N.S.
Cereals	0.05	0.19	0.01	0.83		N.S.
Low-fiber bread	−0.06	0.10	0.01	0.77		N.S.
High-fiber bread	0.16	<0.001	0.12	0.001	0.13	0.001
Rice and pasta	0.06	0.09	0.05	0.19		N.S.
Nuts	0.10	0.008	0.08	0.03	0.07	0.06
Fermented milk	0.03	0.36	−0.02	0.53		N.S.
Tea	0.05	0.15	0.04	0.28		N.S.
Coffee	0.03	0.41	0.03	0.40		N.S.
Alcohol	0.10	0.007				
Wine	0.09	0.01	0.08	0.04		N.S.
Beer	0.07	0.07	0.03	0.37		N.S.
Spirits	0.003	0.94	−0.04	0.28		N.S.
BMI	−0.11‖	0.004				
Body fat (%)	−0.14‖	<0.001			−0.14	<0.001

*Adjusted for age, date of blood collection, method version, and total energy.
†Minimally adjusted model with food variables included simultaneously.
‡Stepwise backward linear regression model with smoking status, educational status, and leisure-time physical activity included in the model, adjusted for age categories, date of blood collection, method version, and total energy; N.S. is an eliminated variable with $P > 0.10$.
§Model not adjusted for fiber-rich foods.
‖Adjusted for age and date of blood collection.

ERα (+)/ERβ (−) and ERα (+)/ERβ (+) breast cancers (P = 0.08). Using a joint effect model, the heterogeneity test had a P value of 0.24 for ERα and a P value of 0.02 for ERβ. However, a marked underdispersion was found.

Discussion

This study identified fruit and berries and high-fiber bread as the major dietary determinants for enterolactone concentration in plasma in this population. Current smokers and individuals with obesity had lower enterolactone concentrations, and high leisure-time physical activity and high educational status were associated with high enterolactone concentrations. High enterolactone concentrations were associated with reduced breast cancer risk compared with low enterolactone concentrations. The reduced breast cancer risk with high compared with low concentration was observed for ERα (+) and ERβ (−) tumors.

Dietary and lifestyle determinants of enterolactone concentration have been described in several populations (22-25]). The major strength of the present study is the dietary data of high relative validity. Vegetables, fruit and berries, high-fiber bread, nuts, and wine were correlated to enterolactone concentration, and these foods are the major lignan-containing food groups (3). However, only fruit and berries and high-fiber bread were significantly associated with enterolactone concentration when mutually adjusted for other food variables and lifestyle factors. Smoking and obesity have been suggested to influence enterolactone directly but also indirectly because they influence food habits. Educational level does not directly influence enterolactone concentrations but is associated with other socioeconomic factors and many health behaviors and food choices. Thus, a multifaceted complex of many factors arises, and it is hard to single out and identify specific predictors of enterolactone levels. The differences in means across quartiles of physical activity could in a similar way be explained by other health related factors because physical activity is associated with various health behaviors and food choices. We aimed to estimate predictors of enterolactone concentration, and it is obvious that enterolactone is associated with various health related factors. Our conclusion is that this study supports the suggestion that enterolactone is a marker of healthy lifestyle.

The determinants of enterolactone concentrations were similar compared with other studies among Nordic women. A Danish nested case-control study with 857 postmenopausal women using an FFQ showed that whole grains, cabbage, leafy vegetables, and coffee were the major dietary determinants of enterolactone concentration. They also identified BMI, smoking, and frequency of bowel movements as predictors of enterolactone concentration (24). Kilkkinen et al. (23) found associations between enterolactone concentration and age, BMI, smoking, and consumption of vegetables among 1,212

Table 4. Enterolactone concentrations, fiber intakes, and odds ratios (95 % CI) for breast cancer across quartiles of plasma enterolactone concentration in women from the MDC cohort, 1991 to 2004

