Plasma enterolactone and incidence of endometrial cancer in a case–cohort study of Danish women

Julie Aarestrup[1], Cecilie Kyrø[1], Knud E. B. Knudsen[2], Elisabete Weiderpass[3,4,5,6], Jane Christensen[1], Mette Kristensen[7], Anne M. L. Würtz[1], Nina F. Johnsen[1], Kim Overvad[8], Anne Tjønneland[1] and Anja Olsen[1]*

[1]*Danish Cancer Society Research Center, Strandboulevarden 49, DK-2100 Copenhagen Ø, Denmark*
[2]*Department of Animal Science, Aarhus University, Foulum, Denmark*
[3]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden*
[4]*Department of Community Medicine, University of Tromsø, Tromsø, Norway*
[5]*Cancer Registry of Norway, Oslo, Norway*
[6]*Samfundet Folkhälsan, Helsinki, Finland*
[7]*Department of Human Nutrition, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark*
[8]*Section of Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark*

Abstract

The phyto-oestrogen enterolactone has been hypothesised to protect against hormone-dependent cancers, probably through its anti-oestrogenic potential. We investigated whether a higher level of plasma enterolactone was associated with a lower incidence of endometrial cancer in a case–cohort study in the 'Diet, Cancer and Health' cohort. The cohort study included 29 875 women aged 50–64 years enrolled between 1993 and 1997. Information on diet and lifestyle was provided by self-administrated questionnaires and blood was drawn from each participant. Time-resolved fluoroimmunoassay was used for biochemical determination of plasma enterolactone. A total of 173 cases and 149 randomly selected cohort members were included. We estimated incidence rate ratio (IRR) and 95 % CI by a Cox proportional hazards model. A 20 nmol/l higher plasma concentration of enterolactone was associated with a non-significant lower risk of endometrial cancer (IRR 0·93, 95 % CI 0·84, 1·04). When excluding women with low enterolactone concentrations (quartile 1) due to potential recent antibiotic use, the association became slightly stronger, but remained non-significant (IRR 0·90, 95 % CI 0·79, 1·02). Menopausal status, hormone replacement therapy or BMI did not modify the association. In conclusion, we found some support for a possible inverse association between plasma enterolactone concentration and endometrial cancer incidence.

Key words: Enterolactone: Endometrial cancer: Case–cohort studies: Phyto-oestrogens

Worldwide, endometrial cancer is the sixth most common cancer in women, being more frequent in high-income countries[1]. The majority of risk factors are related to oestrogenic exposure, where oestrogen-stimulated endometrial cell proliferation increases risk if not counterbalanced sufficiently by progesterone[2]. It is biologically plausible that dietary phyto-oestrogens may protect against hormone-dependent cancers[3]. In Nordic countries, plant lignans contribute to the largest phyto-oestrogen concentration[4]. Lignans are ubiquitously found in plants, predominantly in the fibre layers, and rich sources include seeds, whole-grain cereals, legumes, vegetables and fruits[5]. Plant lignans are converted to enterolignans by microbial fermentation in the colon, and the concentration is primarily determined by the intake of plant lignans and the capacity of the intestinal microflora[3].

Owing to their chemical similarities with oestrogens, enterolactone may compete with oestrogens for binding to oestrogen receptors and thereby disrupt oestrogen signalling, an effect that could vary according to the level of endogenous oestrogen. Thus, enterolactone might reduce a woman's lifetime exposure to bioavailable oestrogens and thereby decrease risk of endometrial cancer. Furthermore, enterolactone may decrease endometrial cancer risk by stimulating the synthesis of sex hormone-binding globulin, inhibiting

aromatase and 17-β-hydroxysteroid dehydrogenase, as well as exerting antioxidative effects, inducing apoptosis and inhibiting cancer cell growth[6].

