Published in final edited form as:
Breast Cancer Res Treat. 2013 June ; 139(3): 801–809. doi:10.1007/s10549-013-2586-y.

Plasma Enterolactone and Breast Cancer Risk in the Nurses' Health Study II

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Abstract

Background—Lignans are plant-based phytoestrogens with both estrogenic and anti-estrogenic properties that may be important for breast carcinogenesis. Retrospective studies have observed decreased breast cancer risk associated with high circulating enterolactone concentrations, a biomarker of lignan intake, but results from prospective studies are conflicting.

Methods—To prospectively examine this association, we measured plasma enterolactone levels in 802 breast cancer cases and 802 matched controls nested among predominantly premenopausal women in the Nurses' Health Study II (NHSII) cohort. We used conditional logistic regression and polytomous logistic regression models, adjusting for known breast cancer risk factors, to calculate relative risks (RR) and 95% confidence intervals (CI).

Results—Compared to women with enterolactone concentrations ≤ 4nmol/L, the multivariate adjusted RRs for breast cancer were 1.18 (95% CI: 0.86–1.62), 0.91 (95% CI: 0.66–1.25), and 0.96 (95% CI: 0.70–1.33) for women with enterolactone levels in the 2nd to the 4th quartiles, respectively; P_{trend}=0.60. Results were similar across tumors defined by estrogen and progesterone receptor status. Among premenopausal women with follicular estradiol levels below the median (<47 pg/mL), women in the highest category of enterolactone levels had a 51% lower breast cancer risk compared to those in the lowest category (95% CI: 0.27–0.91); P_{trend}=0.02. No association was observed among women with high follicular estradiol levels (≥47 pg/mL), (comparable RR=1.39, 95% CI: 0.73–2.65; $P_{interaction}$=0.02).

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Conflict of interest: The authors declare that they have no conflict of interest. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript.

Conclusions—We did not observe an overall association between plasma enterolactone and breast cancer risk in a large nested case-control study of US women. However, a significant inverse association was observed among premenopausal women with low follicular estradiol levels, suggesting that enterolactone may be important in a low estrogen environment. This should be confirmed in future studies.

Keywords

enterolactone; lignan; breast cancer; biomarker; prospective study; premenopausal

Introduction

Higher levels of both endogenous estrogens (e.g., circulating estradiol) and exogenous estrogens (e.g., post-menopausal hormone use) have been associated with increased risk of breast cancer among postmenopausal women [1,2]. Among premenopausal women, a positive association of endogenous estrogen with breast cancer risk is suggestive but not conclusive [3–6]. Enterolactone is a phytoestrogen that may be important in breast carcinogenesis.

Enterolactone is an enterolignan formed by intestinal microbiota from lignan precursors that are contained mainly in vegetables, whole grain products, berries, and flaxseeds [7]. Other factors influencing enterolactone concentrations in plasma include smoking; obesity; constipation; antibiotics use; intake of fiber, fruits, coffee, or tea; and post-menopausal hormone use [8–11]. Since enterolactone is the major lignan metabolite with the highest concentrations in plasma [12], it is most commonly examined in epidemiologic studies as a biomarker for dietary lignan exposures.

Enterolactone has an aromatic structure similar to estradiol and is weakly estrogenic, such that it can stimulate proliferation of breast cancer cells [13,14]. Conversely, anti-estrogenic properties also have been observed; cell-based studies suggest that enterolactone competitively binds the estrogen receptor (ER) and inhibits estradiol-induced breast cancer cell growth [15–17]. Several mechanisms for enterolactone action in breast cancer development have been proposed. First, it may stimulate production of sex hormone-binding globulin (SHBG), which binds to estradiol and lowers estradiol bioactivity [13]. Second, high concentrations of enterolactone may inhibit aromatase, an enzyme that converts androgens to estrogens in fat, muscle and other tissue, and a primary source of estrogens in postmenopausal women [18–20]. Third, it may act as a strong antioxidant [21]. Finally, it may have preferential activation of estrogen receptors [22–24,17].

