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Prospective study of plasma enterolactone and prostate cancer risk (Sweden)

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Abstract

Objectives: Enterolactone, a phytoestrogen produced by the intestinal microflora from precursors in plant foods, has been postulated to protect against hormone-dependent cancers. We studied the association between plasma enterolactone and risk of prostate cancer.
Methods: In the Northern Sweden Health and Disease Cohort, enterolactone concentrations were measured by time-resolved fluoroimmunoassay in plasma taken from 265 men who were diagnosed with prostate cancer at a mean time of 5 years after blood collection, and in plasma from 525 control men, matched for age and date of blood collection.
Results: There was no significant association between quartiles of plasma enterolactone and risk of prostate cancer. Odds ratios for prostate cancer, estimated by conditional logistic regression for increasing concentrations of enterolactone in quartiles were 1.00 (referent), 0.81 (95% confidence interval 0.52–1.27), 1.03 (0.67–1.58), and 1.22 (0.80–1.86). Adjustments for body mass index (BMI), smoking status and stratification for age, lag time, storage time and tumour characteristics did not materially alter risk estimates. Men with very low enterolactone levels, however, had significantly higher risk of prostate cancer, odds ratio for bottom decile *versus* all other deciles was 1.68 (1.03–2.74).
Conclusions: Our results do not support the hypothesis that enterolactone formed from dietary lignans protects against prostate cancer.

Introduction

It has been suggested that phytoestrogens protect against cancer by altering levels of growth-promoting hormones, sex hormones (or their binding to cell receptors), cell adhesion, proliferation, apoptosis, and angiogenesis [1–7]. Lignans are the predominant type of phytoestrogens in a Western diet, whereas the intake of isoflavonoids, mostly from soy products, is high especially in Southeast Asia [1]. Lignans are present in flax seed, whole grain cereals, particularly in rye, but also in other seeds, berries, some vegetables and fruits [4, 8]. Enterolactone is the most abundant circulating lignan in human subjects and is produced in the conversion of plant lignan glycosides by the intestinal microflora. Circulating concentrations of enterolactone are related to the ingested amount of dietary precursors and also to the efficiency of biotransformation by the microflora and intestinal uptake [9].

Rats and mice fed on a rye diet had high urinary excretion levels of enterolactone and reduced take and growth rates of prostate tumour implants compared to those animals on a control diet [5, 6]. In a recent, small,

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randomized trial in men with conservatively treated prostate cancer, a high intake of rye-bran-bread was associated with increased apoptosis in biopsies from the prostate, although the increase in apoptosis did not correlate to levels of plasma enterolactone [10].

In the two previously published prospective studies on circulating enterolactone and prostate cancer risk, no significant association was found [11, 12]. Previously, data from three Scandinavian cohorts, The Janus serum bank in Oslo, Norway, The Helsinki heart study in Helsinki, Finland, and The Northern Sweden health and disease cohort in Umeå, Sweden was pooled in a collaborative study within the Nordic biologic specimen bank's working group on cancer causes & control [11]. The result in that study was largely driven by the Norwegian study group, who made up more than two thirds of the study base and the median level of enterolactone in the Norwegian men (7 nmol/l) was about half of that found in men in the Finnish (16 nmol/l) and Swedish (14 nmol/l) subgroups. We speculated that the enterolactone concentrations in that study may have been too low to show a protective effect on enterolactone on prostate cancer development. No information was available in that analysis for smoking status, and data on body mass index (BMI) was only available for the Finnish and Swedish groups. However, these factors may be associated with enterolactone levels and may influence the association between enterolactone and risk.

In the present report, we describe the results of an extension of the study within the Northern Sweden Health and Disease cohort on the association between levels of plasma enterolactone and risk of prostate cancer. We also relate enterolactone to obesity, smoking, and other factors associated with dietary life-style and hormonal status.

