

Serum Enterolactone Concentration Is Not Associated with Prostate Cancer Risk in a Nested Case-Control Study

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Abstract

The lignan enterolactone, produced by the intestinal microflora from dietary precursors, has been hypothesized to protect against hormone-dependent cancers and cardiovascular diseases. We conducted a nested case-control study to examine the relationship between serum enterolactone concentration and prostate cancer. Enterolactone concentrations were measured by time-resolved fluoroimmunoassay in serum collected at baseline in the α -Tocopherol, β -Carotene Cancer Prevention Study from 214 men with prostate cancer diagnosed during a 6-year follow-up and from 214 controls matched by age, date of baseline blood collection, intervention group, and local study area. Mean serum enterolactone concentration (in nmol/liter) did not differ significantly between case and control subjects [15.9 (SD, 15.2) versus 16.9 (SD, 14.9), respectively ($P = 0.42$)]. Odds ratios for prostate cancer risk estimated by conditional logistic regression for increasing quartiles of enterolactone concentration were 1.00 (referent), 0.72 [95% confidence interval (CI), 0.43–1.23], 0.98 (95% CI, 0.58–1.68), and 0.71 (95% CI, 0.42–1.21). Our findings do not support the hypothesis that enterolactone is involved in the development of prostate cancer.

Introduction

Prostate cancer is the leading cancer in males in most Western populations (1). Despite this, its etiology remains largely unknown, with age, race, and family history of disease being the only established risk factors (2). The observations that the incidence of latent prostate cancer is similar worldwide but the

rate of invasive disease varies markedly between populations (1) support an important role for environmental factors, including diet, in the progression and clinical manifestation of this malignancy. Several dietary components have been studied with inconsistent findings (3, 4), emphasizing some of the current challenges in the epidemiology of prostate cancer.

Lignan phytoestrogens have received particular attention during the past decade. Enterolactone, the most abundant lignan in humans, is produced by the intestinal microflora from plant lignans present in flaxseed and other seeds, whole grain cereals, berries, vegetables, and fruits (5–8). *In vitro* studies suggest that enterolactone possesses a broad spectrum of biological properties including but not limited to antioxidant activity and inhibition of several enzymes involved, for example, in steroid hormone metabolism, giving it the potential to reduce the risk of hormone-dependent cancers (9–12). A diet rich in lignan-containing foods or pure plant lignans has retarded the development of experimental cancer in animal models (13–16). Only a few epidemiological studies have been published, and the results are inconclusive (17–19). Our study extends the previous research by examining the association between serum enterolactone concentration and prostate cancer in a prospective design.

Materials and Methods

Study Population. The ATBC⁵ Study was a randomized, double-blind, placebo-controlled prevention trial to determine whether daily supplementation with α -tocopherol, β -carotene, or both would reduce the incidence of lung and other cancers. A total of 29,133 Finnish male smokers aged 50–69 years were recruited in 1985–1988 and followed until the end of the trial or until death, whichever occurred earlier (median follow-up, 6.1 years). The study design, methods, and primary trial results have been described in detail elsewhere (20).

A total of 246 prostate cancers were diagnosed during the trial period between May 1985 and April 1993. These cancers were identified primarily through the Finnish Cancer Registry and the Register of Causes of Deaths. Medical records were reviewed centrally by two study oncologists to confirm diagnoses. Cases with available histology or cytology (98%) were also reviewed by pathologists. For each case, a control matched by age (± 1 year), date (± 28 days) of baseline blood collection, intervention group, and local study area was selected. A blood sample for serum enterolactone analysis was available for 233 cases and 222 controls, leaving 214 case-control pairs for the analysis.

The ATBC Study was approved by the institutional review

Received 4/22/03; revised 7/1/03; accepted 7/21/03.

Grant support: The ATBC Study was supported by a contract with the United States National Cancer Institute (N01-CN-45165). The present study was supported in part by the Sigrid Jusélius Foundation and the Folkhälsan Research Foundation.