Epidemiologic studies that examined enterolactone and breast cancer risk have yielded inconsistent results, as discussed in a recent meta-analysis [25]. Retrospective studies have reported a decreased breast cancer risk with higher circulating enterolactone concentrations [26–30], but results from prospective studies have been conflicting [31–41]. However, previous studies suggested an inverse association between high enterolactone levels and postmenopausal breast cancer risk [29,37,38]. Thus, we prospectively examined the association of plasma enterolactone concentrations and breast cancer risk in a large study (802 cases and 802 matched controls) of predominantly premenopausal women nested within the Nurses' Health Study II (NHSII) cohort for whom we have detailed information on case characteristics (e.g., estrogen and progesterone receptor (ER/PR) status) as well as dietary intake and circulating estradiol concentrations in the follicular and luteal phases.

Methods

Study Population

The NHSII is a prospective cohort established in 1989, when 116,430 U.S. female nurses, 25 to 42 years of age completed an initial questionnaire. Participants have been followed by biennially mailed questionnaires to update exposure information, lifestyle factors, and ascertain non-fatal incident diseases. Women completed a semi-quantitative food frequency questionnaire (FFQ) every 2–4 years since 1991. Deaths were reported by family or postal authorities. We also searched for names of non-responders in the National Death Index [42,43].

Between 1996 and 1999, 29,611 NHS II participants (aged 32–54) provided blood samples and completed a short questionnaire recording their weight, menstrual cycle length, first day of the menstrual cycle, and the time, date, and number of hours since the last meal for each blood sample (details in [44,45]). Among these, 18,521 premenopausal women who had not used oral contraceptives, been pregnant, or breastfed within 6 months provided timed blood samples within the menstrual cycle. Blood samples were drawn on the 3rd to 5th day of their menstrual cycle (follicular) and 7 to 9 days before the anticipated start of their next cycle (luteal). The remaining 11,090 women (e.g., postmenopausal, using hormonal contraception, or unwilling to give timed samples) provided an untimed sample. Women had their blood drawn and shipped overnight on ice to our laboratory, where the blood was processed and separated into plasma, red blood cells, and white blood cells. Samples were stored in liquid nitrogen freezers (≤–130 °C) after collection.

Measurement of exposure and laboratory assays

To measure enterolactone levels, frozen plasma was sent to the Analytical Biochemistry Shared Resource of the University of Hawaii Cancer Center, Honolulu, HI in 2011 and 2012. Extraction and analysis of enterolactone was performed from freshly thawed samples by liquid-liquid extraction after enzymatic hydrolysis followed by isotope dilution liquid chromatography electrospray ionization tandom mass spectrometry as described in detail previously [46] except for the replacement of diethyl ether by methyl tertiary butyl ether during extraction and the replacement of arylsulfatase (which was discontinued by Boehringer Mannheim) by using 30 uL arylsulfatase isolated from Helix pomatia at 2,000 U/mL in aqueous solution (Sigma, St. Louis, MO, # S9751).

Laboratory personnel were blinded to case-control and quality control status and matched cases and controls (ordered randomly within each set) were assayed in the same laboratory batch. Samples were sent in 2 batches and we assayed the luteal sample for women with timed samples. The detection limit of enterolactone was 1nmol/L. The inter-assay coefficient of variation (CV) from quality control samples was 14.1% and the intra-assay CV was 5.4%.

Assessment of covariates

Information on covariates was collected from the biennial questionnaires and the questionnaire completed at blood collection. Participants provided information on height, weight at age 18, and age at menarche at baseline. Dietary information was taken from the 1999 FFQ, since it was the questionnaire closest to the time of blood collection. Smoking, current weight, antibiotics use, menopausal status, and postmenopausal hormone use (among postmenopausal women) were ascertained at the time of blood collection. Physical activity, history of benign breast disease, parity, age at first birth, oral contraceptive use, and family history of breast cancer were assessed on the 1997 questionnaire. We measured both

follicular and luteal estradiol levels among women providing timed samples previously and details can be found elsewhere [3].

Identification of breast cancer cases

We identified incident breast cancer cases by self-report on biennial questionnaires, and from reports of death from family members, the National Death Index, and the US Postal Service. For reported breast cancer cases, we obtained medical records or records from cancer registries for confirmation and to obtain information on invasiveness and estrogen and progesterone receptor (ER, PR) status. We identified 804 breast cancer cases after blood collection but before June 2009. Each case was matched to one control on race/ethnicity, age at blood draw, time of day of blood draw and fasting status for follicular and luteal samples (or the one untimed sample), month/year of blood draw, menopausal status at blood draw and diagnosis, and postmenopausal hormone use at blood draw. For women with timed samples, we additionally matched on the luteal day of the blood draw (date of next period minus date of blood draw). Two cases were excluded because the plasma samples of the matched controls were not available for assay. Therefore, 802 cases of breast cancer and 802 matched controls were included in the analyses. The Institutional Review Board of the Brigham and Women's Hospital approved this analysis. Informed consent was implied by receipt of completed questionnaires and blood samples.

