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ORIGINAL REPORT

Serum Enterolactone and Prognosis of Postmenopausal Breast Cancer

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ABSTRACT

Purpose
Lignans—plant-derived compounds with estrogen-dependent and -independent anticarcinogenic properties—have been associated with postmenopausal breast cancer risk, but data are limited regarding their effect on survival. Dietary lignans are metabolized to enterolignans, which are subsequently absorbed and become bioavailable.

Patients and Methods
We assessed the prognosis of 1,140 postmenopausal patients with breast cancer age 50 to 74 years who were diagnosed between 2002 and 2005. Vital status through the end of 2009 was ascertained via local population registries, and deaths were verified by death certificates. Information on recurrences and secondary tumors was verified by clinical records and attending physicians. Associations of postdiagnostic serum enterolactone (a biomarker for dietary lignans) with overall survival and distant disease-free survival were assessed by using Cox proportional hazards models stratified by age at diagnosis and adjusted for prognostic factors.

Results
Median enterolactone levels for deceased patients and those still alive were 17.0 and 21.4 nmol/L, respectively. During a median of 6.1 years of follow-up after diagnosis, 162 deaths were confirmed. Higher serum enterolactone levels were associated with significantly reduced hazard ratios (HRs) for death (HR per 10 nmol/L increment, 0.94; $P = .04$; HR for the highest quartile, 0.58; 95% CI, 0.34 to 0.99). For distant disease, HR was 0.94 per 10 nmol/L increment ($P = .08$) and 0.62 (95% CI, 0.35 to 1.09) for the highest quartile. The highest quartile of serum enterolactone was associated with a significantly reduced risk of death only for estrogen receptor–negative tumors (HR, 0.27; 95% CI, 0.08 to 0.87) but not for estrogen receptor–positive tumors (HR, 0.91; 95% CI, 0.45 to 1.84: P for heterogeneity = .09).

Conclusion
Postmenopausal patients with breast cancer who have high serum enterolactone levels may have better survival.

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INTRODUCTION

Lignans are plant-derived substances that make up one class of phytoestrogens with estrogen-dependent and -independent anticarcinogenic properties.[1,2] Lignans are thought to be the major source of phytoestrogens in Western populations and are present in seeds, grains, fruits, and vegetables.[3,4] In humans, they are metabolized by the gut microflora into enterolignans, with enterolactone as the main metabolite.[5-7]

Because of the structural features they share with mammalian estrogens, lignans can bind to estrogen receptors (ERs) and thereby prevent the binding of endogenous estrogens. This may result in a rise in sex hormone–binding globulin and a subsequent inhibition of 17β-hydroxysteroid dehydrogenases, leading to an increased clearance of steroids from blood circulation and less growth stimulation of breast cancer cells.[2,8] In animal studies, lignans were also shown to exert ER-independent mechanisms such as inhibition of angiogenesis, tumor growth, and metastasis, as well as stimulation of apoptosis.[9-11]

A recent meta-analysis[12] showed that dietary lignans are associated with postmenopausal breast cancer risk in women from Western countries, although no association for circulating enterolactone levels was found. Epidemiologic evidence of the potential effects of lignans and enterolignans on breast

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cancer prognosis is limited. So far, two studies in the United States[13,14] assessed the association between dietary lignans and breast cancer prognosis. Dietary lignans were found to be associated with decreased mortality of postmenopausal women in one study[13] but not in the other.[14] Both studies reported a nonsignificantly increased mortality associated with higher intakes of dietary lignans in premenopausal women.

In contrast to dietary assessments, biomarkers of phytoestrogens account for interindividual variation in metabolism of dietary phytoestrogens by intestinal microflora and subsequent absorption of phytoestrogens.[15,16] To the best of our knowledge, this is the first study to investigate whether postdiagnostic serum levels of the enterolignan enterolactone are associated with overall survival (OS) and distant disease-free survival (DDFS) in postmenopausal patients with breast cancer. Further, we investigated whether this association differs by characteristics of the tumor (ie, ER status, tumor size, and grade).

PATIENTS AND METHODS

Study Population

Our study population comprised patients with breast cancer who participated in a population-based case-control study conducted in two study regions (Hamburg and Rhine-Neckar-Karlsruhe) in Germany.[17] Patients were diagnosed between August 1, 2002, and July 31, 2005, with a histologically confirmed primary invasive (stages I to IV) or in situ breast tumor and were age 50 to 74 years. Cases were identified through frequent monitoring of participating clinics. Women were defined to be postmenopausal if they reported their last menstrual bleeding was at least 12 months before diagnosis or if they had a bilateral oophorectomy. Patients older than age 55 years with unclear menopausal status because of hysterectomy or hormone use were also considered postmenopausal. For this study, we included 1,164 of the 1,559 recruited postmenopausal patients from one study region (Rhine-Neckar-Karlsruhe). Eighty-one patients were excluded because of a previous diagnosis of cancer (except for in situ carcinoma or basal and squamous cell skin carcinoma) or missing information on prior cancer. Patients without a serum sample or a hemolytic sample (n = 314) were also excluded.