Due to its oestrogenic potential, enterolactone has been evaluated in relation to several hormone-dependent cancers with inconsistent findings[3]. To our knowledge, however, only one currently published study has investigated the association between circulating levels of enterolactone and incidence of endometrial cancer[7]. This nested case–control study did not find an association when evaluating enterolactone using ordered categorical variables in a population with a relatively low concentration and variation of circulating enterolactone. Two other case–control studies have estimated intake of plant lignans in relation to endometrial cancer[8,9], where one found a protective effect[8].

The aim of the present study was to evaluate whether plasma enterolactone concentrations were associated with the incidence of endometrial cancer among Danish middle-aged women in a case–cohort study nested within a large prospective cohort. Furthermore, we evaluated if the association between enterolactone and endometrial cancer differed in different hormonal environments. This was done by testing for potential effect modification by menopausal status, use of hormone replacement therapy (HRT) and BMI.

Materials and methods

Cohort

The 'Diet, Cancer and Health' study is a prospective cohort study established with the primary aim of evaluating the aetiological role of diet in cancer development[10]. Between December 1993 and May 1997, 79 729 Danish women living in greater Copenhagen and Aarhus were invited to participate, the inclusion criteria being age 50–64 years, born in Denmark and not registered with a previous cancer diagnosis in the Danish Cancer Registry. Subjects were identified by a unique ten-digit personal identification number assigned to all citizens from the Civil Registration System. A total of 29 875 women were enrolled in the study corresponding to approximately 37 % of those invited. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethical Committees on Human Studies in Copenhagen and Aarhus. All cohort participants provided written informed consent.

All participants attended one of the two study centres and filled in a validated 192-item FFQ[11] and a life-style questionnaire. Information on parity, menopausal status, use of HRT and smoking was obtained from the life-style questionnaire. Anthropometric measurements were taken by professional staff from where information on BMI was gained. A total of 30 ml blood was drawn in a non-fasting state and samples were spun and divided into 1 ml tubes (citrated and plain Venojects) of plasma, serum, erythrocytes and buffy coat. All samples were processed and frozen within 2 h at −20°C and stored in liquid N_2 vapour with a maximum temperature of −150°C at the end of the day.

Definition of study group

Of the initial 29 875 women, we excluded 335 who were later reported to the Danish Cancer Registry with a diagnosis of cancer before study entry. Furthermore, another 1291 women with no or incomplete blood samples were excluded, leaving 28 249 women for the study. These were linked to the Central Population Registry to obtain information on vital status and migration.

Each woman was followed from baseline (i.e. date of visit at a study centre) until diagnosis of any cancer (except non-melanoma skin cancer), hysterectomy, death, emigration or end of follow-up (i.e. 27 April 2006), whichever came first. Information on endometrial cancer occurrence was obtained through record linkage to the Danish Cancer Registry (ICD7 172.0-172.2, 472.0, 872.0) and to the Danish Pathology Databank (T84000 and M8-9). Furthermore, the women were linked to the Hospital Discharge Registry to gain information on hysterectomy.

A total of 188 women were diagnosed with endometrial cancer during follow-up, and a random subcohort sample of 188 women was chosen, corresponding to a case–cohort design. Coincidentally, none of the subcohort members were cases. One woman was excluded due to suspected registry error, as were two cases concurrently diagnosed with endometrial and cervical cancer. Furthermore, ten cases and four subcohort members without enterolactone measurements and one case with an unrealistic enterolactone concentration (i.e. above 1100 nmol/l) were excluded. One subcohort member not handing in the life-style questionnaire, three cases and four subcohort members with missing information on potential confounders and thirty-one subcohort members with a hysterectomy before baseline were excluded. A total of 149 women were ultimately included as subcohort members and 173 women as cases in the final statistical analyses.

Biological analysis

The standard analytical procedure for time-resolved fluoroimmunoassay was used for determinations of plasma enterolactone concentrations[12,13]. The principle of the analysis relies on a method where goat anti-rabbit IgG-coated wells bind rabbit anti-enterolactone IgG (Labmaster Limited). Fluorescence was read in the DELFIA Victor multilabel counter (Wallac Oy). Duplicate internal control plasma samples were included in each batch and placed at the beginning and end of the plates. The plasma concentrations of enterolactone in the controls were 9·3 and 37·6 nmol/l, respectively, and the intra- and inter-assay CV were 13·5 and 15·0 %, respectively, at the low concentration and 8·1 and 9·2 %, respectively, at the high concentration. We have previously found the assay CV to be virtually independent of the concentration of enterolactone in plasma in the range 0–140 nmol/l (KEB Knudsen, unpublished results).

