Plasma Enterolactone and Breast Cancer Incidence by Estrogen Receptor Status

Anja Olsen,[1] Knud Erik Bach Knudsen,[4] Birthe L. Thomsen,[1] Steffen Loft,[2] Connie Stripp,[1] Kim Overvad,[5] Susanne Møller,[3] and Anne Tjønneland[1]

[1]Institute of Cancer Epidemiology, Danish Cancer Society; [2]Institute of Public Health, University of Copenhagen; [3]Danish Breast Cancer Co-operative Group, Rigshospitalet, Copenhagen, Denmark; [4]Department of Animal Nutrition and Physiology, Research Centre Foulum, Tjele, Denmark; and [5]Department of Clinical Epidemiology, Aalborg Hospital and Aarhus University Hospital, and Department of Epidemiology and Social Medicine, University of Aarhus, Aarhus, Denmark

Abstract

The phytoestrogen enterolactone has been hypothesized to prevent breast cancer. Because one of the biological effects of enterolactone is probably estrogenic, it is possible that the preventive effect on breast cancer differs with the estrogen receptor (ER) α status of the tumor. The objective of this study was to investigate whether high plasma levels of enterolactone are associated with breast cancer risk and whether the ERα status of the tumor influences this relation. The cohort study Diet, Cancer and Health included 29,785 women, ages 50 to 64 years, between 1993 and 1997. Information about diet and life-style factors was obtained by questionnaire, and blood was drawn from each participant. We matched 381 postmenopausal breast cancer cases to 381 controls and analyzed the concentration of enterolactone in plasma with a time-resolved fluoroimmunoassay. Associations between plasma concentrations of enterolactone and breast cancer were analyzed by logistic regression. The incidence rate ratio (IRR) for all breast cancer was 0.93 [95% confidence interval (CI), 0.86-1.01] per 20 nmol/L higher plasma concentration of enterolactone. For ERα-positive cancers (n = 273) only a weak association was seen (IRR, 0.97; 95% CI, 0.88-1.06), whereas for ERα-negative cancers (n = 80; IRR, 0.71; 95% CI, 0.53-0.94) a protective effect was seen per 20 nmol/L higher plasma enterolactone. In accordance with earlier research, we found a tendency toward a lower risk for breast cancer with higher concentrations of enterolactone, which was restricted almost entirely to ERα-negative breast cancer. (Cancer Epidemiol Biomarkers Prev 2004; 13(12):2084–9)

Introduction

Phytoestrogens are biologically active compounds that affect hormone metabolism, intracellular enzymes, protein synthesis, growth factors, malignant cell proliferation, and angiogenesis (1). The most potent group of phytoestrogens, the isoflavonoids, is primarily found in soy and red clover. These foods, however, are eaten in very low amounts in the northern part of Europe. The main phytoestrogens in the northern European diet are lignans. Plant lignans occur as glycosides in whole grain, seeds, nuts, vegetables, berries, and beverages such as tea and coffee (2). In contrast to isoflavonoids, plant lignans do not have inherent estrogenic activity but are converted to the weakly estrogenic compounds enterolactone and enterodiol by microflora in the large intestine (3-5). Until recently, it was believed that the plant lignans secoisolariciresinol and matairesinol were the only plant lignans to be converted to enterolactone and enterodiol, but recent studies have identified several plant lignans (pinoresinol, syringaresinol, and lariciresinol) as potential new precursors that can be converted to enterolactone and enterodiol *in vitro* and *in vivo* (6, 7). Lignan products are mainly found as enterolactone in mammalian feces, plasma, and urine, and the concentrations are determined primarily by intake of dietary precursors and the capacity of the intestinal microflora (4).