Statistical Analyses

We did not identify any statistical outliers for enterolactone levels using the generalized extreme Studentized deviate many-outlier detection approach [47]. Nine samples had values below the limit of detection; these were set to be half the limit of detection or 0.5 nmol/L. We calibrated the enterolactone levels in one laboratory batch to the other one, R^2=0.98 and categorized enterolactone levels into quartiles based on the distribution in controls. We used conditional logistic regression models to estimate relative risks (RR) and 95% confidence intervals (CI) of breast cancer. The models were conditioned on matching factors and adjusted for covariates including family history of breast cancer (yes, no), history of benign breast disease (yes, no), oral contraceptive use (never, >0–1 yrs, >1–5 yrs, and >5 yrs), BMI at age 18 (continuous), weight change since age 18 (continuous), parity and age at first birth (nulliparous, 1–2 children and <25 y, 1–2 children and 25–29 y, 1–2 children and ≥30 y, ≥3 children and <25 y, ≥3 children and ≥25 y), age at menarche (continuous), antibiotics use within last month (yes, no), alcohol consumption (continuous), smoking (never, past and <10 y since quitting, past and ≥10 y since quitting, current), and physical activity (0–3.8, >3.8–11.1, >11.1–26.4, >26.4 MET-hrs/wk). Tests for trend were conducted by modeling natural log transformed quartile median concentrations and calculating the Wald statistic.

In stratified analyses by menopausal status at blood draw, menopausal status at diagnosis, median plasma estradiol level in the follicular phase, and median plasma estradiol level in the luteal phase, we used unconditional logistic regression, additionally adjusting for matching factors, since results were essentially the same from multivariate conditional and unconditional logistic regression models. Our previous analysis of plasma estradiol levels and breast cancer risk has been published elsewhere [3,6]. We used likelihood ratio tests to assess effect modification by menopausal status and estradiol levels. We also examined if the association varied by invasive versus in situ breast cancer as well as by ER/PR status using polytomous logistic regression [48]. All *P* values were based on two-sided tests and were considered statistically significant if ≤0.05. We used SAS 9.3 software (SAS Institute, Cary, NC, USA) and STATA 12.1 software (StataCorp. College Station, TX, USA).

Results

Our analysis included 802 incident cases of breast cancer and 802 matched controls. There were 553 invasive breast cancer cases and 218 in situ cases, 373 ER+/PR+, 89 ER−/PR−, and 55 ER+/PR− breast cancer cases, and 408 premenopausal cases, 302 postmenopausal cases, and 92 cases with unknown menopausal status. The average time interval between blood draw and time of diagnosis was 6.2 years with a standard deviation of 3.4 years. Compared to those in the lowest quartile of enterolactone, controls in the highest quartile of enterolactone had higher average intakes of vegetables (27.8 versus 25.4 cups/week, respectively), whole grains (10.4 versus 8.7 cups/week), fiber (6.1 versus 5.2 g/day), caffeine (241.2 versus 205.4 mg/day), and tofu (0.5 versus 0.2 oz/day) (Table 1). Further, those in the highest versus lowest quartile of enterolactone had a lower average BMI (24.5 kg/m^2 versus 27.7 kg/m^2) and weight change between age 18 and blood draw (21.6 lb versus 38.7 lb). Comparing cases to controls, the median levels (10^{th}–90^{th} percentiles) of enterolactone were 11 (1–38) nmol/L among cases and 11 (1–40) nmol/L controls respectively (Supplemental Table 1).

Overall, plasma enterolactone concentrations were not associated with risk of breast cancer (Table 2). Compared to women in the first quartile of enterolactone concentration (≤ 4 nmol/L), the multivariate adjusted RRs for the 2^{nd} to 4^{th} quartiles were 1.18 (95% CI: 0.86–1.62), 0.91 (95% CI: 0.66–1.25), and 0.96 (95% CI: 0.70–1.33), respectively. No significant linear trend was observed (P_{trend}=0.60).