Statistical methods

Baseline descriptive characteristics for the included women are presented as median values (5th and 95th percentiles)

for continuous variables, while categorical variables are presented as fractions (%).

The statistical analyses were performed according to the principles described by Prentice[14] and suggested by Barlow et al.[15], where a modified Cox proportional hazards model was used to evaluate the association between enterolactone concentration and endometrial cancer incidence. Age was used as the underlying time scale. Two-sided 95 % CI for incidence rate ratio (IRR) was calculated based on Wald's test of the Cox regression parameter, i.e. on the log rate ratio scale for testing the null hypothesis. All P values were two-sided, and the statistical significance level was set to 0·05.

The associations were evaluated with and without adjustment for baseline values of potential confounders, they being established risk factors for endometrial cancer and obtainable from the questionnaires: parity (yes or no), number of births (n, continuous), age at first birth (continuous), menopausal status (premenopausal or postmenopausal), use of HRT (current, former or never), type of HRT among users (unopposed oestrogen, combination or other), smoking (current, former or never) and BMI (kg/m², continuous).

The association between enterolactone and endometrial cancer is presented as IRR associated with a 20 nmol/l increment in enterolactone concentration. The continuous analyses were supplemented with categorical analyses, where women were categorised into quartiles based on the distribution of enterolactone among subcohort members.

Possible interactions between enterolactone and the potential effect modifiers, menopausal status, use of HRT and BMI, were investigated. We tested whether the slopes for enterolactone at each level of the potential effect modifiers differed significantly from a single common slope using the likelihood ratio test.

All quantitative variables were entered linearly into the models, as this is biologically more reasonable than the step function corresponding to categorisation and furthermore increases the statistical power of the analyses[16,17].

Administration of antibiotics reduces the intestinal microflora and thereby the plasma concentration of enterolactone[18]. Women with a relatively low enterolactone concentration may therefore comprise a heterogeneous group of women with a long-term low-enterolactone concentration and women with a short-term low-enterolactone concentration due to recent use of antibiotics. Thus, the second quartile was used as a reference group. A possible deviation from a linear association was evaluated with linear splines, with one boundary placed at the quartile 1 border based on the distribution among the subcohort. When including all women, a significant deviation from linearity ($P = 0.001$) was present, which, however, disappeared when excluding women in the lowest quartile ($P = 0.37$).

SAS version 9.1 (SAS Institute, Inc.) on a TextPad/Unix platform was used for statistical analyses.

Table 1. Baseline characteristics of endometrial cancer cases and subcohort members nested within the 'Diet, Cancer and Health' cohort

(Medians and 5th and 95th percentiles; percentages)

Characteristics	Subcohort (n 149)		Cases (n 173)	
	Median	5th–95th percentiles	Median	5th–95th percentiles
Enterolactone (nmol/l)	31	4–157	29	4–112
Age at baseline (years)	56	50–63	57	51–64
Parity				
No. of births*	2	1–4	2	1–4
Nulliparous				
Fraction (%)	8		16	
Age at first birth* (years)	23	18–32	23	18–30
Menopausal status				
Premenopausal				
Fraction (%)	19		17	
Postmenopausal				
Fraction (%)	81		83	
Use of HRT				
Never				
Fraction (%)	54		45	
Present				
Fraction (%)	33		35	
Former				
Fraction (%)	13		20	
Use of unopposed oestrogen†				
Fraction (%)	6		7	
BMI (kg/m²)	24·9	20·9–36·6	25·4	19·9–34·9
Smoking at baseline (present)				
Fraction (%)	31		25	

HRT, hormone replacement therapy.
* Among parous women.
† Among users.