The possible protective effect of enterolactone and other phytoestrogens on breast cancer has been ascribed primarily to their antiestrogenic effects. Owing to their chemical structure, phytoestrogens can compete with endogenous estrogens for binding with estrogen receptors (ER), thereby plausibly reducing the hormonal effect of endogenous estrogens, which are much more potent than phytoestrogens (8). Enterolactone has also been shown to have antioxidative effects (9, 10), which can be another mechanism behind its potential effects protective to breast cancer.

The relation among dietary intake of lignans, concentrations of enterolactone in urine or blood, and development of breast cancer has been evaluated in seven epidemiologic studies (11-17). In the first study, Adlercreutz et al. (11) found that postmenopausal women with breast cancer excreted significantly less enterolactone in their urine than did healthy postmenopausal women. Four case-control studies followed the study of Adlercreutz et al. Two found decreased risks for breast cancer with increasing concentrations of enterolactone in urine or

Received 12/10/03; revised 7/6/04; accepted 7/14/04.

Grant support: Danish Cancer Society and Europe against Cancer: European Prospective Investigation into Cancer and Nutrition.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Requests for reprints: Anja Olsen, Institute of Cancer Epidemiology, Danish Cancer Society, Strandboulevarden 49, DK-2100 Copenhagen Ø, Denmark. Phone: 45-35257606; Fax: 45-35257731. E-mail: anja@cancer.dk

serum (12, 15), whereas the third showed no association between dietary intake of lignans and breast cancer (14). The fourth study found a protective effect of dietary intake of lignans among premenopausal but not among postmenopausal women with breast cancer (17). Use of the case-control design to study the association between enterolactone and breast cancer can, however, be affected by bias. When blood or urine is obtained after diagnosis of cancer, the concentration of enterolactone might have been influenced by metabolic consequences of the disease (13), and reports of dietary lignan intake might be affected by recall bias (18). Therefore, a prospective design must be regarded as preferable for studying associations between lignans or enterolactone and risk for breast cancer. The results of only two prospective studies have been reported (13, 16). den Tonkelaar et al. (13) found no association between urinary excretion of enterolactone and breast cancer risk among postmenopausal women, whereas Hulten et al. (16), in a cohort consisting of both premenopausal and postmenopausal women, found an inexplicable U-shaped association, with increased risks for breast cancer among both women with low plasma concentrations and women with high plasma concentrations of enterolactone.

If enterolactone affects the risk for breast cancer through ERs, it might be expected to relate mainly to ER-positive tumors. This hypothesis was addressed in one of the two prospective studies on enterolactone and breast cancer, although no substantial difference in risk was seen when the analyses were restricted to ER-positive breast cancer cases (13). The power of that study, however, was relatively low, with only 55 known receptor-positive cases among a total of 88 cases.

The purpose of the present study was to investigate associations between plasma concentrations of enterolactone and breast cancer incidence rates in a case-control study nested within a large cohort of postmenopausal Danish women, with prospectively collected blood samples and questionnaire data. In particular, we focused on possible differences in risk with respect to ERα expression of breast cancer.

Materials and Methods

Cohort. The Diet, Cancer and Health study is a prospective cohort study, established with the primary aim of studying the etiologic role of diet on cancer risk. Between December 1993 and May 1997, 79,729 women were invited to participate in the study. We invited all women who lived in greater Copenhagen and Aarhus and who fulfilled the following criteria: ages between 50 and 64 years, born in Denmark, and not registered with a previous diagnosis of cancer in the Danish Cancer Registry. A total of 29,875 women, corresponding to ~37% of those invited, were enrolled into the cohort.

The Diet, Cancer and Health study and the present substudy were approved by the regional ethical committees on human studies in Copenhagen and Aarhus and by the Danish Data Protection Agency.

All cohort members attended one of two established study centers, and each participant filled in a food frequency questionnaire and a life-style questionnaire. The life-style questionnaire included questions about reproductive factors, health status, social factors, and life-style habits. From this questionnaire, we obtained information about years of school education (short, ≤7 years; medium, 8-10 years; or long, >10 years), parity (parous/nulliparous, number of births, and age at birth of first child), use of hormone replacement therapy (HRT; never, past, current), duration of HRT, and present smoking (yes/no). Anthropometric data were obtained by professional staff members. Body mass index was calculated as weight (kg) per height (m) squared. Information on alcohol intake was obtained from a 192-item food frequency questionnaire.