The study was approved by the ethics committee of both the University of Heidelberg and the University of Hamburg and was conducted in agreement with the Helsinki Declaration. Written informed consent was provided by all participants at baseline and during follow-up.

At baseline, in-person interviews were performed to collect information on demographic factors, established and suggested breast cancer risk factors, and possible prognostic factors. Data on prognostic factors were obtained from clinical and pathologic records.

Blood Samples and Laboratory Measurements

Nonfasting serum samples provided at recruitment by the patients were stored at −80°C. Median time between diagnosis and blood collection was 101 days (range, 2 to 1,112 days; standard deviation, 179 days).

Enterolactone was measured by using time-resolved fluoroimmunoassays (TR-FIA; Labmaster, Turku, Finland) according to the validated methods.[18] This consisted of hydrolysis and liquid extraction steps followed by the TR-FIA analysis. In brief, 150 μL of serum was hydrolyzed with an acetate buffer containing beta-glucuronidase and sulfatase. Diethyl ether was used for the extraction of free enterolactone and hydrolyzed conjugates. The dried ether phases were dissolved in assay buffer and measured by using (europium-labeled) TR-FIA. All batches were analyzed blinded. The first 160 samples were measured in duplicate to assess intra-assay coefficients of variation (CVs), which were adequately low. Therefore, the remaining samples were analyzed in single measurements. Two quality control samples (mean concentrations, 33.2 and 33.0 nmol/L) at various locations on the plates were measured. The mean intra-assay and interassay CVs were 7.2% and 14.6%, respectively. Twenty-four samples with serum enterolactone below the detection limit were excluded, resulting in a sample of 1,140 patients used for the statistical analyses.

Outcome Assessment

For all patients, vital status through the end of 2009 was ascertained via local population registries, which were complete for all patients. Causes of death were verified by death certificates and were coded according to the International Classification of Diseases, 10th Revision (ICD-10) classifications. Recurrences and secondary tumors were collected from self-reports during a follow-up telephone interview conducted from May to September 2009 with additional verification of the self-reported events by using clinical records and attending physicians or directly via clinical records and attending physicians when self-reports were not available. The primary and secondary end points of interest were OS and DDFS, respectively, specified according to the proposed standardized definitions.[19] Only patients with early-stage disease (stages 0 to IIIA) were included for the analysis of DDFS, thereby excluding 178 patients with late-stage (IIIB, IIIC, and IV) disease, with neoadjuvant chemotherapy (NACT; therefore, stage unknown), or with unknown status on distant recurrences. Women without an event of interest were censored either at date of last information or at the end of 2009.

Statistical Analysis

The differences in enterolactone levels between deceased patients and those who were still alive were tested by using the Kruskal-Wallis test.

Kaplan-Meier curves were used to visualize the association of serum enterolactone quartiles with OS and DDFS. Cox proportional hazards models were used for estimating hazard ratios (HRs) and 95% CIs for enterolactone levels as a continuous variable (per 10 nmol/L increment) and in quartiles by using the lowest quartile as the reference category. Median follow-up time was calculated as the time between diagnosis and the event of interest or censoring by using the reverse Kaplan-Meier estimation.[20]

All analyses were stratified by age at diagnosis (in 1-year categories). Multivariate analyses were adjusted for the traditional prognostic factors (ie, tumor size of < 2, 2 to 5, or ≥ 5 cm; growth into chest wall; NACT-treated carcinoma; and in situ carcinoma), nodal status (0, 1 to 3, 4 to 9, ≥ 10; NACT-treated carcinoma; and in situ carcinoma), metastasis (yes, no and in situ carcinoma), grade (1 to 2, 3; NACT-treated carcinoma; and in situ carcinoma), and ER/progesterone receptor (PR) status (ER-positive/PR-positive, ER-positive/PR-negative or ER-negative/PR-positive, ER-negative/PR-negative; NACT-treated carcinoma; and in situ carcinoma). Further covariates in multivariate models were selected by using a backward elimination procedure based on $P < .05$ in the likelihood ratio test for the covariate or a more than 10% change in HR for enterolactone. Adult body mass index (< 18.5, 18.5 to 24.9, 25 to 29.9, or ≥ 30 kg/m^2), mode of detection (physician detected by routine investigation, mammography, or ultrasound; or self detected by palpation, secretion, or pain), hormone replacement therapy use at diagnosis (current, never/past), diabetes (yes, no), and leisure time physical activity since the age of 50 years (< 28 metabolic equivalent h/wk, ≥ 28 metabolic equivalent h/wk) were thus included in the final multivariate models. Cardiovascular disease, human epidermal growth factor receptor 2 (HER2/neu) status, radiotherapy, chemotherapy, tamoxifen use, alcohol consumption, smoking habits, energy intake, occupational status, phytoestrogen supplementation use, and time between diagnosis and blood collection were tested but not included. Tests for trend were performed by using continuous values of the serum enterolactone levels. The proportional hazards assumption was tested by using the Grambsch and Therneau[21] test and was found to hold for the final model.