Table 2. Baseline characteristics of women within the 'Diet, Cancer and Health' cohort according to the enterolactone levels among the subcohort members

(Medians and 5th and 95th percentiles; percentages)

	Quartiles of enterolactone concentration (nmol/l)*							
	Q1 (≤14)		Q2 (>14 and ≤31)		Q3 (>31 and ≤63)		Q4 (>63)	
	Median	5th−95th percentiles	Median	5th−95th percentiles	Median	5th−95th percentiles	Median	5th−95th percentiles
Cases/subcohort (*n*)	37/38		58/37		45/37		33/37	
Characteristics								
Age at baseline (years)	56	51−63	58	50−64	56	50−63	56	50−62
Parity								
No. of births†	2	1−4	2	1−4	2	1−4	2	1−3
Nulliparous								
Fraction (%)	15		8		16		11	
Age at first birth† (years)	22	17−32	23	19−31	23	18−31	24	18−31
Menopausal status								
Premenopausal								
Fraction (%)	19		14		26		16	
Postmenopausal								
Fraction (%)	81		86		74		84	
Use of HRT								
Never								
Fraction (%)	53		48		48		49	
Present								
Fraction (%)	25		37		37		36	
Former								
Fraction (%)	21		15		16		16	
Use of unopposed oestrogen‡								
Fraction (%)	7		6		9		4	
BMI (kg/m²)	27·2	19·9−38·0	25·8	20·6−38·7	24·0	20·4−32·5	23·7	20·0−30·1
Smoking at baseline (present)								
Fraction (%)	40		23		30		17	

HRT, hormone replacement therapy.
* Cut-point quartiles (nmol/l) based on distribution among subcohort.
† Among parous women.
‡ Among users.

Results

The median time from baseline to endometrial cancer diagnosis was 5·5 years, with a range from 0 to 11·8 years. The baseline characteristics of subcohort members and cases are presented separately in Table 1 for plasma enterolactone concentration and selected potential and established risk factors for endometrial cancer. The concentration of enterolactone was slightly lower among cases than among women in the subcohort, while cases were moderately older. Number of births and age at first birth among parous women did not differ, while a larger fraction of cases was nulliparous than subcohort members. More cases were postmenopausal and a greater proportion was present or former users of HRT than subcohort members, and among present users of HRT, a slightly higher fraction of cases used unopposed oestrogen. Furthermore, cases had a moderately higher BMI and fewer were present smokers at baseline than subcohort members. The distribution of risk factors among the included and excluded cases was rather similar, with the exception of smoking, where a lower proportion of the excluded cases was smokers at baseline (results not shown).

The baseline characteristics of all women are presented in Table 2 according to the enterolactone levels among the subcohort members. The risk factors for endometrial cancer do not seem to differ systematically according to the levels of enterolactone.

When evaluated linearly, a 20 nmol/l higher level of enterolactone was associated with a statistical borderline lower incidence of endometrial cancer (IRR 0·93, 95 % CI 0·85, 1·01). Adjustment for potential confounders did not change the IRR, but the CI became slightly wider (IRR 0·93, 95 % CI 0·84, 1·04) (Table 3).

Women in quartile 2 were used as a reference when evaluating enterolactone categorically, but none of the point estimates differed statistically significantly from the reference. When excluding women in the lowest quartile, every 20 nmol/l increase in plasma enterolactone concentration was associated with a 13 % lower risk of endometrial cancer when unadjusted (IRR 0·87, 95 % CI 0·77, 0·98). However, the association was no longer statistically significant after adjustment for potential confounders (IRR 0·90, 95 % CI 0·79, 1·02) (Table 3).

Potential effect modification by menopausal status (postmenopausal or premenopausal), use of HRT (never, former or present) and BMI (≤25, 25–30 or ≥30 kg/m²) on the association between plasma enterolactone concentration and endometrial cancer risk was evaluated after excluding women in quartile 1, but no modifications were observed (all P ≥ 0·08) (Table 4).