In the study centers, 30 mL of blood (nonfasting, collected in citrated and plain Venojects) were drawn from each participant. The samples were spun and divided into 1-mL tubes of plasma, serum, RBCs, and buffy coat. All samples were processed and frozen within 2 hours at −20°C. At the end of the day of collection, all samples were stored in liquid nitrogen vapor (maximum temperature, −150°C).

Of the initial 29,875 women, we excluded 326 who later were reported to the Danish Cancer Registry with a cancer diagnosed before the visit to the study clinic. In addition, eight women were excluded from the study because they did not fill in the life-style questionnaire. Because the present analysis aimed at women who were postmenopausal at study entry, we further excluded 4,844 women, including 4,798 who were considered premenopausal because they had reported at least one menstruation <12 months before entry and no use of HRT, 9 women who gave a lifetime history of no menstruation, and 37 women who did not answer the questions about current or previous use of HRT, leaving 24,697 postmenopausal women for study.

Cohort members were identified from their unique personal identification number, which is allocated to every Danish citizen by the Central Population Registry. All the postmenopausal cohort members were linked to the Central Population Registry to obtain information on vital status and immigration. Information on cancer occurrence among cohort members was obtained through record linkage to the Danish Cancer Registry, which collects information on all cases of cancer in Denmark (19). Linkage was done by use of the personal identification number. Each cohort member was followed up for breast cancer occurrence from date of entry, that is, date of visit to the study center until the date of diagnosis of any cancer (except for nonmelanoma skin cancer), date of death, date of emigration, or December 31, 2000, whichever came first. Incident breast cancer was diagnosed in 434 women during the follow-up period.

A registry exclusively about breast cancer also exists in Denmark and information on ERα status was obtained by linkage with the Danish Breast Cancer Co-operative Group, which holds records on a range of details for ~90% of breast cancers diagnosed in Denmark (20). A standardized immunohistochemical method was used in all medical centers. The cutoff level used to define positive ERα status was ≥10% positive cells. Information on ERβ and progesterone receptor status is not registered consequently in the register; thus, it was not possible to consider these receptors.

Matching of Cases and Controls. In view of the size of the cohort, plasma concentrations of enterolactone could not be determined for all of the women. We therefore used

a nested case-control design, with one control selected for each of the 434 cases. The control was cancer free at the exact age at diagnosis of the case and was further matched on age at inclusion into the cohort (half-year intervals), certainty of postmenopausal status (known/probably postmenopausal), and use of HRT at inclusion into the cohort (current/former/newer). The probably postmenopausal group includes women that were hysterectomized or used HRT at baseline such that postmenopausal status could not be established based on information on menstrual bleedings (use of HRT can cause vaginal bleedings, although there is no hormone production in the ovaries). We assumed that these women had gone into menopause, because HRT is rarely given to women without menopausal inconveniences. Furthermore, the median (5-95%) age at diagnosis (or at censoring for the controls) in the probably postmenopausal group was 60 years (range, 53-68 years), making it very likely that the women had gone into menopause. Of the 434 pairs (866 women; 434 cases, and 434 controls, including 2 cases), 11 pairs were excluded owing to lack of blood sample for case or control or problems with the biological analyses and 42 pairs were excluded because information was missing for either the case or the control about one or more of the potentially confounding variables [reproductive events (number and ages at births), duration of HRT use, length of school education, alcohol intake, body mass index, or present smoking, leaving 381 case-control pairs for the study].