Methods

Men in The Northern Sweden Health and Disease Cohort (NSHDC) were recruited through the Västerbotten Intervention Project (VIP), and the Northern Sweden part of the WHO study for Monitoring of Trends and Cardiovascular Disease Study (MONICA) [13–15]. VIP is a population-based intervention study which started in 1985 and is still ongoing. Each year, all residents of Västerbotten county who turn 40, 50, and 60 years of age are invited by letter to participate in a health promoting project with the aim of reducing cardiovascular disease and cancer by advocating a healthy diet and lifestyle to the general public. The MONICA study includes 2704 men, recruited in 1986, 1990, and 1994, and it is also a population-based sample from the counties of Västerbotten and Norrbotten. In both projects, subjects were asked to complete a self-administered questionnaire that included questions about demographic, medical, and lifestyle characteristics including smoking habits. In addition, we recorded the subjects' height and weight (recorded to the closest 0.2 cm and kg, respectively) and drew a 20 ml blood sample from each subject at study entry. Together these two projects had recruited a total of 37,776 men by July 2001. For the large majority of participants, blood collection took place in the morning, and all plasma samples are stored at −80 °C. All participants signed an informed consent form at the time of recruitment, and the study was approved by the local Research Ethical Committee at Umeå University Hospital.

Case ascertainment and control selection

All incident cases of prostate cancer and all cases of death were identified through linkage with the regional cancer registry, using a nation-wide individual identification number as the identity link. If several samples were available from the same case subject, the first sample was chosen. The first linkage in 1997 identified 87 cases of prostate cancer who were included in the previous study [11]. A subsequent linkage in 2001 identified 178 additional cases of prostate cancer. Data from medical records in hospitals that provided diagnostic work-up and care for the cases in this study were extracted by a research nurse, verified by the treating physician and reported to the prostate cancer registry at Oncological Center, Umeå University Hospital, which includes more than 98% of all cases of prostate cancer diagnosed in the region. Data in the registry includes date of diagnosis, mode of diagnosis, TNM classification, tumor differentiation, serum level of prostate specific antigen (PSA), and primary treatment. For cases diagnosed before 1992, the same protocol was applied and data was directly entered to the study file. No formal screening programme has been or currently is in operation in the catchment area of the cohort. Since 2000, the cause for work-up leading to the diagnosis of prostate cancer is registered in the registry. Approximately 10% of cases were detected in health check-ups in 2000–2001, indicating little exposure for opportunistic PSA screening for early detection of prostate cancer. Two controls were randomly selected within sets of subjects from the cohort, including all members who were alive and free of cancer at the time of diagnosis of the case, the index case being matched on age (± 6 months) and date (± 2 months) of the blood sampling.

Biochemical assays

The samples were transported on dry ice to the Institute for Preventive Medicine, Nutrition and Cancer, Folkhälsan Research Center, Helsinki. The concentration of enterolactone was measured with time-resolved fluoroimmunoassay (TR-FIA), a method which has been described in detail earlier with regard to sensitivity, specificity, accuracy, and precision [16, 17]. The protocol used in the second study group was identical to that used in our previous pooled study [11]. In brief, 100 μl of plasma was diluted with buffer and the enterolactone conjugates were hydrolyzed with β-glucuronidase and sulfatase, the hydrolyzed enterolactone was extracted twice with diethyl ether, the dry residue was then dissolved in methanol after evaporation of the ether, followed by mixing and evaporation. Assay buffer was added to the tubes and TR-FIA of the solution was performed in duplicate in IgG coated microtitration wells, adding antiserum, tracer, and enhancement solution, and fluorescence was measured in a multi-label counter. The working range of the assay was 8.9–3218 pg/μl, corresponding to plasma levels of 1.5–540 nmol/l. All the batches of samples included three quality control samples. The mean inter-assay coefficient of variation calculated from these samples was 11.8%. Samples pertaining to matched study-subjects were always analyzed together in the same batch (*i.e.,* on the same day and within the same run). The laboratory personnel were not given access to the case–control status of the samples. Measurements of other variables that had been used in previous reports were related to the concentrations of enterolactone. These measurements included blood pressure, plasma concentrations of IGF-I, IGFBPs-1, -2, -3, insulin [15], leptin [18], sex hormone binding globuline (SHBG), testosterone [19], blood glucose, triglycerides, and cholesterol [14].