Discussion

In models adjusted for potential confounders, we found a non-significant lower risk of endometrial cancer associated with a higher concentration of plasma enterolactone (IRR 0·93, 95 % CI 0·84, 1·04 for every 20 nmol/l). After excluding the women with a low enterolactone level (≤14·3 nmol/l), the association became slightly stronger but remained non-significant (IRR 0·90, 95 % CI 0·79, 1·02 for every 20 nmol/l). The association between enterolactone and endometrial cancer did not differ between different categories of BMI, use of HRT or menopausal status.

The primary strengths of the present study include the prospective study design, the objective measure of and relatively large variation in exposure as well as detailed information on potential confounders. Selection bias, information bias and confounding were not considered problematic, as revealed by the prospective study design, a nearly complete follow-up (99·5 %) and the objective measure of exposure along with valid and complete registries for outcome measurement[19]. However, the present study is limited by the relatively small number of endometrial cancer cases, making it difficult to detect a small or moderate effect of enterolactone, if one exists. The study has 80 % power to detect a 40 %

Table 3. Endometrial cancer incidence per every 20 nmol/l increment in plasma concentration of enterolactone and when comparing women across quartiles in the 'Diet, Cancer and Health' cohort

(Incidence rate ratios (IRR) and 95 % confidence intervals)

Enterolactone	Cases/subcohort (n)	Crude model			Adjusted model*		
		IRR	95 % CI	P_trend	IRR	95 % CI	P_trend
Per 20 nmol/l increment†	173/149	0·93	0·85, 1·01		0·93	0·84, 1·04	
Quartiles‡							
Q1 (≤14)	37/38	0·67	0·36, 1·25		0·66	0·32, 1·38	
Q2 (>14 and ≤31)	58/37	1·00	Reference		1·00	Reference	
Q3 (>31 and ≤63)	45/37	0·86	0·46, 1·61		0·83	0·41, 1·68	
Q4 (>63)	33/37	0·62	0·33, 1·18		0·66	0·33, 1·32	
				0·10			0·31
Per 20 nmol/l increment§	136/111	0·87	0·77, 0·98		0·90	0·79, 1·02	

HRT, hormone replacement therapy.

* Adjusted for baseline values of menopausal status (post or pre), use of HRT (never, former or present), type of HRT (unopposed oestrogen, combination or other), parity (number of births, age at first pregnancy or nulliparity), BMI (kg/m²) and smoking (present, former or never).
† Including all women.
‡ Including all women. Cut-point quartiles (nmol/l) based on distribution among subcohort.
§ Including women with enterolactone >14·3 nmol/l.

Table 4. Endometrial cancer incidence per every 20 nmol/l increment in plasma concentration of enterolactone stratified by menopausal status, use of hormone replacement therapy (HRT) and BMI, respectively, in the 'Diet, Cancer and Health' cohort

(Incidence rate ratios (IRR) and 95 % confidence intervals)

		Crude model			Adjusted model*		
	Cases/subcohort (n)	IRR	95 % CI	$P_{\text{interaction}}$	IRR	95 % CI	$P_{\text{interaction}}$
Menopausal status							
Premenopausal	22/23	0·53	0·22, 1·24		0·49	0·21, 1·16	
Postmenopausal	114/88	0·89	0·79, 1·00		0·93	0·83, 1·05	
				0·23			0·15
Use of HRT†							
Never	42/39	0·85	0·71, 1·02		0·88	0·73, 1·06	
Former	20/11	1·07	0·77, 1·48		1·30	0·95, 1·77	
Present	52/38	0·87	0·71, 1·07		0·86	0·69, 1·08	
				0·48			0·08
BMI							
Normal (≤ 25 kg/m^2)	69/62	0·91	0·81, 1·02		0·95	0·84, 1·07	
Overweight ($25{-}30$ kg/m^2)	41/36	0·82	0·56, 1·21		0·78	0·50, 1·22	
Obese (≥ 30 kg/m^2)	26/13	0·67	0·32, 1·38		0·59	0·26, 1·36	
				0·65			0·44

* Adjusted for baseline values of menopausal status (post or pre), use of HRT (never, former or present), type of HRT (unopposed oestrogen, combination or other), parity (number of births, age at first pregnancy or nulliparity), BMI (kg/m^2) and smoking (present, former or never). Menopausal status, use of HRT and BMI were not adjusted for in the analyses stratified for these variables.
† Among postmenopausal women.

decrease in risk. This means that a true effect of a more moderate magnitude may be missed.