Fractional polynomials were used to assess the functional form of the enterolactone levels in the Cox model in which the continuous serum enterolactone variable was entered transformed by a set of defined functions (x^{-2}, x^{-1}, $x^{-0.5}$, $\log(x)$, $x^{0.5}$, x^1, x^2, x^3) allowing maximally two terms.[22] The model with the best fit based on the -2 log likelihood was selected. The concordance (c) index and R^2 were used to assess the predictive discriminatory capability and internal validity of the multivariate model, respectively.[23,24] The measures were calculated from 1,000 bootstrap samples and the mean values and 95% CIs of all samples are presented.

Several sensitivity analyses with the OS end point were performed: subgroup analyses were restricted to patients with stages I to IIIA disease, by median time between diagnosis and blood collection, and for blood collection

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Table 1. Descriptive Covariates With Univariate HRs for Overall Survival of 1,140 Postmenopausal Patients With Breast Cancer*

Variable	Total Population		Univariate			Enterolactone Levels (nmol/L)	
	No.	%	HR†	95% CI	*P*	Median	IQR
All patients	1,140					20.8	34.6
Deaths	162					17.0	27.9
Tumor size, cm							
< 2	564	49.5	1.00 (Ref)		(Ref)	23.6	35.8
2-5	365	32.0	3.42	2.27 to 5.14	< .01	19.3	32.2
≥ 5	39	3.4	6.45	3.35 to 12.40	< .01	18.3	24.3
Growth into chest wall	34	3.0	9.46	5.13 to 17.45	< .01	13.9	25.4
NACT	68	6.0	7.80	4.53 to 13.41	< .01	12.9	25.9
In situ	67	5.9	0.52	0.13 to 2.17	.37	25.9	48.3
Nodal status							
0	662	58.1	1.00 (Ref)		(Ref)	21.9	35.7
1-3	222	19.5	1.88	1.22 to 2.92	.01	18.4	32.5
4-9	64	5.6	2.95	1.61 to 5.40	< .01	17.8	26.3
≥ 10	53	4.7	11.36	7.04 to 18.33	< .01	22.2	31.6
NACT	68	6.0	6.13	3.68 to 10.20	< .01	12.9	25.9
In situ	67	5.9	0.40	0.10 to 1.63	.20	25.9	48.3
Metastasis							
No	1,019	90.3	1.00 (Ref)		(Ref)	19.5	33.8
Yes	44	3.9	10.53	6.79 to 16.31	< .01	31.8	41.4
In situ	67	5.9	0.24	0.06 to 0.99	.05	25.9	48.3
Grade							
1-2	726	63.7	1.00 (Ref)		(Ref)	22.4	34.5
3	275	24.1	2.67	1.90 to 3.77	< .01	17.6	30.2
NACT	68	6.0	4.98	3.05 to 8.13	< .01	12.9	25.9
In situ	67	5.9	0.31	0.08 to 1.28	.11	25.9	48.3
ER/PR status							
ER-positive/PR-positive	616	54.0	1.00 (Ref)		(Ref)	22.9	33.3
ER-positive/PR-negative or ER-negative/ PR-positive	203	17.8	1.59	1.03 to 2.45	.04	20.1	39.1
ER-negative/PR-negative	185	16.2	2.68	1.78 to 4.03	< .01	15.7	30.9
NACT	68	6.0	4.92	2.99 to 8.10	< .01	12.9	25.9
In situ	67	5.9	0.31	0.08 to 1.26	.10	25.9	48.3
HER2/neu status							
Positive	202	17.7	1.44	0.95 to 2.17	.09	16.3	30.4
Negative	756	66.3	1.00 (Ref)		(Ref)	21.5	35.0
NACT	68	6.0	1.17	0.70 to 1.93	.55	12.9	25.9
In situ	67	5.9	0.24	0.06 to 0.99	.05	25.9	48.3
HRT use at diagnosis							
Past/never	675	59.2	1.00 (Ref)		(Ref)	17.8	33.2
Current	454	39.8	0.48	0.34 to 0.70	< .01	23.9	35.1
Chemotherapy							
No	617	54.1	1.00 (Ref)		(Ref)	24.9	37.1
Adjuvant	445	39.0	2.50	1.76 to 3.57	< .01	16.5	30.9
Neoadjuvant	68	6.0	6.13	3.66 to 10.25	< .01	12.9	25.9
Radiotherapy							
No	272	23.9	1.00 (Ref)		(Ref)	21.9	36.2
Yes	866	76.0	0.53	0.38 to 0.74	< .01	20.1	33.6
Tamoxifen use							
No	346	30.4	1.00 (Ref)		(Ref)	18.7	34.4
Yes	682	59.8	0.83	0.58 to 1.18	.29	22.3	33.7
Aromatase inhibitor use							
No	558	49.0	1.00 (Ref)		(Ref)	19.9	34.9
Yes	529	46.4	1.10	0.78 to 1.55	.59	21.9	33.3
Phytoestrogen supplement use							
No	1,100	99.4	1.00 (Ref)		(Ref)	20.9	34.6
Yes	7	0.6	2.52	0.31 to 20.63	.39	9.9	18.0