Biological Analysis. The analytic procedure for time-resolved fluoroimmunoassay of enterolactone in plasma (21, 22) is as follows. A 200-μL aliquot of plasma was mixed with 200 μL β-glucuronidase and sulfatase in acetate buffer and incubated overnight at 37°C. Diethyl ether was used to extract unconjugated enterolactone after hydrolysis. The hydrolyzed extract in buffer was then pipetted into prewashed goat anti-rabbit immunoglobulin G microstrips. Simultaneously, antiserum (dilution, 1:250,000) in bovine serum albumin-Tris buffer and europium-labeled enterolactone (dilution 1:400,000) were added to the microstrips. After incubation and shaking of the strips slowly in a DELFIA plate washer (Wallac, Turku, Finland) at room temperature for 90 minutes, the strips were washed with a DELFIA plate washer (Wallac). Subsequently, DELFIA enhancement solution (Wallac) was added to each well, and the strips were shaken slowly for an additional 5 minutes. Fluorescence was read in a DELFIA Victor multilabel counter (Wallac). Duplicate control plasma samples were processed through the procedure for each batch and placed at the beginning and end of the plates. The concentration of enterolactone in the control plasma was 19.7 nmol/L, and the intra-assay and interassay coefficients of variation were <10%. Results of a previous study[6] further showed that the assay coefficients of variation were virtually independent of the concentration of enterolactone in plasma in the range 0 to 140 nmol/L.

We used plasma levels of enterolactone to measure exposure because this biomarker is considered more stable than urinary excretion due to possible enterohepatic recirculation (23). The plasma concentration is relatively constant within an individual, with a reliability constant of 0.55 over a 2-year period (24). Accordingly, it should be reasonably representative of the levels throughout the study period.

Statistical Methods. We did conditional logistic regression analyses, which led to estimates of breast cancer incidence rate ratios (IRR) corresponding to a Cox proportional hazards model with age as the time axis, owing to the sampling design with perfect matching on age at cancer diagnosis (25). The associations were evaluated with and without adjustment for baseline values of potential confounders. The adjusting variables were chosen because they are established risk factors for breast cancer and data could be obtained from the questionnaires: parity (entered as two variables; the categorical variable parous/nulliparous and the quantitative variable number of births), age at birth of first child, length of school education (low, medium, high), duration of HRT use, body mass index, alcohol intake, and present smoking (yes/no).

All quantitative variables were entered linearly into the logistic model because this is biologically more reasonable than the step functions corresponding to categorization; furthermore, it increases the power of the analyses (26). The linearity of the associations was evaluated graphically by linear splines with three boundaries placed at the quartile cutoff points according to the exposure distribution among cases (27). None of the associations showed signs of deflection or threshold values.

Administration of antibiotics temporarily reduced the concentration of enterolactone in urine and plasma due to inhibition of intestinal microflora (5, 28). Women with a low enterolactone concentration may therefore comprise a heterogeneous combination of women with long-term low plasma concentration and women with short-term low plasma concentrations due to recent use of antibiotics. If a high habitual concentration of enterolactone is associated with a lower breast cancer rate, then an inverted U-shaped relation with the single measurements taken at baseline could be expected. This possible deviation from a log-linear association between total and ERα-specific breast cancer and plasma enterolactone was evaluated with linear splines with one boundary. The pattern for the association was illustrated by the average rate ratios when the women were categorized according to the quartiles for the enterolactone concentrations. Because of the expected heterogeneity of the lowest quartile group, we used the second quartile group as reference group.

Two-sided 95% confidence intervals (95% CI) for the IRR were calculated with χ^2 tests of the regression variable (i.e., on the log IRR scale). The procedure PHREG in SAS (release 6.12, SAS Institute, Inc., Cary, NC) on Unix platform was used for statistical analyses.

Results

The median age at entry into the cohort for the 381 pairs was 57 years (range, 50-65 years). The median (1st-99th percentiles) length of follow-up for the 762 postmenopausal women was 4.3 years (range, 0.1-6.8 years). Information about the ERα status of tumors was obtained for 353 (93%) cases of breast cancer, with 273 of the

[6] K.E. Bach Knudsen, unpublished study.

observed tumors reported to be ERα positive and 80 tumors ERα negative. Information about ERα status was not obtained for the remaining 28 cases, 8 of which were *in situ* tumors and 20 of which could not be located in the registry.