No significant associations were observed when we examined at invasive and *in situ* tumors separately. For invasive tumors, the RR comparing the top versus bottom quartile of enterolactone levels was 1.16 (95% CI: 0.83–1.61); P_{trend}=0.38 and for in situ tumors, the comparable RR was 0.82 (95% CI: 0.53–1.26); P_{trend}=0.22 ($P_{heterogeneity}$=0.21). Similarly no associations were observed for ER+/PR+ tumors (comparable RR=1.23, 95% CI: 0.84–1.80; P_{trend}=0.28), ER−/PR− tumors (RR=1.05, 95% CI: 0.56–1.97; P_{trend}=0.76), or ER+/PR− tumors (RR=0.77, 95% CI: 0.32–1.82; P_{trend}=0.46; $P_{heterogeneity}$=0.56). When additionally adjusting for fiber, coffee, tofu, total vegetables, and whole grains individually in the multivariate model, similar results were observed (results not shown).

In stratified analyses, we observed that the association differed by levels of estradiol (stratified at the median of 47 pg/mL) during the follicular phase, ($P_{interaction}$=0.02) (Table 3). Among women with low follicular estradiol levels (<47 pg/mL), the multivariate adjusted RRs were 1.32 (95% CI: 0.75–2.32), 0.64 (95% CI: 0.36–1.15), and 0.49 (95% CI: 0.27–0.91) for the 2^{nd} to 4^{th} quartile of enterolactone respectively, compared with the 1^{st} quartile; P_{trend}=0.02. However, among women with follicular estradiol levels ≥ 47 pg/mL, the comparable RRs (95% CI) were 1.23 (0.67–2.27), 1.03 (0.55–1.93), and 1.39 (0.73–2.65); P_{trend}=0.44. No significant interactions were observed by BMI, fiber intake, alcohol consumption, menopausal status at blood draw, menopausal status at diagnosis, or estradiol levels during the luteal phase ($P_{interaction}$ > 0.15, data not shown).

Discussion

This is the largest prospective study to date examining plasma enterolactone concentrations in relation to breast cancer risk, and it is the first study examining the association stratified by follicular and luteal phase estradiol levels among premenopausal women. The median enterolactone level measured in our study is in accordance with the range of previously published circulating enterolactone concentrations in the US and Europe [49,29,50,36,37,30,51,52,28,53,54,33,55], but lower than that in Asia [56]. We did not observe an association between plasma enterolactone, a biomarker of lignan intake, and

breast cancer risk either overall or by hormone receptor status. However, a significant inverse association was observed among premenopausal women with lower estradiol levels during the follicular phase.

Inconsistent results regarding the enterolactone-breast cancer association have been observed between retrospective studies and prospective studies, with case-control studies generally observing inverse association [26–28,31–34,41,35–38,29,30,39,40]. It is possible that breast cancer or its treatment could result in altered circulating enterolactone levels (e.g. due to changes in dietary intake post-diagnosis, or changes in gut flora as a result of breast cancer treatment), making retrospective case-control study results less reliable. On the other hand, results from 10 prospective studies, with a range of sample sizes, menopausal status of participants, and biospecimen types, have been inconsistent [31–40]. Seven reported an overall null association, while two studies reported inverse associations [37,40], and one reported an increased breast cancer risk for either very low or very high plasma enterolactone concentrations [32]. Two studies have reported inverse associations among either ER− or ER+ subgroups [33,37]. The variation in results may be due, in part, to relatively small number of cases in 6 out of the 10 studies (range 88–300), but more importantly due to heterogeneity of the study populations. Several studies observed an inverse association between enterolactone levels and breast cancer risk among postmenopausal women [29,37,38], and the other study that observed an inverse association only included women with palpable cysts and adjusted for cyst type in the analysis [40]. Further, three studies measured enterolactone in urine [31,36,38] and eight studies measured enterolactone either in serum or plasma [32–37,39,40], although a strong correlation between enterolactone in urine and blood has been reported (r=0.94, $P<0.001$) [41]. Also the studies varied in the assay types. Seven studies used time-resolved fluoroimmunoassay [31–34,37,39,40] and three studies used mass spectrometry [35,36,38].