	Quartiles of ENL concentration				*P* for trend
	1	2	3	4	
ENL concentration (nmol/L), median (range)*	4.9 (0.3-8.4)	11.6 (8.5-16.1)	21.1 (16.1-26.1)	36.8 (26.3-334.4)	
Fiber intake (g/d), median*	17.6	17.2	18.9	19.7	<0.001
All women					
Age (y), median	56.4	56.3	56.7	55.4	0.83
Cases/controls	100/183	104/183	78/185	84/182	
OR† (95 % CI)	1.00	1.06 (0.74-1.51)	0.77 (0.53-1.11)	0.84 (0.58-1.20)	0.16
OR‡ (95 % CI)	1.00	1.03 (0.70-1.52)	0.70 (0.46-1.06)	0.81 (0.55-1.20)	0.14
Women above 50 years + exclusion of cases with diagnosis within 1 y					
Cases/controls	73/145	80/133	66/134	57/136	
OR† (95 % CI)	1.00	1.23 (0.82-1.86)	0.99 (0.65-1.50)	0.84 (0.55.1.27)	0.30
OR‡ (95 % CI)	1.00	1.25 (0.78-2.00)	0.89 (0.55-1.45)	0.73 (0.45-1.19)	0.13
OR‡ (95 % CI; 2nd quartile as reference)	0.80 (0.50-1.28)	1.00	0.71 (0.44-1.15)	0.59 (0.35-0.97)	0.04§

Abbreviation: OR, odds ratio; ENL, enterolactone.
*Estimated for all women (n = 1099).
†Crude analysis.
‡Adjusted for weight, height, educational status, smoking habits, leisure-time physical activity, household activities, alcohol habits, age at menopause, parity, age at birth of first child, and current use of menopausal hormone therapy.
§Test of trend from quartiles 2 to 4.

Finnish women using a 38–food item questionnaire. These results further support the use of enterolactone as a biomarker of a healthy lifestyle with a diet containing high amounts of whole grains, fruits, and vegetables, without smoking and obesity.

It is known that the capacity of the gut microflora is a very influential factor for the formation of enterolactone from plant lignans (53). The use of antibiotics is known to reduce the amount of bacteria in the gut and subsequently the concentrations of enterolactone in the blood (26). Enterolactone may even be a marker of the capacity of the microflora. Therefore, a potential weakness is that we may have only captured very few antibiotics users, that is, individuals reporting regular use of antibiotics and use of antibiotics during the 7 days after the blood were drawn. A Finnish study indicated that the blood concentrations of enterolignans can be influenced by antibiotic use up to 12 to 16 months before the blood collection (26). The correlations between lignan-containing foods and enterolactone concentrations would potentially have been higher if we had been able to exclude all antibiotic users during the year preceding blood collection.

An advantage of this study is the prospective design. The blood was drawn before cancer diagnosis, and the enterolactone concentrations were therefore not influenced by the diagnosis. However, the relation between enterolactone concentration and breast cancer was most likely attenuated because all users of antibiotics may not have been identified. Olsen et al. (14) found an inverted U-shaped curve for enterolactone and breast cancer in Danish postmenopausal women. They suggested that the lowest quartile also included individuals with low concentrations due to use of antibiotics and used the 2nd quartile as reference category. We observed almost identical risk estimates compared with this Danish study when restricting our analyses to women above 50 years at baseline and excluded cases with diagnosis within 1 year after blood collection, with a significant decreased risk in the 4th quartile compared with the 2nd quartile. A nested case-control study in the north of Sweden found the opposite relation and observed increased risks for breast cancer at very high and very low serum levels of enterolactone (15). However, most women with high enterolactone concentrations were premenopausal. Thus, other factors (e.g., genetic factors) may play a greater role in that subgroup of women in that study. Enterolactone concentrations were not related to breast cancer risk in a recent study conducted in a Dutch cohort of postmenopausal women (18), and a nested case-control study with 206 breast cancer cases conducted in Finland did not show a protective effect of high enterolactone concentrations (16). To interpret these results, information of dietary intakes would have been valuable because it seems to be the dietary fiber complex, including the associated lignans, rather than the lignans per se, that protects against diseases (5).