The baseline characteristics of the cases and controls are presented in Table 1. Cases had slightly longer use of HRT, higher intake of alcohol, and longer education than controls, whereas cases were less often present smokers. Age at baseline and present/ever use of HRT were identical for cases and controls due to the matching procedure.

The breast cancer risk factors showed no visible confounding when the association between plasma concentrations of enterolactone and IRR for breast cancer was analyzed in the linear model (Table 2). We found a tendency toward lower rates with higher concentrations (IRR, 0.93; 95% CI, 0.86-1.01) per 20 nmol/L higher plasma enterolactone.

Plasma concentrations of enterolactone were also evaluated with ERα-specific breast cancer as the outcome (Table 2). No association was seen with ERα-positive breast cancer, but a 29% reduction in the rate of ERα-negative breast cancer was seen per 20 nmol/L higher plasma enterolactone (IRR, 0.71; 95% CI, 0.53-0.94). To evaluate whether ever use of HRT, HRT use at blood sampling, or postmenopausal status (known/possible postmenopausal) influenced the associations, we conducted the analyses from Table 2 stratified according to these factors. No signs of different effects were seen (results not shown).

The hypothesized, inverted U-shaped deviation from a log-linear association between total and ERα-specific breast cancer rates and plasma enterolactone was evaluated with a linear spline with one boundary. We tried three different placings of the boundary (at the lowest quartile, at the lower tertile, or at the median) and found signs of an inverted U shape, but the deviation from linearity was not significant (all Ps $\geq$ 0.29). As an illustration of the association between plasma concentrations of enterolactone and breast cancer, we present the average IRRs for total and ERα-specific breast cancer when the women are categorized according to the quartiles for plasma concentrations of enterolactone (Table 3), suggesting an inverted U-shaped pattern about total breast cancer, as well as when the cases were stratified according to the ERα status of their tumor.

Table 1. Baseline characteristics of 381 breast cancer cases and their 381 matched control subjects at baseline

Characteristics	Median (5th-95th percentiles) or fraction	
	Controls	Cases
Age at baseline (y)	57 (51-64)	57 (51-64)
Duration of HRT use (y)*	5 (1-21)	6 (1-20)
Age at first birth (y)	23 (18-31)	23 (18-32)
No. births	2 (0-4)	2 (0-3)
Body mass index (kg/m^2)	25 (20-33)	25 (20-34)
Alcohol intake (g/d)	10 (1-43)	11 (0-44)
Schooling (y)		
≤7%	34	29
8-10%	49	48
≥11%	18	23
Use of HRT at inclusion (% yes)	51	51
Ever use of HRT (% yes)	65	65
Parous (% yes)	87	87
Present smoking (% yes)	37	33

NOTE: Values are medians (5th and 95th percentiles) or fraction (%) in each category.
*Among ever users of HRT.

Table 2. IRRs and 95% CIs according to plasma levels of enterolactone for total breast cancer (381 pairs), ER-positive breast cancer (273 pairs), and ER-negative breast cancer (80 pairs)

Breast cancer	Enterolactone per 20 nmol/L increment			
	IRR (95% CI)*	*P*	IRR (95% CI)†	*P*
All	0.94 (0.87-1.01)	0.09	0.93 (0.86-1.01)	0.09
ER positive	0.97 (0.89-1.06)	0.45	0.97 (0.88-1.06)	0.45
ER negative	0.73 (0.56-0.95)	0.02	0.71 (0.53-0.94)	0.02

*Estimates are adjusted for age and use of HRT (through matching).
†Further adjusted for baseline values of school education, intake of alcohol, parity (parous/nulliparous, number of births, and age at first birth), duration of HRT use, body mass index, and present smoking.