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Table 1. Descriptive Covariates With Univariate HRs for Overall Survival of 1,140 Postmenopausal Patients With Breast Cancer* (continued)

Variable	Total Population		Univariate			Enterolactone Levels (nmol/L)	
	No.	%	HR†	95% CI	*P*	Median	IQR
Mode of detection							
Self detected	670	58.8	1.00 (Ref)		(Ref)	17.8	33.3
Physician detected	464	40.7	0.32	0.21 to 0.47	< .01	23.7	35.9
Surgery type							
Ablation	30	2.6	1.00 (Ref)		(Ref)	39.9	48.8
Ablation + axilla	301	26.4	1.70	0.68 to 4.27	.26	17.8	30.9
BCT	130	11.4	0.24	0.07 to 0.85	.03	24.5	44.8
BCT + axilla	667	58.5	0.71	0.28 to 1.78	.47	21.4	34.1
Age at diagnosis, years							
50-54	89	7.8	1.00 (Ref)		(Ref)	13.0	24.3
55-59	236	20.7	1.24	0.59 to 2.61	.57	16.6	33.2
60-64	362	31.8	1.36	0.67 to 2.76	.40	24.0	35.1
65-69	308	27.0	1.58	0.78 to 3.22	.21	22.3	35.2
70-74	145	12.7	1.86	0.87 to 3.97	.11	26.8	37.3
BMI, kg/m^2							
< 18.5	26	2.3	1.77	0.77 to 4.09	.18	39.0	52.5
18.5-24.9	818	71.8	1.00 (Ref)		(Ref)	22.3	33.7
25-29.9	249	21.8	1.27	0.88 to 1.83	.20	15.6	30.4
≥ 30	47	4.1	1.94	1.02 to 3.68	.04	16.3	41.6
Smoking history							
Never	716	62.8	1.00 (Ref)		(Ref)	22.0	35.3
Past	268	23.5	1.04	0.70 to 1.54	.85	22.1	35.1
Current	156	13.7	1.60	1.02 to 2.50	.04	15.8	32.5
Leisure physical activity (age 50)							
< 28 MET h/wk	400	35.1	1.00 (Ref)		(Ref)	18.5	35.5
≥ 28 MET h/wk	735	64.5	0.71	0.52 to 0.98	.03	21.6	34.1
Cardiovascular disease							
No	556	48.8	1.00 (Ref)		(Ref)	21.2	33.6
Yes	584	51.2	1.39	1.00 to 1.94	.05	20.1	35.5
Diabetes							
No	1,019	89.4	1.00 (Ref)		(Ref)	20.0	34.0
Yes	120	10.5	1.39	0.89 to 2.15	.15	24.4	37.1

Abbreviations: BCT, breast conservation therapy; BMI, body mass index; ER, estrogen receptor; HER2/neu, human epidermal growth factor receptor 2; HR, hazard ratio; HRT, hormonal replacement therapy; IQR, interquartile range; MET, metabolic equivalent; NACT, neoadjuvant chemotherapy; PR, progesterone receptor; Ref, reference.

*Numbers do not always add up to total because of missing values.

†Stratified by age at diagnosis.

before (including no chemotherapy) versus after start of adjuvant chemotherapy. In addition, possible effect modification by tumor characteristics (ER status: positive, negative; ER/PR status: ER-positive or PR-positive, ER-negative/PR-negative; tumor size: < 5 cm, ≥ 5 cm; and tumor grade: < 2, 3) was assessed, while excluding patients with unknown status for these characteristics. Heterogeneity was tested by using the *Q* statistic.

All tests were two-sided with a significance level of 0.05 and were performed by using R, version 2.9.2 (R Foundation for Statistical Computing, Vienna, Austria)[25] and SAS, version 9.2 (SAS Institute, Cary, NC) statistical software. Because of the exploratory nature of the study, no formal adjustment for multiple testing was performed.

RESULTS

Phytoestrogen Levels

Overall median level of enterolactone was 20.8 nmol/L (interquartile range, 34.6 nmol/L), and the levels differed significantly between deceased patients (median, 17.0 nmol/L) and those who were still alive (median, 21.4 nmol/L; $P = .04$).