Statistical analyses

Spearman's coefficients of correlation adjusted for age, BMI and case–control status were used to examine cross-sectional relationships between enterolactone and other variables. Pair-wise *t*-tests were used to test for differences in analytes between the case and the mean value for the two matched controls. Geometric mean was used as description of central tendency for enterolactone as the distribution was skewed. Odds ratios (ORs) for disease were calculated by conditional logistic regression for quartile levels of enterolactone. Cut-off points were determined on variable distribution of combined cases and controls. Confidence intervals (95%) were computed using the standard errors of the pertinent regression coefficients, assuming a normal probability distribution for the estimated coefficients. Reported *p*-values are two sided. The logistic regression analyses were performed using the 'PHREG' procedure for proportional hazards regression of the Statistical Analysis System (SAS) [20].

Results

Baseline characteristics and cross-sectional interrelationships

Plasma samples had been stored for a mean time of 7.1 years (range 5.2 months–14.4 years) before measurement of enterolactone. The mean time between blood collection and diagnosis for cases was 4.9 years (range

Table 1. Base-line characteristics for cases with prostate cancer and matched controls in The Northern Sweden Health and Disease Cohort[a]

	Cases n = 265	Cases n = 525	p_{diff}
Median age at recruitment, years	59.9	59.9	Matched
	Mean (SD)[b]		
Storage time (years)[c]	7.12 (2.71)	7.11 (2.73)	0.77
Lag time (years)[d]	4.9 (2.8)	–	
Height (cm)	176.0 (6.4)	175.3 (6.0)	0.11
Weight (kg)	81.1 (10.3)	82.0 (12.6)	0.26
BMI (kg/m^2)	26.2 (2.8)	26.6 (3.8)	0.04
Enterolactone (nmol/l), geometric mean(10, 90% percentile)	15.07 (4.8–42.0)	15.02 (3.4–48.2)	0.66
	Number and (percentage) of cases and controls		
Smoking status			
Current smoker	48 (19%)	106 (21%)	
Ex-smoker	80 (32%)	148 (30%)	
Complete non-smoker	123 (49%)	241 (49%)	0.73[e]

[a] The number of case–control triplets included into the analysis were 257 for height and 252 for weight. Geometric mean was used for description of central tendency of enterolactone because of the scewed distribution.

[b] Standard deviation.

[c] Time between date of blood sampling and date of biochemical analysis.

[d] Time between date of blood sampling and date of diagnosis for cases.

[e] The difference in the frequency of smoking between cases and controls was evaluated by Fisher exact Chi-square test.

[f] A complete non-smoker being someone who has never smoked regularly.

13 days–13.2 years). Mean levels of plasma enterolactone were virtually the same in cases and controls, Table 1. For cross-sectional relationships, we combined cases and controls. Enterolactone levels were significantly and inversely associated to BMI, and current smokers had significantly lower levels of enterolactone than ex-smokers and those who had never smoked (to be referred to as complete non-smokers), Figure 1. The mean BMI for current smokers (25.6 kg/m^2, 95% CI = 21.0–31.2) was significantly lower than for ex-smokers (27.0 kg/m^2, 22.1–33.2) and complete non-smokers (26.4 kg/m^2, 21.6–32.2). Concentrations of plasma enterolactone were slightly, but significantly higher in older men and in those men who had fasted over-night before the blood draw. The geometric mean of enterolactone was 13.9 nmol/l in men younger than median age, and 16.2 nmol/l in men over median age. For men with fasting times of less than eight hours (n = 259), and of more than eight hours (n = 531), *i.e.,* over night fasting, mean levels of enterolactone were 13.0 and 16.1 nmol/l, respectively. Cross-sectional correlations of plasma enterolactone to blood pressure and plasma levels of blood lipids, blood glucose, anabolic hormones and their binding proteins are shown in Table 2.