Discussion

In accordance with most earlier research, we found a tendency (IRR, 0.93; 95% CI, 0.86-1.01 per 20 nmol/L higher concentration) toward a lower risk for breast cancer with higher plasma concentrations of enterolactone. The tendency toward a preventive effect was confined almost entirely to ERα-negative breast cancer.

Selection bias, information bias, and confounding can to some degree be excluded: follow-up was nearly complete (99.8%), the results for the blood samples are objective, and the potential confounders included in the models did not affect the estimates of the association between plasma enterolactone and breast cancer incidence rate. Our relatively short follow-up period can be viewed as a weakness because it is likely that the possible effect of enterolactone on the tumor occurs years before diagnosis; however, excluding pairs in which the case was diagnosed within the first year of follow-up did not alter the results.

Another weakness is the limited number of ERα-negative cases. We found only 80 ERα-negative breast cancer cases, and our limited power to study this group must be considered when the results are interpreted. In addition, we had information only on ERα, and it has been shown previously that the dietary phytoestrogens genistein and coumesterol have higher binding affinity for β-receptors than for α-receptors (29-31). Unfortunately, we do not have information on ERβ in the present study. The binding affinity for enterolactone has to our knowledge not been evaluated, but it is reasonable to believe that the mechanism of its biological action is similar to that of genistein and coumesterol, although its binding affinity is probably lower. In animals, the protective effect of enterolactone against dimethylbenz (*a*)anthracene-induced mammary tumors seemed to be unrelated to antiestrogenic effects, and enterolactone had no effect in assays for estrogen activity *in vivo* (32).

Table 3. IRRs and 95% CIs according to plasma levels of enterolactone for total (381 pairs), ER-positive (273 pairs), and ER-negative (80 pairs) breast cancer

Cases	Quartiles of serum enterolactone concentration (nmol/L)*			
	0.1-14.4	14.5-28.1	28.2-47.9	48.0-454.6
All	0.77 (0.51-1.15; n = 94)	1 (n = 109)	0.80 (0.53-1.21; n = 96)	0.55 (0.36-0.85; n = 82)
ER-positive	0.77 (0.48-1.24; n = 65)	1 (n = 73)	0.95 (0.59-1.53; n = 71)	0.67 (0.41-1.08; n = 64)
ER-negative	0.61 (0.25-1.53; n = 21)	1 (n = 28)	0.43 (0.16-1.15; n = 17)	0.26 (0.09-0.77; n = 14)

NOTE: Values are presented as IRR (95% CI).
*Quartiles for plasma enterolactone among all women. Estimates are adjusted for age and use of HRT (through matching).

In the present study, we analyzed the blood samples with intra-assay and interassay coefficients of variations ~10%, as in other studies (21, 22, 33). We had single determinations among nonfasting individuals. In a recent intervention study in which pigs were given high-lignan and low-lignan diets, a low diurnal intra-animal variation was found (7). This is because a substantial portion of absorbed enterolactone undergoes enterohepatic circulation, a situation that can also be expected in humans as shown for phenolic estrogen (34). The enterohepatic circulation ensures that it takes time until a certain concentration is reached in the blood, and the body pool has a buffering effect on daily fluctuation. The plasma concentration of enterolactone is consequently not sensitive to the time of sampling (fed or fasted stage) but is primarily a reflection of the intake of enterolactone precursors and the microbial capacity to convert plant lignans to mammalian lignans (7).