Patients with higher enterolactone levels tended to have a higher proportion of tumors with smaller tumor size, a lower tumor grade, hormone receptor–positive tumors, and physician-detected tumors compared with patients with lower enterolactone levels (Table 1). They were also more likely to have used hormone replacement therapy at diagnosis, not received chemotherapy, have a lower body mass index, and have a never/past smoking history. In addition, enterolactone levels were higher for older women than for younger women. Variable distribution by quartile of enterolactone can be found in Appendix Table A1 (online only).

Prognostic Associations of Phytoestrogens

Mean age of the patients included in the study was 62.8 ± 5.6 years. During a median follow-up time of 6.1 years after diagnosis (range, 0.2 to 7.7 years), 162 deaths occurred in 1,140 patients. Besides 124 deaths (76.5%) from breast cancer, causes of death included other cancers (8.0%; n = 13), cardiovascular diseases (9.3%; n = 15), and other causes (6.2%; n = 10). In the 962 patients with early-stage

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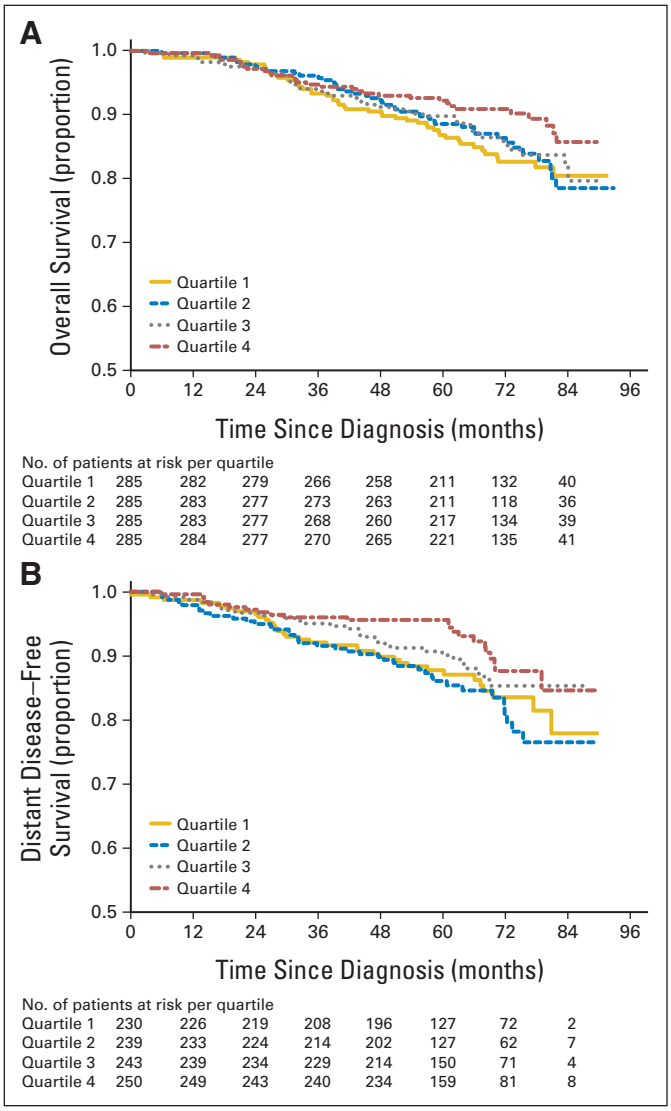

Fig 1. Unadjusted (A) overall survival and (B) distant disease–free survival in postmenopausal patients with breast cancer by quartiles of serum enterolactone concentration, including patients at risk, per 12-month time intervals.

disease included for the DDFS analysis, 124 distant recurrences or deaths occurred.

Higher serum enterolactone levels were associated with a significantly reduced HR for overall mortality (multivariate HR per 10 nmol/L increment, 0.94; 95% CI, 0.88 to 1.00; $P = .04$), and the highest versus the lowest enterolactone quartile was associated with a significantly reduced risk for death (multivariate HR, 0.58; 95% CI, 0.34 to 0.99; Fig 1; Table 2). Serum enterolactone was also associated with a similarly reduced yet nonsignificant HR for distant disease (multivariate HR was 0.94; 95% CI, 0.87 to 1.01; $P = .08$ per 10 nmol/L increment and 0.62; 95% CI, 0.35 to 1.09 for the highest quartile). Modeling with fractional polynomials resulted in linear associations between the log HR and enterolactone levels for OS and DDFS. The predictive discriminatory capability of the multivariate model was found to be high (c index = 0.809; 95% CI, 0.808 to 0.811), and $R^2 = 0.190$ (95% CI, 0.189 to 0.191) documents internal validity.