Similar to many large-scale epidemiologic studies, one nonfasting sample was drawn during baseline from each participant. It is therefore important to examine carefully the reliability of using one sample when classifying individuals according to their blood concentration. Moreover, it is important to estimate the variability of biomarkers in each study population in which an epidemiologic study will be done because the variability across studies is not always comparable. We have previously estimated intraclass correlation coefficient for nonfasting samples to be 0.48 over a month (45). Another study with nonfasting blood has shown plasma enterolactone concentrations to be relatively stable over a 2-year period with an intraclass correlation coefficient of

Table 5. Distribution of ERα and ERβ status of the tumors among incident breast cancer cases in the MDC cohort, 1991 to 2004

	ERα (−)	ERα (+)	ERα unknown	Total
ERβ (−)	31	152	2	185
ERβ (+)	21	157	3	181
Total	52	309	5	366

0.55 (54). Biomarkers with low intraclass correlation coefficient often result in an attenuation of the relation between the exposure and disease. In this study, we also used information from the reproducibility study to correct risk estimates for within-person variation during a month (52), and we observed stronger risk estimates after the correction (that is, an odds ratio of 0.55 for high enterolactone concentration compared with low enterolactone concentration).

Only 52 (14%) of the breast cancers in our study were ERα (−), and consequently, we had limited power to examine whether enterolactone concentrations were associated differently with breast cancer among ERα (−) compared with ERα (+) breast cancers. A Danish study observed a protective effect of enterolactone with ERα (−) tumors (14). However, that study also suffered from limited power because only 80 cases with ERα (−) tumors were identified. Results from the Swedish mammography cohort showed no heterogeneity in the association between lignan intakes and breast cancer across ERα or progesterone receptor subtypes (55). However, a large prospective study conducted in France, including 1,180 cases with known ER and progesterone receptor status, showed that the inverse association between the risk for breast cancer and lignan intakes calculated from food records was only observed among ERα (+) and progesterone receptor (+) tumors (56).

A potential misclassification of ER status of the tumors may influence the results. The tissue microarray technique used in this study is a well-documented method for high-throughput tissue screening and is now the preferable method to evaluate large tumor materials (57, 58). Potential inter- and intraobserver variation about classification of tumors were probably limited because the receptor status of all breast tumors was evaluated twice in a standardized way by one person.

We are not aware of any study that has examined the association between enterolactone and ERβ-defined breast cancer. ERβ was recently discovered (33), and its biological function and prognostic role is still not fully understood (34). There is considerable variation in the reported efficacy of ERβ antibodies for immunohistochemical evaluation (59), which contributes to the controversial view of ERβ. The ERβ antibody used in this study has been validated by comparing the immunohistochemical method with Western blot (35). In the present study, we found a decreased breast cancer risk with high enterolactone concentration among cases with tumors expressing ERα but not ERβ. ERβ has been suggested to have a regulatory role in ERα activity (36), and new insights on the interaction between the two ERs reveal that ERβ acts by antagonizing ERα on a very specific subset of estrogen-stimulated genes and actively prevents ERα stimulated cell growth (60). A recent study showed that enterolactone has preference for ERα (10). Our results might be explained by the lack of ERβ-mediated inhibitory effects in ERα (+)/ERβ (−) tumors, thus making tumors more susceptible for the antiestrogenic influence of enterolactone.

In conclusion, enterolactone is positively correlated to a high-fiber diet, and this study supports other studies suggesting that enterolactone is a marker of a healthy lifestyle. The protective association between enterolactone and breast cancer is more evident in tumors that express ERα but not ERβ. Thus, fiber-rich diets and lifestyles linked to high levels of enterolactone are associated with lower risk for tumors [that is, ERα (+)] that are common in industrialized countries of today,

Table 6. Odds ratios (95 % CI) for ER-defined breast cancer across plasma enterolactone concentration (low/high) in women from the MDC cohort, 1991 to 2004

	ENL concentration		*P* for heterogeneity
	Low	High	
ENL concentration (nmol/L), median (range)	8.4 (0.3-16.1)	26.3 (16.1-334.4)	
Cases/controls	204/366	162/367	
OR (95% CI)	1.00	0.75 (0.58-0.98)	
ERα (−) tumors			
Cases/controls	27/366	25/367	
OR (95% CI)	1.00	0.86 (0.48-1.54)	
ERα (+) tumors			
Cases/controls	175/366	134/367	
OR (95% CI)	1.00	0.73 (0.55-0.97)	0.62*
ERβ (−) tumors			
Cases/controls	113/366	72/367	
OR (95% CI)	1.00	0.60 (0.43-0.85)	
ERβ (+) tumors			
Cases/controls	91/366	90/367	
OR (95% CI)	1.00	0.94 (0.67-1.33)	0.04 †
ERα (+)/ERβ (−) tumors			
Cases/controls	93/366	59/367	
OR (95% CI)	1.00	0.59 (0.41-0.86)	
ERα (+)/ERβ (+) tumors			
Cases/controls	82/366	75/367	
OR (95 % CI)	1.00	0.90 (0.63-1.28)	0.08 ‡