Enterolactone and prostate cancer risk

There was no significant association between levels of enterolactone in quartiles and prostate cancer risk. The estimated ORs over quartiles of increasing concentrations of enterolactone from lowest to highest quartile were 1.00 (referent), 0.81 (95% CI = 0.52–1.27), 1.03 (0.67–1.58), and 1.22 (0.80–1.86), Table 3. Adjustment for obesity, smoking status, and fasting times separately and combined did not materially influence the risk

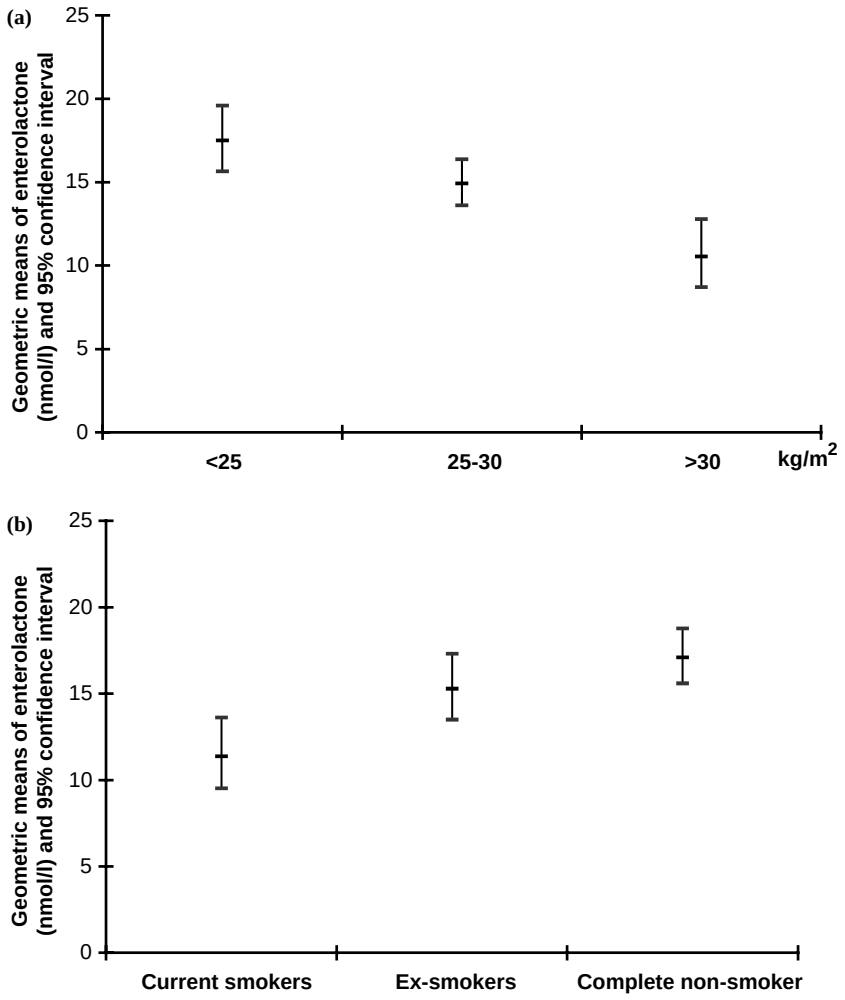

Fig. 1. Geometric mean levels of enterolactone (95% confidence interval) by category of (a) BMI; (b) smoking status.

Table 2. Cross-sectional interrelationships between enterolactone and age, BMI, blood pressure, blood lipids, hormones and binding proteins (Spearman correlations)

		Adjusted for age and BMI
Age	0.12**	
BMI	−0.19**	
Systolic blood pressure	−0.07*	−0.04
Diastolic blood pressure	−0.10*	−0.05
Triglycerides	−0.18**	−0.13*
Cholesterol	−0.06	−0.06
HDL	−0.02	−0.07
Leptin	−0.17**	−0.06
Blood glucose	−0.04	−0.01
Insulin	−0.08	−0.02
IGFBP-1	0.14*	0.06
IGFBP-2	0.05	−0.02
SHBG	0.07*	−0.006
Testosterone	0.08*	0.007
IGF-I	−0.01	−0.03

* < 0.05.
** < 0.001.

estimates. No significant association between enterolactone and risk was found in analyses stratified for age, lag time, *i.e.,* time between date of blood sampling and diagnosis, storage time, or tumour characteristics such as serum levels of PSA above 10 ng/ml at the time of diagnosis, or advanced disease, Table 4. In the group of advanced cases a non-significant increase in risk was seen for the top quartile of enterolactone.