In the subgroup of 902 early-stage (stages I to IIIA) patients with breast cancer who receive more standardized adjuvant treatment, the HR for death associated with the highest versus the lowest enterolactone levels was not substantially different compared with that for the total population (P for heterogeneity = .94) albeit not statistically significant (multivariate HR, 0.95; 95% CI, 0.88 to 1.02 per 10 nmol/L increment and HR, 0.56; 95% CI, 0.27 to 1.14 for the highest quartile; Appendix Table A2, online only).

The association between enterolactone and overall mortality was not significantly heterogeneous for time between diagnosis and blood collection below or above the median, with HRs for death for the highest compared with the lowest quartile of 0.60 (95% CI, 0.27 to 1.32) and 0.59 (95% CI, 0.26 to 1.34), respectively (P for heterogeneity = .98). There was also no significant heterogeneity between patients who did not receive chemotherapy or with blood collected before chemotherapy (73%; n = 827) and patients with blood collected after the start of chemotherapy (26%; n = 295; multivariate HR, 0.56; 95% CI, 0.25 to 1.25 and HR, 0.38; 95% CI, 0.16 to 0.90 for the highest quartile; P for heterogeneity = .57).

When assessed according to ER status, the association between enterolactone and overall mortality was statistically significant only for ER-negative tumors in the highest compared with the lowest quartile (HR, 0.27; 95% CI, 0.08 to 0.87) and not for ER-positive tumors (HR, 0.91; 95% CI, 0.45 to 1.84; P for heterogeneity = .09; Table 3). Similar associations were observed by ER/PR status, although none of the HRs was significant (Appendix Table A3, online only). Effect heterogeneity by tumor size or by grade was also not observed (data not shown).

DISCUSSION

Our study provides, for the first time (to the best of our knowledge), evidence for an association between high postdiagnostic serum enterolactone levels and increased OS in postmenopausal patients with breast cancer.

The two studies that assessed the association between dietary lignans and breast cancer prognosis were inconsistent. The Western New York Exposures and Breast Cancer (WEB) Study[13] reported that dietary lignan intake was associated with significantly reduced hazard ratios for all-cause and breast cancer–specific mortality in postmenopausal women, comparing the highest (> 318 μg/d) to the lowest (< 155 μg/d) category of intake. Their risk estimate for all-cause mortality associated with high dietary lignans (HR, 0.49) is similar to that observed in our study population. In the Long Island Breast Cancer Study Project (LIBCSP), the highest (≥ 9 mg/d) compared with the lowest (≤ 2.2 mg/d) quintile of dietary lignan intake was not associated with all-cause mortality in postmenopausal women.[14]

The discrepancy in results may be partly due to differences in the estimation of dietary lignan intake. The WEB Study estimated intake of four lignans from diet by using a phytoestrogen database,[7] whereas the LIBCSP reported on total lignan intake that was estimated by using the lignan content of foods extracted from the literature. The range of lignan intake was higher in the LIBCSP (0 to > 9 mg/d) when compared with that in the more recent WEB Study (mean, approximately 245 μg/d). In our study, median intake of total plant lignans was 1,043 μg/d, and median enterolactone intake was 245 μg/d. However, correlation between estimated dietary and serum enterolactone levels was

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Table 2. Prognostic Associations of Serum Enterolactone Levels With Overall Survival and Distant Disease in 1,140 Postmenopausal Patients With Breast Cancer