NOTE: Adjusted for age, date of blood collection, weight, height, smoking status, educational status, and current use of menopausal hormone therapy.
*Test of heterogeneity between ERα (−) and ERα (+) tumors.
†Test of heterogeneity between ERβ (−) and ERβ (+) tumors.
‡Test of heterogeneity between ERα (+)/ERβ (−) and ERα (+)/ERβ (+) tumors.

especially when the antagonistic influence of ERβ is absent. The findings will need to be confirmed by other studies, especially the associations observed between enterolactone and breast cancer risk among ERβ (−) tumors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Mattisson I, Wirfalt E, Johansson U, et al. 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;90:122–7.
2. Milder IE, Arts IC, van de Putte B, Venema DP, Hollman PC. Lignan contents of Dutch plant foods: a database including lariciresinol, pinoresinol, secoisolariciresinol and matairesinol. Br J Nutr 2005;93:393–402.
3. Mazur W. Phytoestrogen content in foods. Baillieres Clin Endocrinol Metab 1998;12:729–42.
4. Heinonen S, Nurmi T, Liukkonen K, et al. *In vitro* metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. J Agric Food Chem 2001;49:3178–86.
5. Adlercreutz H. Lignans and human health. Crit Rev Clin Lab Sci 2007;44:483–525.
6. Low YL, Dunning AM, Dowsett M, et al. Phytoestrogen exposure is associated with circulating sex hormone levels in postmenopausal women and interact with ESR1 and NR1I2 gene variants. Cancer Epidemiol Biomarkers Prev 2007;16:1009–16.
7. Brooks JD, Thompson LU. Mammalian lignans and genistein decrease the activities of aromatase and 17beta-hydroxysteroid dehydrogenase in MCF-7 cells. J Steroid Biochem Mol Biol 2005;94:461–7.
8. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology 1998;139:4252–63.
9. Mueller SO, Simon S, Chae K, Metzler M, Korach KS. Phytoestrogens and their human metabolites show distinct agonistic and antagonistic properties on estrogen receptor alpha (ERalpha) and ERbeta in human cells. Toxicol Sci 2004;80:14–25.
10. Penttinen P, Jaehrling J, Damdimopoulos AE, et al. Diet-derived polyphenol metabolite enterolactone is a tissue-specific estrogen receptor activator. Endocrinology 2007;148:4875–86.
11. Prasad K. Antioxidant activity of secoisolariciresinol diglucoside-derived metabolites, secoisolariciresinol, enterodiol, and enterolactone. Int J Angiol 2000;9:220–5.
12. Ingram D, Sanders K, Kolybaba M, Lopez D. Case-control study of phyto-oestrogens and breast cancer. Lancet 1997;350:990–4.
13. Pietinen P, Stumpf K, Mannisto S, et al. Serum enterolactone and risk of breast cancer: a case-control study in eastern Finland. Cancer Epidemiol Biomarkers Prev 2001;10:339–44.
14. Olsen A, Knudsen KE, Thomsen BL, et al. Plasma enterolactone and breast cancer incidence by estrogen receptor status. Cancer Epidemiol Biomarkers Prev 2004;13:2084–9.
15. Hulten K, Winkvist A, Lenner P, et al. An incident case-referent study on plasma enterolactone and breast cancer risk. Eur J Nutr 2002;41:168–76.
16. Kilkkinen A, Virtamo J, Vartiainen E, et al. Serum enterolactone concentration is not associated with breast cancer risk in a nested case-control study. Int J Cancer 2004;108:277–80.
17. den Tonkelaar I, Keinan-Boker L, Veer PV, et al. Urinary phytoestrogens and postmenopausal breast cancer risk. Cancer Epidemiol Biomarkers Prev 2001;10:223–8.
18. Verheus M, van Gils CH, Keinan-Boker L, et al. Plasma phytoestrogens and subsequent breast cancer risk. J Clin Oncol 2007;25:648–55.
19. Grace PB, Taylor JI, Low YL, et al. Phytoestrogen concentrations in serum and spot urine as biomarkers for dietary phytoestrogen intake and their relation to breast cancer risk in European prospective investigation of cancer and nutrition-norfolk. Cancer Epidemiol Biomarkers Prev 2004;13:698–708.
20. Ward HA, Chapelais G, Kuhnle GG, et al. Breast cancer risk in relation to urinary and serum biomarkers of phytoestrogen exposure in the EPIC-Norfolk study. Breast Cancer Res 2008;10:R32.
21. Lampe JW. Isoflavonoid and lignan phytoestrogens as dietary biomarkers. J Nutr 2003;133 Suppl 3:956–64S.