When examining extreme levels of enterolactone, we found that men with very low enterolactone levels had a significant increase in prostate cancer risk. ORs of prostate cancer for the bottom decile of enterolactone *versus* all other deciles combined was 1.68 (95% CI = 1.03–2.74), $p = 0.04$. Men with very high levels (top decile) of enterolactone had a non-significant increase in risk compared to men in all other deciles combined, OR of 1.23 (0.76–2.02), $p = 0.39$). Geometric mean levels of enterolactone were 1.9 nmol/l (95% CI = 0.6–4.0) in the bottom decile and 59.2 nmol/l (46.5–86.5) in the top decile.

Discussion

In this prospective study, we found no significant association between quartiles levels of plasma enterolactone and prostate cancer risk in accordance with the two previous, prospective studies [11, 12].

Mean levels for cases and controls in the present study were more than twice as high as those in our previous, pooled study, which was largely dominated by Norwegian subjects who had very low levels of serum enterolactone and who also had very long lag times between blood donation and tumour diagnosis. In the present study, however, men had higher enterolactone concentration levels of plasma, levels that correspond rather well to a recent cross-sectional study of 1168 Finnish men between 25–64 years old, in which the median enterolactone levels were also around 14 nmol/l [21]. Furthermore, the lag time between blood donation and tumour diagnosis in the current study was much shorter compared to our previous, pooled study. In spite of these differences, we still observed no significant protective effect of circulating enterolactone, and in fact we saw a slight, non-significant increase in risk in all analyses for the highest quartile of enterolactone except in the strata of young men. There was a significant increase in prostate cancer risk for men in the bottom decile of plasma enterolactone levels and a non-significant increase for the top decile. This U-shaped risk pattern is in accordance with observations in a previous prospective study in the same cohort on plasma enterolactone and risk of breast cancer [22].

Table 3. Odds ratios and (95% confidence interval) of prostate cancer by quartiles of plasma levels of enterolactone in the whole study population

	Quartiles of plasma enterolactone				
	1 (referent)	2	3	4	$p_{\text{for trend}}$[a]
Cut-off (nmol/l)	< 9.38	9.38–17.64	17.64–28.31	≥28.31	
Cases/controls	65/132	59/139	68/129	73/125	
Univariate model	1	0.81 (0.52–1.27)	1.03 (0.67–1.58)	1.22 (0.80–1.86)	0.21
Adjusted for BMI[b]	1	0.78 (0.49–1.23)	1.01 (0.65–1.57)	1.07 (0.68–1.68)	0.5
Adjusted for smoking[c]	1	0.75 (0.47–1.20)	1.00 (0.64–1.57)	1.23 (0.78–1.93)	0.1
Adjusted for fasting[d]	1	0.83 (0.53–1.31)	1.02 (0.66–1.58)	1.22 (0.80–1.86)	0.23
Adj BMI, smoking and fasting	1	0.74 (0.45–1.20)	0.95 (0.60–1.52)	1.05 (0.65–1.69)	

Odds ratios and (95% confidence interval) in conditional logistic regression models.
[a] Linear trends in odds ratios for original values of enterolactone.
[b] Model adjusted for body mass index (BMI) as a continuous variable.
[c] Model adjusting for smoking coded as current smoker, ex-smoker and complete non-smoker.
[d] Model adjusting for fasting time coded as less than or more than 8 h.

Table 4. Odds ratios and (95% confidence interval) of prostate cancer by quartiles of plasma levels of enterolactone stratified for age, lag time, storage time and tumour characteristics