Our results are consistent with results from most prospective studies, in that no overall significant association was observed, including by ER status. In contrast with previous prospective studies, we were able to evaluate the association between enterolactone and breast cancer by premenopausal estradiol levels. With our large sample size and the collection of timed samples within menstrual cycle in premenopausal women, we observed a differential association by follicular estradiol levels, with an inverse association between enterolactone levels and breast cancer risk among women with low follicular estradiol levels. We did not observe a similar difference by luteal phase estrogens, even though enterolactone levels were measured in the luteal phase. Although no studies have examined whether plasma enterolactone levels differ by phase of the menstrual cycle, one study observed similar urinary levels in the follicular and luteal phases [57], such that a measure of luteal enterolactone likely represents exposure in the follicular phase. Our data suggest that enterolactone could have a protective effect only in a low-estrogen environment. While we did not observe similar effect modification by luteal phase estradiol, the median estradiol level is two to three times higher than that during the follicular phase [3]. We also did not observe an inverse association among postmenopausal women, who generally have low hormone levels. This may be because we had few postmenopausal women (N=222), and over 85% of those were using postmenopausal hormones at blood draw; thus, we did not have power to separately examine postmenopausal women not using hormones. Interestingly, other prospective studies that reported inverse associations support the hypothesis that enterolactone may only be important in a low-estrogen environment. First, the participants in the Sonestedt, et al., Goodman, et. al., and Zaineddin et. al. studies were mostly postmenopausal women whose endogenous hormone production was lower than that in premenopausal women [29,37,38]. Adjusting for postmenopausal hormone use strengthened the risk estimates in the Sonestedt et. al. study [37]. Second, the Boccardo et al. study observed that high serum enterolactone concentrations were associated with a reduced

breast cancer risk. They also evaluated associations by cyst type, and observed a suggestive inverse association only among type II cysts [40]. However, the Boccardo et al. study had only 383 participants and 18 breast cancer cases, which may have limited power. Experimental studies reported that estradiol concentrations are substantially lower in type II cysts [58]. This evidence suggests that it is necessary to either adjust for or stratify by estrogen environment to reveal a potential protective association between enterolactone and breast cancer risk.

Enterolactone is a phytoestrogen with complex and potentially contradicting properties, as it can act in both an estrogenic and antiestrogenic capacity [13,15]. Based on these results, it is possible that a protective effect of enterolactone may be important when mammary epithelium proliferation is low. Evidence suggests that mammary epithelium proliferation is significantly lower during the follicular phase, compared to that in the luteal phase [59,60], in part because estrogens, which are higher in the luteal phase, increase mammary epithelial proliferation and mitotic activity [61]. Another possibility is that enterolactone is important when estrogen receptor expression is low; some studies suggest that ER expression is lower during the follicular phase [62], though other studies observed constant expression of estrogen receptor status over time [63,64]. We observed no significant difference by ER status; however prior prospective studies in postmenopausal women have reported stronger inverse associations for ER- breast cancer [29,33]. Since our study is the first to report this potential interaction by follicular phase estradiol levels, replication in other large case-control studies nested in prospective cohort studies and more biologic studies exploring enterolactone mechanisms are warranted.

A strength of our study is that we used isotope dilution mass spectrometry to assay for enterolactone, which provided higher specificity and sensitivity than time-resolved fluoroimmunoassay [65]. Although the assay CVs were modest (~15%), this is likely because many women in our population had relatively low enterolactone concentrations. This measurement error may have modestly attenuated the association. Another strength of our study is the comprehensive collection of dietary and lifestyle information, as well as information on breast cancer subtypes. The prospective design of our study and the large number of incident breast cancer cases is another advantage, although we had limited statistical power to examine case subgroups. A limitation in our study is that we only measured plasma enterolactone concentration at one point in time, while the true exposure of interest is the long-term average level of enterolactone. The reliability study conducted within our cohort has shown that stability of circulating enterolactone was fair over 2 to 3 years (ICC=0.44; 95% CI: 0.22–0.68) [45]. In addition, any measurement error would be non-differential misclassification with respect to case-control status, and would have biased our results toward the null.

Conclusions

Overall, we did not observe an association between a biomarker of lignan intake, plasma enterolactone, and breast cancer risk in a large nested case-control study of primarily premenopausal US women. However, the association varied by follicular phase estradiol levels, with a significant inverse association observed among premenopausal women with low estradiol levels. This suggests that enterolactone may influence breast carcinogenesis only in a low estrogen environment. Further prospective studies with sufficient sample sizes, long follow-up, and accounting for circulating estrogen levels are needed to confirm our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This project was supported by the National Institutes of Health (NIH) R01 CA67272, R01 CA50385, and P30 CA71789. J.X. is supported by scholarships from Harvard University. M.S.R. is supported by NIH T32 CA09001. We thank participants of the Nurses' Health Study II for their longstanding contributions and support to this study. Laurie Custer, University of Hawai'i Cancer Center, is acknowledged for her skillful performance of the LCMS assay. We thank the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, and WY.