Enterolactone was measured in a single non-fasting baseline sample regarded to reflect long-term exposure, which might be reasonable due to a relatively slow elimination, precursors in many commonly consumed food items[20] and enterohepatic circulation[21]. Mean elimination half-life for enterolactone is 12·6 h, while mean residence time is 35·8 h for enterolactone[22]. However, studies disagree on the suitability of using a single non-fasting sample[23,24], which might introduce exposure measurement error, potentially causing an attenuation of the association. Additionally, information on administration of antibiotics was not available, probably contributing further to exposure measurement errors. Quartile 2 was therefore used as a reference in the categorical analyses, and continuous analyses were presented both with and without women from quartile 1. Unexpectedly, the IRR of quartile 1 was of similar magnitude to the IRR of quartile 4. An IRR corresponding to a weighted average of quartiles 2, 3 and 4 would have been more biologically plausible.

Only one previous study has investigated circulating enterolactone concentrations in relation to risk of endometrial cancer[7]. The nested case–control study included 153 cases, aged 56–70 years, from cohorts in New York, Sweden and Italy. Enterolactone was not associated with a lower endometrial cancer incidence, neither when the full study population was evaluated nor after stratifying by menopausal status. The authors only presented results for a categorical evaluation of enterolactone, where risk is assumed to be the same within categories, which can be seen as a limitation. Risk estimates were only adjusted for BMI, as further adjustment did not affect the results, while type of HRT was not evaluated. The study evaluated effect modification by cohort and results were pooled even though the test was borderline significant ($P = 0{\cdot}06$). The relatively small number of cases

might have limited the power to detect small or moderate effects in both the present and the previously published study. The median level (5–95 %) of enterolactone was lower in the previous case–control study (cases: 19 nmol/l (2–68), controls: 19 nmol/l (3–56)) than in the present study (Table 1), whereby it might have been more difficult to identify a potential association in the previous study due to a relatively homogeneous exposure level. Estimation of lignan intake by FFQ in the two American case–control studies[8,9], including 482 and 417 cases, respectively, is limited by intake measurement methodologies and problems associated with estimating the precise content of lignans in foods included in the FFQ[25]. When comparing studies using plant lignan intake as an exposure to studies using circulating enterolactone, it must be regarded that enterolactone quantifies a combination of plant lignan intake and microflora capacity.

The primary consideration in relation to generalisability in the present study is the limited selected age range as well as potential differences among participants and non-participants, where participants might be healthier in general, partly explained by a higher socio-economic status[10].

In conclusion, the findings from the present substudy provide some support for a possible inverse association between plasma enterolactone concentration and endometrial cancer incidence, although further studies with higher statistical power and information on antibiotic use are required to confirm the associations.

Acknowledgements

References

1. Globocan 2008 (2008) Cancer Incidence, Mortality and Prevalence Worldwide in 2008. International Agency for Research on Cancer (IARC). World Health Organization (WHO). http://www.iarc.fr (accessed March 2012).

2. Kaaks R, Lukanova A & Kurzer MS (2002) Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. *Cancer Epidemiol Biomarkers Prev* **11**, 1531–1543.

3. Adlercreutz H (2007) Lignans and human health. *Crit Rev Clin Lab Sci* **44**, 483–525.

4. Peeters PH, Slimani N, van der Schouw YT, *et al.* (2007) Variations in plasma phytoestrogen concentrations in European adults. *J Nutr* **137**, 1294–1300.