	Quartiles of plasma enterolactone				
	1 (referent)	2	3	4	p_{trend}[a]
< Median age[b]	1	0.47 (0.25–0.88)[b]	0.80 (0.42–1.53)	0.81 (0.44–1.50)	0.96
> Median age	1	1.14 (0.61–2.13)	1.18 (0.65–2.15)	1.57 (0.88–2.81)	0.14
< Median lag time[c]	1	0.69 (0.37–1.30)	0.90 (0.50–1.62)	1.18 (0.66–2.11)	0.40
> Median lag time	1	0.83 (0.43–1.58)	1.08 (0.58–2.02)	1.30 (0.70–2.41)	0.27
< Median storage time[d]	1	0.71 (0.37–1.34)	0.99 (0.56–1.75)	1.19 (0.66–2.13)	0.35
> Median storage time	1	1.00 (0.55–1.85)	1.08 (0.56–2.05)	1.38 (0.74–2.55)	0.29
Exclusion less than 1 year follow-up[e]	1	0.91 (0.56–1.47)	1.16 (0.74–1.83)	1.30 (0.83–2.04)	0.14
PSA > 10 ng/ml[f]	1	0.93 (0.50–1.73)	0.83 (0.46–1.53)	1.30 (0.73–2.32)	0.45
Cases–controls	37/72	35/74	33/76	43/67	
PSA < 10	1	0.82 (0.41—1.62)	1.43 (0.76–2.71)	1.50 (0.78–2.86)	0.09
Cases–controls	25/58	22/61	33/50	31/53	
Advanced cases[g]	1	1.13 (0.43–2.97)	0.87 (0.32–2.35)	2.20 (0.83–5.84)	0.15
Cases–controls	13/32	15/31	12/34	21/25	
Non advanced cases	1	0.74 (0.44–1.23)	0.98 (0.61–1.58)	1.12 (0.70–1.80)	0.39
Cases–controls	52/99	44/108	53/99	55/97	
Extended study[h]	1	0.55 (0.28–1.10)	0.93 (0.51–1.68)	1.30 (0.72–2.36)	0.14
Cases–controls	31/63	23/72	32/62	39/56	

[a] Linear trends in odds ratios for original values of enterolactone. Odd ratios and 95% confidence interval in conditional logistic regression analysis stratified for:

[b] Age at the time of blood sampling.

[c] Lagtime between date of blood sampling and date of diagnosis.

[d] Time between date of blood sampling and biochemical analysis.

[e] 234 cases with a lag time between date of blood retrieval and date of diagnosis more than one year.

[f] Presence of significant disease defined as a serum level of prostate specific antigen (PSA) > 10 ng/ml at time of diagnosis.

[g] Advance ed disease defined as locally extensive tumour (T3, T4) and/or with bone metastasis on bone scan (M1) and/or lymph node metastasis at surgical exploration of fossa obturatorius (N1), and/or a serum PSA above 50 ng/ml at the time of diagnosis.

[h] Subgroup of new cases identified in linkage in 2001 and their matched controls.

Factors related to plasma enterolactone levels

Levels of circulating enterolactone depend on dietary intake and their biotransformation by the microflora in the gut, as well as on absorption rates of enterolactone from the gut. In a recent randomized intervention study, mean plasma levels of enterolactone were twice as high in men who had had a very high intake of rye-bran-bread, a rich source of enterolactone, compared to men with an equally large intake of wheat bread [10]. However, despite very little variation in the intake of rye-bran within the intervention group, the plasma levels differed more than 10-fold between the men in that group, thus indicating that factors other than diet are equally important as determinants of plasma enterolactone levels, the most likely being rates of biotransformation and absorption.

The biotransformation and absorption of enterolactone is decreased by a high fat intake [21, 23], which is one possible determinant of excess weight, and high fat intake might explain our observation of an inverse relationship of enterolactone with BMI. We also found that smokers had lower enterolactone levels compared to former smokers and complete non-smokers, even though smokers had a significantly lower BMI than former and complete non-smokers. The mechanism underlying this relationship of smoking with low enterolactone levels is unclear, but might be due to a lower lignan intake or decreased lignan biotransformation and absorption of enterolactone among smokers. We also observed that enterolactone levels were weakly but significantly inversely related to a number of factors related to the metabolic syndrome such as high blood pressure, high blood lipids, and high levels of leptin. However, after adjustment for age and BMI these correlations were no longer significant, except for a weak but significant inverse relationship to triglycerides.

Antibiotics have also been shown to influence enterolactone levels, in likelihood by affecting the microflora in the gut [24]. One hypothesis on prostate cancer aetiology states that inflammation in the prostate predisposes to tumour development and progression [25]. If this is true, one would expect that cases may have been more likely to have had episodes of prostatitis and would have received antibiotic treatments more frequently. However, cases

and controls had virtually the same mean levels of enterolactone, and there are no indications that the use of antibiotics was different in cases compared to controls in our study.