22. Horner NK, Kristal AR, Prunty J, et al. Dietary determinants of plasma enterolactone. Cancer Epidemiol Biomarkers Prev 2002;11:121–6.
23. Kilkkinen A, Stumpf K, Pietinen P, et al. Determinants of serum enterolactone concentration. Am J Clin Nutr 2001;73:1094–100.
24. Johnsen NF, Hausner H, Olsen A, et al. Intake of whole grains and vegetables determines the plasma enterolactone concentration of Danish women. J Nutr 2004;134:2691–7.
25. Milder IE, Kuijsten A, Arts IC, et al. Relation between plasma enterodiol and enterolactone and dietary intake of lignans in a Dutch endoscopy-based population. J Nutr 2007;137:1266–71.
26. Kilkkinen A, Pietinen P, Klaukka T, et al. Use of oral antimicrobials decreases serum enterolactone concentration. Am J Epidemiol 2002;155:472–7.
27. Riboli E, Elmstahl S, Saracci R, Gullberg B, Lindgarde F. The Malmo Food Study: validity of two dietary assessment methods for measuring nutrient intake. Int J Epidemiol 1997;26 Suppl 1:S161–73.
28. Elmstahl S, Riboli E, Lindgarde F, Gullberg B, Saracci R. The Malmo Food Study: the relative validity of a modified diet history method and an extensive food frequency questionnaire for measuring food intake. Eur J Clin Nutr 1996;50:143–51.
29. Dontu G, El-Ashry D, Wicha MS. Breast cancer, stem/progenitor cells and the estrogen receptor. Trends Endocrinol Metab 2004;15:193–7.
30. Althuis MD, Fergenbaum JH, Garcia-Closas M, et al. Etiology of hormone receptor-defined breast cancer: a systematic review of the literature. Cancer Epidemiol Biomarkers Prev 2004;13:1558–68.
31. Kushi LH, Potter JD, Bostick RM, et al. Dietary fat and risk of breast cancer according to hormone receptor status. Cancer Epidemiol Biomarkers Prev 1995;4:11–9.
32. Suzuki R, Ye W, Rylander-Rudqvist T, et al. Alcohol and postmenopausal breast cancer risk defined by estrogen and progesterone receptor status: a prospective cohort study. J Natl Cancer Inst 2005;97:1601–8.
33. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. Proc Natl Acad Sci U S A 1996;93:5925–30.
34. Murphy LC, Watson PH. Is oestrogen receptor-beta a predictor of endocrine therapy responsiveness in human breast cancer? Endocr Relat Cancer 2006;13:327–34.
35. Borgquist S, Holm C, Stendahl M, et al. Oestrogen receptors alpha and beta show different associations to clinicopathological parameters and their co-expression might predict a better response to endocrine treatment in breast cancer. J Clin Pathol 2008;61:197–203.
36. Saji S, Hirose M, Toi M. Clinical significance of estrogen receptor beta in breast cancer. Cancer Chemother Pharmacol 2005;56 Suppl 1:21–6.
37. Duffy MJ. Estrogen receptors: role in breast cancer. Crit Rev Clin Lab Sci 2006;43:325–47.
38. Manjer J, Carlsson S, Elmstahl S, et al. The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. Eur J Cancer Prev 2001;10:489–99.
39. Berglund G, Elmstahl S, Janzon L, Larsson SA. The Malmo Diet and Cancer Study. Design and feasibility. J Intern Med 1993;233:45–51.
40. Fuqua SA, Schiff R, Parra I, et al. Expression of wild-type estrogen receptor beta and variant isoforms in human breast cancer. Cancer Res 1999;59:5425–8.
41. Pero RW, Olsson A, Berglund G, et al. The Malmo biological bank. J Intern Med 1993;233:63–7.
42. Pero RW, Olsson A, Bryngelsson C, et al. Quality control program for storage of biologically banked blood specimens in the Malmo Diet and Cancer Study. Cancer Epidemiol Biomarkers Prev 1998;7:803–8.
43. Adlercreutz H, Wang GJ, Lapcik O, et al. Time-resolved fluoroimmunoassay for plasma enterolactone. Anal Biochem 1998;265:208–15.
44. Stumpf K, Uehara M, Nurmi T, Adlercreutz H. Changes in the time-resolved fluoroimmunoassay of plasma enterolactone. Anal Biochem 2000;284:153–7.
45. Sonestedt E, Ericson U, Gullberg B, et al. Variation in fasting and non-fasting serum enterolactone concentrations in women of the Malmo Diet and Cancer cohort. Eur J Clin Nutr 2008;62:1005–9.
46. Callmer E, Riboli E, Saracci R, Akesson B, Lindgarde F. Dietary assessment methods evaluated in the Malmo food study. J Intern Med 1993;233:53–7.
47. Parvez S, Malik KA, Ah Kang S, Kim HY. Probiotics and their