The possible preventive effect of enterolactone against breast cancer has been ascribed primarily to two different biological mechanisms: an antiestrogenic effect and an antioxidative effect. If the effect of enterolactone is antiestrogenic, this would be expected to affect ER-positive breast cancer by preventing ER-positive breast cancer or by slowing the disease progression. It is not known whether ER-negative breast cancer is a progressed form of ER-positive breast cancer or whether ER-positive and ER-negative breast cancers are two biologically different diseases with different risk factors (35). The prognosis of survival from ER-negative breast cancer is poorer than that from ER-positive breast cancer, and tumors are known to lose their estrogen dependence during progression, which indicates that ER-negative breast cancer may be a more progressed state of ER-positive breast cancer. An investigation of this problem, however, gave inconsistent results (35). If ER-negative tumors are a progressed form of ER-positive tumors, an antiestrogenic effect may lead to an association in which a higher enterolactone concentration is associated with a stable or decreased total breast cancer rate with a decreased number of ER-negative tumors as observed in the present study. In contrast to our results, den Tonkelaar et al. (13) found a tendency toward increased risk with high urinary concentrations of enterolactone, and an analysis restricted to 55 cases of ER-positive breast cancer gave a similar result.

Administration of antibiotics, which inhibit intestinal microflora, reduces plasma and urine enterolactone dramatically, and the effect may persist for some time (5, 28). We expected that the women with a low enterolactone concentration at the baseline examination comprised a heterogeneous combination of women with long-term biologically low plasma concentration (due to stable low production in the gut and/or low dietary intake of lignan) and women with short-term low plasma concentrations due to recent use of antibiotics. If a habitually high concentration of enterolactone decreases the breast cancer rate, then a lower average rate would be expected in this heterogeneous group. The observed pattern in the present study was in accordance with this theory, but the deviation from a dose-response type association was not statistically significant.

Although this study is the largest prospective study on enterolactone and breast cancer conducted, our findings must be confirmed in other prospective studies. Future studies of the association with ERα-specific breast cancer should be larger, in view of the relatively small fraction of ERα-negative breast cancers.

Acknowledgments

We thank Katja Boll and Jytte Fogh Larsen for collection of data and Winnie Østergaard, Hanne M. Purup, and Janne Adamsen for excellent technical assistance.

References

1. Adlercreutz H, Mazur W. Phyto-oestrogens and Western diseases. Ann Med 1997;29:95–120.
2. Mazur W. Phytoestrogen content in foods. Baillieres Clin Endocrinol Metab 1998;12:729–42.
3. Setchell KD, Lawson AM, Borriello SP, et al. Lignan formation in man-microbial involvement and possible roles in relation to cancer. Lancet 1981;2:4–7.
4. Kilkkinen A, Stumpf K, Pietinen P, Valsta LM, Tapanainen H, Adlercreutz H. Determinants of serum enterolactone concentration. Am J Clin Nutr 2001;73:1094–100.
5. Adlercreutz H. Phyto-oestrogens and cancer. Lancet Oncol 2002;3:364–73.
6. 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.
7. Bach Knudsen KE, Serena A, Kjaer AK, et al. Rye bread in the diet of pigs enhances the formation of enterolactone and increases its levels in plasma, urine and feces. J Nutr 2003;133:1368–75.
8. Ganry O. Phytoestrogen and breast cancer prevention. Eur J Cancer Prev 2002;11:519–22.
9. Kitts DD, Yuan YV, Wijewickreme AN, Thompson LU. Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. Mol Cell Biochem 1999;202:91–100.
10. Prasad K. Antioxidant activity of secoisolariciresinol diglucoside-derived metabolites, secoisolariciresinol, enterodiol, and enterolactone. Int J Angiol 2000;9:220–5.