Other factors that may have contributed to the lack of significant result in our study include the possibility that the result from a single analysis of enterolactone concentration in plasma may not be a reliable measurement of long time exposure. However, from a comparison between enterolactone measurements in three repeated blood collections taken over a time period of two years, the estimated reliability coefficient was 0.55 [26]. This level of reliability indicates a reasonable degree of stability of enterolactone levels within individuals over time, and indicates a sufficient degree of reliability of one analysis of enterolactone concentration in plasma from a single blood sample as a measurement of individuals' long-term average levels to allow their use for epidemiological studies, although relative risks would certainly be substantially attenuated.

The biological effects of enterolactone have been attributed to its estrogenic activity [1], but relatively little is known about the estrogenic and antiestrogenic effects of mammalian lignans. Some studies have shown an agonistic effect of lignan on estrogen receptors, other studies have shown antagonistic effects and it has been speculated that the effect may depend on the level of estrogens present in the system under study [1, 3, 27–29].

Conclusions

Our study provides no support for the hypothesis that high levels of circulating enterolactone protect against prostate cancer. However, our results show that plasma enterolactone is inversely related to obesity and smoking, thus supporting the view that enterolactone is a marker of a healthy life-style, including high intake of whole grain cereal, fruits, and vegetables. Our data is congruent with the fact that obesity is not a strong risk factor for prostate cancer [30–32] and that prostate cancer has not been strongly linked to diet [33, 34].

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References

1. Adlercreutz H, Mazur W (1997) Phyto-oestrogens and Western diseases. *Ann Med* **29**: 95–120.
2. Bingham SA, Atkinson C, Liggins J, Bluck L, Coward A (1998) Phytoestrogens; where are we now? *Br J Nutr* **79**: 393–406.
3. Saarinen NM, Warri A, Makela SI, *et al.* (2000) Dietary phytoestrogens and their role in hormonally dependent disease. *Nutr Cancer* **36**: 207–216.
4. Adlercreutz A (1998) Human health and phytoestrogens. In: Korach KS ed. *Reproductive and Developmental Toxicology*. New York, pp. 299–371.
5. Landström M, Zhang JX, Hallmans G, *et al.* (1998) Inhibitory effects of soy and rye diets on the development of Dunning R3327 prostate adenocarcinoma in rats. *Prostate* **36**: 151–161.
6. Bylund A, Zhang JX, Bergh A, *et al.* (2000) Rye bran and soy protein delay growth and increase apoptosis of human LNCaP prostate adenocarcinoma in nude mice. *Prostate* **42**: 304–314.
7. Demark-Wahnefried W, Price DT, Polascik TJ, *et al.* (2001) Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features. *Urology* **58**: 47–52.
8. Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H (2000) Identification of lignans as major components in the phenolic fraction of olive oil. *Clin Chem* **46**: 976–988.
9. Heinonen S, Nurmi T, Liukkonen K, *et al.* (2001) *In vitro* metabolism of plant lignans: new precursors of mammalian lignans enterolactone and enterodiol. *J Agric Food Chem* **49**: 3178–3186.
10. Bylund A, Lundin E, Zhang JX, *et al.* (2003) Randomised controlled short-term intervention pilot study on rye bran bread in prostate cancer. *Eur J Cancer Prev* **12**: 407–415.
11. Stattin P, Adlercreutz H, Tenkanen L, *et al.* (2002) Circulating enterolactone and prostate cancer risk: a Nordic nested case–control study. *Int J Cancer* **99**: 124–129.
12. Kilkkinen A, Virtamo J, Virtanen MJ, Adlercreutz H, Albanes D, Pietinen P (2003) Serum enterolactone concentration is not associated with prostate cancer risk in a nested case–control study. *Cancer Epidemiol Biomarkers Prev* **12**: 1209–1212.
13. Weinehall L (1997) Partnership for health on the role of primary health care in a community intervention programme. Umeå University Medical Dissertations ISSN 0346-6612-ISBN 91-7191-388-2. Umeå University, Umeå.
14. Weinehall L, Hallgren CG, Westman G, Janlert U, Wall S (1998) Reduction of selection bias in primary prevention of cardiovascular disease through involvement of primary health care. *Scand J Prim Health Care* **16**: 171–176.
15. Stattin P, Bylund A, Rinaldi S, *et al.* (2000) Plasma insulin-like growth factor-I, insulin-like growth factor-binding proteins, and prostate cancer risk: a prospective study. *J Natl Cancer Inst* **92**: 1910–1917.
16. Adlercreutz H, Wang GJ, Lapcik O, *et al.* (1998) Time-resolved fluoroimmunoassay for plasma enterolactone. *Anal Biochem* **265**: 208–215.
17. Stumpf K, Uehara M, Nurmi T, Adlercreutz H (2000) Changes in the time-resolved fluoroimmunoassay of plasma enterolactone. *Anal Biochem* **284**: 153–157.