5. Mazur W (1998) Phytoestrogen content in foods. *Baillière's Clin Endocrinol Metab* **12**, 729–742.

6. Adlercreutz H, Mousavi Y, Clark J, *et al.* (1992) Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. *J Steroid Biochem Mol Biol* **41**, 331–337.

7. Zeleniuch-Jacquotte A, Lundin E, Micheli A, *et al.* (2006) Circulating enterolactone and risk of endometrial cancer. *Int J Cancer* **119**, 2376–2381.

8. Horn-Ross PL, John EM, Canchola AJ, *et al.* (2003) Phytoestrogen intake and endometrial cancer risk. *J Natl Cancer Inst* **95**, 1158–1164.

9. Bandera E, Williams M, Sima C, *et al.* (2009) Phytoestrogen consumption and endometrial cancer risk: a population-based case–control study in New Jersey. *Cancer Causes Control* **20**, 1117–1127.

10. Tjonneland A, Olsen A, Boll K, *et al.* (2007) Study design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer and Health: a population-based prospective cohort study of 57,053 men and women in Denmark. *Scand J Public Health* **35**, 432–441.

11. Tjonneland A, Overvad K, Haraldsdottir J, *et al.* (1991) Validation of a semiquantitative food frequency questionnaire developed in Denmark. *Int J Epidemiol* **20**, 906–912.

12. Adlercreutz H, Wang GJ, Lapcík O, *et al.* (1998) Time-resolved fluoroimmunoassay for plasma enterolactone. *Anal Biochem* **265**, 208–215.

13. Stumpf K, Uehara M, Nurmi T, *et al.* (2000) Changes in the time-resolved fluoroimmunoassay of plasma enterolactone. *Anal Biochem* **284**, 153–157.

14. Prentice RL (1986) A case–cohort design for epidemiologic cohort studies and disease prevention trials. *Biometrika* **73**, 1–11.

15. Barlow WE, Ichikawa L, Rosner D, *et al.* (1999) Analysis of case–cohort designs. *J Clin Epidemiol* **52**, 1165–1172.

16. Greenland S (1995) Avoiding power loss associated with categorization and ordinal scores in dose–response and trend analysis. *Epidemiology* **6**, 450–454.

17. Greenland S (1995) Dose–response and trend analysis in epidemiology: alternatives to categorical analysis. *Epidemiology* **6**, 356–365.

18. Kilkkinen A, Pietinen P, Klaukka T, *et al.* (2002) Use of oral antimicrobials decreases serum enterolactone concentration. *Am J Epidemiol* **155**, 472–477.

19. Storm HH, Michelsen EV, Clemmensen IH, *et al.* (1997) The Danish Cancer Registry – history, content, quality and use. *Dan Med Bull* **44**, 535–539.

20. Milder IE, Kuijsten A, Arts IC, *et al.* (2007) Relation between plasma enterodiol and enterolactone and dietary intake of lignans in a Dutch endoscopy-based population. *J Nutr* **137**, 1266–1271.

21. Adlercreutz H (2002) Phytoestrogens and breast cancer. *J Steroid Biochem Mol Biol* **83**, 113–118.

22. Kuijsten A, Arts IC, Vree TB, *et al.* (2005) Pharmacokinetics of enterolignans in healthy men and women consuming a single dose of secoisolariciresinol diglucoside. *J Nutr* **135**, 795–801.

23. Hausner H, Johnsen NF, Hallund J, *et al.* (2004) A single measurement is inadequate to estimate enterolactone levels in Danish postmenopausal women due to large intraindividual variation. *J Nutr* **134**, 1197–1200.

24. Zeleniuch-Jacquotte A, Adlercreutz H, Akhmedkhanov A, *et al.* (1998) Reliability of serum measurements of lignans and isoflavonoid phytoestrogens over a two-year period. *Cancer Epidemiol Biomarkers Prev* **7**, 885–889.

25. Lampe JW (2003) Isoflavonoid and lignan phytoestrogens as dietary biomarkers. *J Nutr* **133**, Suppl. 3, 956–964.