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Table 1

Selected characteristics of participants across quartiles of enterolactone levels among controls in the nested case-control study of breast cancer

	Enterolactone (nmol/L)			
	Q1	**Q2**	**Q3**	**Q4**
N	209	197	196	200
Median (10–90 percentile)				
Enterolactone, nmol/L	1.1 (0–4.0)	8.0 (5.0–10.9)	16.0 (12.0–20.0)	34.0 (23.0–83.5)
Enterolactone (premenopausal), nmol/L	1.5 (0–4.0)	7.7 (5.0–10.0)	16.0 (12.0–20.0)	34.0 (23.3–85.0)
Enterolactone (postmenopausal), nmol/L	1.0 (0–4.0)	8.0 (6.0–11.0)	15.5 (12.0–19.0)	40.0 (24.9–66.0)
Mean (SD)				
Age [a], y	45.2 (4.4)	44.8 (4.2)	44.2 (4.8)	45.5 (4.2)
BMI, kg/m^2	27.7 (6.4)	26.0 (5.5)	25.1 (5.2)	24.5 (4.3)
BMI at age 18, kg/m^2	21.3 (3.4)	21.0 (2.9)	21.0 (3.0)	20.9 (2.5)
Weight change since age 18, lb	38.7 (33.9)	29.8 (29.2)	23.1 (25.8)	21.6 (25.3)
Duration of OC use among ever OC users, y	3.8 (3.6)	4.4 (4.2)	3.9 (4.0)	3.4 (3.4)
Parity among parous women	2.9 (1.3)	3.0 (1.3)	3.0 (1.3)	2.9 (1.2)
Alcohol intake, g/d	3.6 (7.5)	4.2 (8.3)	4.9 (8.4)	5.4 (8.3)
Physical activity, MET- hr/week	15.2 (19.1)	21.1 (35.4)	19.0 (22.2)	19.0 (18.8)
Age at menarche, y	12.5 (1.6)	12.6 (1.4)	12.5 (1.3)	12.4 (1.4)
Age at first birth, y	25.6 (4.7)	25.8 (4.4)	26.4 (4.5)	27.3 (4.8)
Luteal day of blood draw [ab]	7.9 (3.6)	7.1 (2.1)	7.5 (2.8)	7.5 (2.6)
Total calorie intake, kcal/d	1796 (564)	1813 (534)	1870 (580)	1805 (510)
Vegetable intake, cup/week	25.4 (14.4)	26.1 (14.5)	25.0 (8.7)	27.8 (10.2)
Whole grains intake, cup/week	8.7 (4.2)	8.4 (4.2)	9.3 (3.9)	10.4 (5.4)
Fiber, g/d	5.2 (2.1)	5.4 (2.2)	5.6 (2.2)	6.1 (2.5)
Caffeine, mg/d	205 (198)	207 (189)	240 (195)	241 (193)
Tofu, g/d	5.7 (22.3)	8.5 (31.2)	11.3 (34.0)	14.2 (31.2)
Estradiol (follicular), pg/mL	52.5 (44.3)	60.4 (43.9)	56.4 (37.1)	54.7 (36.6)
Estradiol (luteal), pg/mL	138 (107)	150 (85)	147 (77)	152 (74)
Percentage				
Caucasian [a]	96.7	99.5	98.5	98.5
Family history of breast cancer	8.6	10.7	11.2	10.0
History of benign breast disease	14.8	19.3	14.3	22.5
Antibiotics use in last month	17.7	11.2	7.7	8.0
Current smokers	12.4	6.1	4.1	4.0
Post-menopausal at blood collection [a]	18.2	10.7	12.2	14.5
Post-menopausal hormone use at blood collection among post-menopausal women [a]	83.6	87.8	87.0	83.3

[a] Matching factors

[b] Date of next menstrual cycle minus date of blood collection

Table 2

Multivariate-adjusted RR (95% CI) for breast cancer by quartiles of plasma enterolactone