Variable	Survival Measure					
	Overall			Distant Disease		
	HR	95% CI	*P* Trend	HR	95% CI	*P* Trend
Univariate*						
Serum enterolactone						
Q1†	1.00 (Ref)			1.00 (Ref)		
Q2†	0.88	0.58 to 1.35		1.16	0.72 to 1.87	
Q3†	0.84	0.55 to 1.28		0.78	0.46 to 1.30	
Q4†	0.58	0.36 to 0.92		0.55	0.32 to 0.95	
Continuous (per 10 nmol/L increment)	0.94	0.89 to 0.99	.01	0.94	0.88 to 1.00	.06
Multivariate‡						
Serum enterolactone						
Q1†	1.00 (Ref)			1.00 (Ref)		
Q2†	0.89	0.56 to 1.40		1.16	0.70 to 1.91	
Q3†	0.78	0.49 to 1.27		0.93	0.54 to 1.62	
Q4†	0.58	0.34 to 0.99		0.62	0.35 to 1.09	
Continuous (per 10 nmol/L increment)	0.94	0.88 to 1.00	.04	0.94	0.87 to 1.01	.08
Covariables in multivariate model†						
Tumor size, cm						
< 2	1.00 (Ref)			1.00 (Ref)		
2-5	1.85	1.16 to 2.95		1.64	1.05 to 2.54	
≥ 5	2.10	0.97 to 4.53		1.98	0.80 to 4.89	
Growth into chest wall	2.67	1.27 to 5.62		—	—	
Neoadjuvant chemotherapy	8.04	4.12 to 15.67		—	—	
In situ	1.06	0.25 to 4.53		1.50	0.57 to 3.95	
Nodal status§						
0	1.00 (Ref)			1.00 (Ref)		
1-3	1.24	0.77 to 2.00		1.08	0.68 to 1.72	
4-9	1.66	0.86 to 3.24		1.44	0.73 to 2.86	
≥ 10	4.64	2.64 to 8.15		—	—	
Metastasis§						
No	1.00 (Ref)			—	—	
Yes	6.66	3.91 to 1.33		—	—	
Grade§						
1-2	1.00 (Ref)			1.00 (Ref)		
3	1.19	0.77 to 1.83		1.61	1.01 to 2.57	
ER/PR status§						
ER-positive/PR-positive	1.00 (Ref)			1.00 (Ref)		
ER-positive /PR-negative or ER-negative/ PR-positive	1.75	1.09 to 2.83		1.45	0.89 to 2.38	
ER-negative /PR-negative	2.29	1.39 to 3.76		1.46	0.85 to 2.50	
Mode of detection						
Self detected	1.00 (Ref)			1.00 (Ref)		
Physician detected	0.60	0.38 to 0.93		0.56	0.35 to 0.88	
BMI, kg/m^2						
< 18.5	1.98	0.79 to 4.96		2.00	0.75 to 5.35	
18.5-24.9	1.00 (Ref)			1.00 (Ref)		
25-29.9	1.03	0.69 to 1.56		1.17	0.74 to 1.87	
> 30	1.15	0.54 to 2.46		1.29	0.58 to 2.89	
HRT use at diagnosis						
Past/never	1.00 (Ref)			1.00 (Ref)		
Current	0.68	0.45 to 1.02		0.78	0.51 to 1.20	
Diabetes						
No	1.00 (Ref)			1.00 (Ref)		
Yes	1.41	0.84 to 2.34		1.52	0.91 to 2.54	

(continued on following page)

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Table 2. Prognostic Associations of Serum Enterolactone Levels With Overall Survival and Distant Disease in 1,140 Postmenopausal Patients With Breast Cancer (continued)

Variable	Survival Measure					
	Overall			Distant Disease		
	HR	95% CI	*P* Trend	HR	95% CI	*P* Trend
Leisure physical activity (age 50)						
< 28 MET h/wk	1.00 (Ref)			1.00 (Ref)		
≥ 28 MET h/wk	0.82	0.57 to 1.17		0.82	0.55 to 1.23	

Abbreviations: BMI, body mass index; ER, estrogen receptor; HR, hazard ratio; HRT, hormone replacement therapy; MET, metabolic equivalent; PR, progesterone receptor; Q, quartile; Ref, reference.

*Stratified by age at diagnosis.

†In analysis of overall survival, median serum levels (nmol/L; min, max) per Q were Q1 = 3.4 (0.2, 7.8), Q2 = 13.9 (7.9, 20.2), Q3 = 29.7 (20.3, 42.1), Q4 = 64.1 (42.3, 300.0); in analysis of distant disease-free survival, Q1 = 3.5 (0.1, 8.0), Q2 = 14.1 (8.0, 20.8), Q3 = 29.3 (20.8, 42.4), Q4 = 65.0 (42.6, 300.0).

‡Stratified by age at diagnosis. For overall survival, adjusted for tumor size, nodal status, metastases, grade, ER/PR status, breast cancer detection type, diabetes, HRT use at diagnosis, BMI, and physical activity. For distant disease–free survival, adjusted for tumor size, nodal status, grade, ER/PR status, breast cancer detection type, diabetes, HRT use at diagnosis, BMI, and physical activity.

§HRs for the categories neoadjuvant chemotherapy and/or in situ are shown in the variable tumor size.

low ($r = 0.11$) in our study. The large difference between dietary intake and biomarker assessments indicates that direct comparison of our study results to results of the studies assessing dietary intake of lignans may not be appropriate. In line with our results, a small study[26] of postmenopausal patients with breast cancer (N = 424) recently reported better survival with high prediagnostic plasma enterolactone levels.

The biologic plausibility of our results is supported by findings from animal studies,[10,11] which have shown that enterolactone can inhibit both breast tumor formation and growth. The enterolactone precursor lariciresinol was also found to inhibit tumor growth and angiogenesis and to induce tumor cell apoptosis.[9] In an intervention study,[27] dietary supplementation with flaxseeds (rich in lignans) was associated with reduced tumor biologic markers (eg, Ki-67 labeling index) and an increased apoptosis in postmenopausal patients with breast cancer.