fermented food products are beneficial for health. J Appl Microbiol 2006;100:1171–85.
48. WHO. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. Geneva (Switzerland): WHO; 2000.
49. Merlo J, Berglund G, Wirfalt E, et al. Self-administered questionnaire compared with a personal diary for assessment of current use of hormone therapy: an analysis of 16,060 women. Am J Epidemiol 2000;152:788–92.
50. Wirfalt E, Mattisson I, Johansson U, et al. A methodological report from the Malmo Diet and Cancer study: development and evaluation of altered routines in dietary data processing. Nutr J 2002;1:3.
51. Hernan MA, Hernandez-Diaz S, Werler MM, Mitchell AA. Causal knowledge as a prerequisite for confounding evaluation: an application to birth defects epidemiology. Am J Epidemiol 2002;155:176–84.
52. White E. Effects of biomarker measurement error on epidemiological studies. In: Toniolo P, editor. Application of biomarkers in Cancer Epidemiology 142. Lyon: IARC Scientific Publications; 1997. p. 73-93.
53. Bowey E, Adlercreutz H, Rowland I. Metabolism of isoflavones and lignans by the gut microflora: a study in germ-free and human flora associated rats. Food Chem Toxicol 2003;41:631–6.
54. Zeleniuch-Jacquotte A, Adlercreutz H, Akhmedkhanov A, Toniolo P. Reliability of serum measurements of lignans and isoflavonoid phytoestrogens over a two-year period. Cancer Epidemiol Biomarkers Prev 1998;7:885–9.
55. Suzuki R, Rylander-Rudqvist T, Saji S, et al. Dietary lignans and postmenopausal breast cancer risk by oestrogen receptor status: a prospective cohort study of Swedish women. Br J Cancer 2008;98:636–40.
56. Touillaud MS, Thiebaut AC, Fournier A, et al. Dietary lignan intake and postmenopausal breast cancer risk by estrogen and progesterone receptor status. J Natl Cancer Inst 2007;99:475–86.
57. Camp RL, Charette LA, Rimm DL. Validation of tissue microarray technology in breast carcinoma. Lab Invest 2000;80:1943–9.
58. Hoos A, Cordon-Cardo C. Tissue microarray profiling of cancer specimens and cell lines: opportunities and limitations. Lab Invest 2001;81:1331–8.
59. Skliris GP, Parkes AT, Limer JL, et al. Evaluation of seven oestrogen receptor beta antibodies for immunohistochemistry, western blotting, and flow cytometry in human breast tissue. J Pathol 2002;197:155–62.
60. Lin CY, Strom A, Li Kong S, et al. Inhibitory effects of estrogen receptor beta on specific hormone-responsive gene expression and association with disease outcome in primary breast cancer. Breast Cancer Res 2007;9:R25.

Enterolactone Is Differently Associated with Estrogen Receptor β–Negative and –Positive Breast Cancer in a Swedish Nested Case-Control Study

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