		Enterola ctone (nmol/L)				
All	**Cases**	**Q1**	**Q2**	**Q3**	**Q4**	***P* trend[a]**
Cases/controls		199/209	227/197	179/196	197/200	
Cut-points[b] (nmol/L)		0–4	>4–11	>11–21	>21	
Simple RR[c]	802	1.00	1.23 (0.92–1.64)	0.97 (0.72–1.30)	1.05 (0.79–1.40)	0.97
RR[d]	802	1.00	1.18 (0.86–1.62)	0.91 (0.66–1.25)	0.96 (0.70–1.33)	0.60
By subtype		Q1	Q2	Q3	Q4	*P* trend[a]
Invasive	553	1.00	1.32 (0.95–1.81)	1.15 (0.83–1.60)	1.16 (0.83–1.61)	0.38
In situ	218	1.00	1.06 (0.70–1.60)	0.72 (0.46–1.13)	0.82 (0.53–1.26)	0.22
ER+ PR+ [e]	373	1.00	1.49 (1.04–2.15)	1.23 (0.84–1.80)	1.23 (0.84–1.80)	0.28
ER- PR- [e]	89	1.00	0.97 (0.51–1.84)	1.18 (0.64–2.19)	1.05 (0.56–1.97)	0.76
ER+ PR- [e]	55	1.00	1.22 (0.56–2.67)	0.74 (0.31–1.75)	0.77 (0.32–1.82)	0.46

[a] Linear trend test across log transformed median enterolactone levels of each quartile using the Wald test.

[b] Cut-points obtained from controls

[c] Conditioning on matching factors.

[d] Conditioning on matching factors and adjusting for family history of breast cancer (yes, no), history of benign breast disease (yes, no), OC use (never, ≤1yr, >1–5yr, >5yr), BMI at age 18 (continuous), weight change since age 18 (continuous), age at first birth and parity (nulliparous, 1–2 children and <25 y, 1–2 children and 25–29 y, 1–2 children and ≥30 y, ≥3 children and <25 y, ≥3 children and ≥25 y), age at menarche (continuous), antibiotics use within last month (yes, no), alcohol consumption (continuous), smoking (never, past and <10 y since quitting, past and ≥10 y since quitting, current), and physical activity (0–3.8, >3.8–11.1, >11.1–26.4, >26.4 MET-hrs/wk).

[e] Polytomous logistic regression adjusting for matching factors and the same covariates as listed above. P-heterogeneity were 0.21 and 0.56 for invasive versus in situ and ER+/PR+, ER−/PR−, ER+/PR− tumors respectively.

Table 3

Multivariate-adjusted breast cancer RR (95% CI) by quartiles of plasma enterolactone from stratified analyses

Stratified by	Cases	Enterolactone (nmol/L) Q1	Q2	Q3	Q4	*P* trend[a]
Pre-menopausal at blood draw	614	1.00	1.21(0.87–1.69)	0.84(0.59–1.19)	0.85(0.60–1.21)	0.26
Post-menopausal at blood draw	110	1.00	1.30(0.50–3.37)	1.94(0.82–4.59)	1.51(0.63–3.64)	0.20
Pre-menopausal at diagnosis	408	1.00	1.23(0.81–1.86)	0.71(0.46–1.09)	0.80(0.51–1.25)	0.15
Post-menopausal at diagnosis	302	1.00	1.25(0.76–2.04)	1.10(0.66–1.85)	1.23(0.75–2.01)	0.44
Estradiol<47 pg/mL (follicular)	229	1.00	1.32(0.75–2.32)	0.64(0.36–1.15)	0.49(0.27–0.91)	0.02
Estradiol≥47 pg/mL (follicular)	220	1.00	1.23(0.67–2.27)	1.03(0.55–1.93)	1.39(0.73–2.65)	0.44
Estradiol<132 pg/mL (luteal)	243	1.00	1.39(0.81–2.38)	1.07(0.62–1.83)	1.09(0.61–1.95)	0.76
Estradiol ≥ 132	228	1.00	0.95(0.53–1.72)	0.53(0.28–1.01)	0.77(0.42–1.44)	0.19

[a]Linear trend test across log transformed median enterolactone levels of each quartile using the Wald test.

P for interactions were 0.26, 0.26, 0.02, and 0.15 for postmenopausal status at blood draw, postmenopausal status at diagnosis, median estradiol during the follicular phase, and median estradiol during the luteal phase respectively.