18. Stattin P, Söderberg S, Hallmans G, *et al.* (2001) Leptin is associated with increased prostate cancer risk. A nested-case-referent study. *J Clin Endocrinol Metab* **86**: 1341–1345.
19. Stattin P, Lumme S, Tenkanen L, *et al.* (2004) High levels of circulating testosterone are not associated with increased prostate cancer risk: a pooled prospective study. *Int J Cancer* **108**: 418–424.
20. Cary E (1989) SAS Institute Inc. SAS/STAT User's Guide Version 6.4.
21. Kilkkinen A, Stumpf K, Pietinen P, Valsta LM, Tapanainen H, Adlercreutz H (2001) Determinants of serum enterolactone concentration. *Am J Clin Nutr* **73**: 1094–1100.
22. Hultén K, Winkvist A, Lenner P, Johansson R, Adlercreutz H, Hallmans G (2002) An incident case–referent study on plasma enterolactone and breast cancer risk. *Eur J Nutr* **41**: 168–176.
23. Stumpf K, Pietinen P, Puska P, Adlercreutz H (2000) Changes in serum enterolactone, genistein, and daidzein in a dietary intervention study in Finland. *Cancer Epidemiol Biomarkers Prev* **9**: 1369–1372.
24. Kilkkinen A, Pietinen P, Klaukka T, Virtamo J, Korhonen P, Adlercreutz H (2002) Use of oral antimicrobials decreases serum enterolactone concentration. *Am J Epidemiol* **155**: 472–477.
25. De Marzo AM, Marchi VL, Epstein JI, Nelson WG (1999) Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol* **155**: 1985–1992.
26. Zeleniuch-Jaccquotte A, Adlercreutz H, Ahmedkhanov A, Toniolo P (1998) Reliability of serum measurements of lignans and isoflavonoid phytoestrogens over a two-year period. *Cancer Epidemiol Biomarkers Prev* **7**: 885–889.
27. Kaaks R, Lukanova A, Sommersberg B (2000) Plasma androgens, IGF-I, body size and prostate cancer risk: a synthetic review. *Prostate Cancer Prostatic Dis* **3**: 157–172.
28. Saarinen NM, Huovinen R, Warri A, *et al.* (2002) Enterolactone inhibits the growth of 7,12-dimethylbenz(a)anthracene-induced mammary carcinomas in the rat. *Mol Cancer Ther* **1:** 869–876.
29. Adlercreutz H, Mousavi Y, Clark J, *et al.* (1992) Dietary phytoestrogens and cancer: *in vitro* and *in vivo* studies. *J Steroid Biochem Molec Biol* **41:** 331–337.
30. Bianchini Saarinen NM, Warri A, *et al.* (2000) Hydroxymatairesinol, a novel enterolactone precursor with antitumor properties from coniferous tree (*Picea abies*). *Nutr Cancer* **36**: 207–216.
31. Kaaks R, Lukanova A, Sommersberg B (2000) Plasma androgens, IGF-I, body size and prostate cancer risk, Kaaks R, Vainio H (2002) Overweight, obesity, and cancer risk. *Lancet Oncol* **3**: 565–574.
32. Calle EE, Rodriguez C, Walker-Thurmond K, Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* **348**: 1625–1638.
33. World Cancer Research Fund /ifcr (1997) *Prostate. Food, Nutrition and the Prevention of Cancer: A Global Perspective*. BANTA Book Group, Menasha, WI, USA, pp. 310–323
34. Key TJ, Allen N, Appleby P, *et al.* (2004) Fruits and vegetables and prostate cancer: No association among 1,104 cases in a prospective study of 130,544 men in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Int J Cancer* **109**: 119–124.