11. Adlercreutz H, Fotsis T, Heikkinen R, et al. Excretion of the lignans enterolactone and enterodiol and of equol in omnivorous and vegetarian postmenopausal women and in women with breast cancer. Lancet 1982;2:1295–9.
12. Ingram D, Sanders K, Kolybaba M, Lopez D. Case-control study of phyto-oestrogens and breast cancer. Lancet 1997;350:990–4.
13. 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.
14. Horn-Ross PL, John EM, Lee M, et al. Phytoestrogen consumption and breast cancer risk in a multiethnic population: the Bay Area Breast Cancer Study. Am J Epidemiol 2001;154:434–41.
15. Pietinen P, Stumpf K, Mannisto S, Kataja V, Uusitupa M, Adlercreutz H. Serum enterolactone and risk of breast cancer: a case-control study in eastern Finland. Cancer Epidemiol Biomarkers Prev 2001;10:339–44.
16. Hulten K, Winkvist A, Lenner P, Johansson R, Adlercreutz H, Hallmans G. An incident case-referent study on plasma enterolactone and breast cancer risk. Eur J Nutr 2002;41:168–76.
17. McCann SE, Moysich KB, Freudenheim JL, Ambrosone CB, Shields PG. The risk of breast cancer associated with dietary lignans differs by CYP17 genotype in women. J Nutr 2002;132:3036–41.
18. Giovannucci E, Stampfer MJ, Colditz GA, et al. Recall and selection bias in reporting past alcohol consumption among breast cancer cases. Cancer Causes Control 1993;4:441–8.
19. Storm HH, Michelsen EV, Clemmensen IH, Pihl J. The Danish Cancer Registry—history, content, quality and use. Dan Med Bull 1997;44:535–9.
20. Fischerman K, Mouridsen HT. Danish Breast Cancer Cooperative Group (DBCG). Structure and results of the organization. Acta Oncol 1988;27:593–6.
21. Stumpf K, Uehara M, Nurmi T, Adlercreutz H. Changes in the time-resolved fluoroimmunoassay of plasma enterolactone. Anal Biochem 2000;284:153–7.
22. Uehara M, Lapcik O, Hampl R, et al. Rapid analysis of phytoestrogens in human urine by time-resolved fluoroimmunoassay. J Steroid Biochem Mol Biol 2000;72:273–82.
23. Adlercreutz H. Phytoestrogens and breast cancer. J Steroid Biochem Mol Biol 2002;83:113–8.
24. 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.
25. Prentice RL, Breslow NE. Retrospective studies and failure time models. Biometrika 1978;65:153–8.
26. Greenland S. Avoiding power loss associated with categorization and ordinal scores in dose-response and trend analysis. Epidemiology 1995;6:450–4.
27. Greenland S. Dose-response and trend analysis in epidemiology: alternatives to categorical analysis. Epidemiology 1995;6:356–65.
28. Kilkkinen A, Pietinen P, Klaukka T, Virtamo J, Korhonen P, Adlercreutz H. Use of oral antimicrobials decreases serum enterolactone concentration. Am J Epidemiol 2002;155:472–7.
29. Kuiper GG, Carlsson B, Grandien K, et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β. Endocrinology 1997;138:863–70.
30. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β. Endocrinology 1998;139:4252–63.
31. Wiseman H, Duffy R. New advances in the understanding of the role of steroids and steroid receptors in disease. Biochem Soc Trans 2001;29:205–9.
32. Saarinen NM, Huovinen R, Warri A, et al. Enterolactone inhibits the growth of 7,12-dimethylbenz(*a*)anthracene-induced mammary carcinomas in the rat. Mol Cancer Ther 2002;1:869–76.
33. Adlercreutz H, Wang GJ, Lapcik O, et al. Time-resolved fluoroimmunoassay for plasma enterolactone. Anal Biochem 1998;265:208–15.
34. Adlercreutz H, Martin F. Biliary excretion and intestinal metabolism of progesterone and estrogens in man. J Steroid Biochem 1980;13:231–44.
35. Zhu K, Bernard LJ, Levine RS, Williams SM. Estrogen receptor status of breast cancer: a marker of different stages of tumor or different entities of the disease? Med Hypotheses 1997;49:69–75.

Plasma Enterolactone and Breast Cancer Incidence by Estrogen Receptor Status

Anja Olsen, Knud Erik Bach Knudsen, Birthe L. Thomsen, et al.

Cancer Epidemiol Biomarkers Prev 2004;13:2084-2089.