The association of enterolactone with OS was significant only for ER-negative tumors. Although the heterogeneity test of ER status was not significant, we may not have had sufficient power to detect a difference since the association with OS was moderate for ER-positive tumors. Thus, enterolactone may have both estrogenic and nonestrogenic effects on breast cancer survival. Several possible estrogen-independent mechanisms of actions of phytoestrogens on breast cancer risk and disease progression have been proposed, including antioxidant effects, induction of apoptosis, inhibition of tumor metastasis, and angiogenesis.[2,8] In experimental studies, flaxseed as well as enterolignans have been shown to inhibit breast cancer growth and metastases and downregulate expression of tumor growth factors, also of ER-negative human breast cancer.[28-32]

Sensitivity analyses were carried out to assess the robustness of our findings. Time between diagnosis and blood collection in our study was variable; however, subgroup analysis by and adjustment for

Table 3. Prognostic Associations of Serum Enterolactone Levels With Overall Survival and Distant Disease in Postmenopausal Patients With Breast Cancer by Estrogen Receptor Status*

ER Status	Overall Survival					Distant Disease				
	No. of Patients	No. of Events	Multivariate HR†	95% CI	*P* for Linear Trend	No. of Patients	No. of Events	Multivariate HR†	95% CI	*P* for Linear Trend
ER-positive										
Q1	179	21	1.00 (Ref)			150	18	1.00 (Ref)		
Q2	199	31	1.19	0.64 to 2.22		172	28	1.29	0.59 to 2.50	
Q3	212	29	0.98	0.51 to 1.89		186	22	1.18	0.59 to 2.36	
Q4	202	18	0.91	0.45 to 1.84		182	16	0.79	0.38 to 1.64	
Continuous (per 10 nmol/L increment)			0.96	0.89 to 1.04	.30			0.96	0.90 to 1.02	.19
ER-negative										
Q1	79	23	1.00 (Ref)			62	13	1.00 (Ref)		
Q2	66	10	0.48	0.17 to 1.30		55	9	0.40	0.11 to 1.40	
Q3	50	11	0.88	0.33 to 2.32		42	7	0.66	0.18 to 2.35	
Q4	49	9	0.27	0.08 to 0.87		42	6	0.41	0.10 to 1.66	
Continuous (per 10 nmol/L increment)			0.91	0.81 to 1.02	.10			0.99	0.83 to 1.18	.90

Abbreviations: BMI, body mass index; ER, estrogen receptor; HR, hazard ratio; HRT, hormone replacement therapy; Q, quartile; Ref, reference.

*Analyses included only women with known ER status and therefore excluded 104 and 71 patients for overall survival and distant disease–free survival analyses, respectively.

†Stratified by age at diagnosis. For overall survival, adjusted for tumor size, nodal status, metastases, grade, breast cancer detection type, diabetes, HRT use at diagnosis, BMI, and physical activity. For Distant Disease, adjusted for tumor size, nodal status, grade, breast cancer detection type, diabetes, HRT use at diagnosis, BMI, and physical activity.

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time between diagnosis and blood collection did not affect the risk estimates. Enterolactone levels may be influenced by chemotherapy, but we did not observe significant heterogeneity by blood collection before or after start of chemotherapy, and the risk estimates hardly changed after adjustment for chemotherapy. Although the timing of blood collection and chemotherapy after diagnosis did not appear to affect the associations, we do not know whether postdiagnostic enterolactone levels reflect prediagnostic levels. Results of the study on prediagnostic enterolactone levels suggest that the association may not be different. Thus, the relevant timing of enterolactone exposure as well as the threshold serum level of enterolactone required for possible protective effects on breast cancer prognosis warrants further investigation.

The strength of our population-based study is that all events of interest were ascertained actively and verified by using death certificates, medical records, and information from attending physicians and, therefore, misclassifications in the outcome variable are unlikely. We also used a serum biomarker, which provides an index of intake, metabolism, and absorption of phytoestrogens and is not prone to recall bias and misclassification. Therefore, it is likely to be a more sophisticated method for detecting associations compared with dietary intake levels. Moreover, good internal validity and high predictive discrimination capability were observed for the identified multivariate model.

However, like most epidemiologic studies, only one measure of enterolactone in nonfasting, postdiagnostic serum samples was used. Antimicrobial use at or before blood collection, which might have influenced the intestinal metabolism of lignans, was not recorded.[33] It is unknown whether chemotherapy may influence the intestinal microflora. These factors might have introduced interindividual variations.[34,35] Serum levels of enterolactone represent short-term (over several days) rather than long-term intake, and repeated measurement of enterolactone in blood samples from various time points could help to reduce these variations. In addition, the sample size may have been limited for assessing heterogeneity by tumor characteristics, and power was insufficient to conduct further subgroup analyses (eg, tamoxifen use or lifestyle factors). Residual confounding resulting from measurement errors or unmeasured variables could not be entirely ruled out, and the results might therefore be due to chance.

In conclusion, this is the first study showing that postdiagnostic enterolactone levels may be related to better survival after postmenopausal breast cancer. Further investigations in large prospective cohorts of patients with breast cancer are required to confirm our findings and to assess potential effect heterogeneity by ER status, adjuvant hormone therapy use, or genetic variants in the metabolism and biosynthesis of sex hormones.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

AUTHOR CONTRIBUTIONS

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