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⁵ The abbreviations used are: ATBC, α -Tocopherol, β -Carotene Cancer Prevention; CI, confidence interval; OR, odds ratio; BMI, body mass index; TR-FIA, time-resolved fluoroimmunoassay; CV, coefficient of variation; PSA, prostate-specific antigen.

boards of the National Public Health Institute of Finland and the United States National Cancer Institute. Written informed consent was obtained from each study participant at baseline.

Data Collection. Baseline information on sociodemographic characteristics, medical history, and diet was collected by questionnaires, and height and weight were measured. BMI was computed as weight (kg) divided by the square of height (m²).

Assay of Serum Samples. Fasting venous samples were taken from subjects at baseline examination. Serum was separated, divided into 1-ml aliquots, and stored in glass vials at -70°C until analysis of enterolactone in the spring of 1998. The enterolactone analysis was performed by TR-FIA (21), with slight modifications (22). The modified method is briefly as follows: 150 µl of serum were incubated with 150 µl of hydrolysis reagent containing 2 units/ml sulfatase and 0.2 unit/ml β-glucuronidase overnight at 37°C. After hydrolysis, the free enterolactone and hydrolyzed conjugates were extracted twice with 1.5 ml of diethyl ether. Diethyl ether was evaporated to dryness in a water bath, after which the dry residue was dissolved in 150 µl of assay buffer. A sample was divided into two subsamples, and enterolactone of both subsamples was analyzed by TR-FIA using the VICTOR 1420 multilabel counter (Wallac Oy, Turku, Finland). Subsamples were analyzed in the same laboratory batch, and the mean value of these two measurements was used. The matched case/control sets were also analyzed in the same laboratory batch, and laboratory personnel were blinded with regard to the case-control status of samples. All of the batches were analyzed with two quality control samples going through the whole method. The concentrations of quality control samples and the respective interassay CVs were 3.2 (CV = 16.9%) and 12.4 nmol/liter (CV = 12.5%). One additional quality control sample with a concentration of 110 nmol/liter and CV = 8.2% controlled for TR-FIA.

PSA was determined by an immunometric method (23).

Statistical Methods. Baseline characteristics of cases and controls were compared by using paired *t* tests for normally distributed variables and the Wilcoxon signed-rank test for vari-

ables with skewed distribution, *i.e.*, for serum enterolactone concentration, age, education, consumption of fruit and berries, and years of smoking. The McNemar test was used to analyze case-control differences in dichotomized variables, including area of residence, history of prostatomegaly, and proportion of subjects with elevated PSA (PSA > 4 ng/ml; Ref. 24). Furthermore, Spearman correlation coefficients between serum enterolactone concentration and background variables were estimated.

The relationship of serum enterolactone concentration to prostate cancer risk for the matched sets was evaluated by conditional logistic regression using ORs with 95% CIs. Men were stratified into quartiles based on their serum enterolactone levels relative to the distribution in controls, and the ORs were tested for linear trends across the quartiles of serum enterolactone concentration. Some subgroup analyses were conducted using serum enterolactone tertiles because of the smaller number of subjects in these subgroups. Cutoff points for serum enterolactone quartiles were 5.9, 12.4, and 24.4 nmol/liter, and cutoff points for serum enterolactone tertiles were 7.7 and 19.5 nmol/liter.

Results

Characteristics of the 214 case-control pairs are presented in Table 1. The participants were all Caucasian men who were smokers at study entry. Because of individual matching, age distributions were similar between the groups, 61.5 years (SD, 5.1 years). Of those who developed prostate cancer, 67% had a serum PSA above 4 ng/ml at baseline compared with 10% of controls (*P* < 0.0001). No significant differences were present between the groups for area of residence, BMI, consumption of lignan-containing foods, education, history of prostatomegaly, or smoking habits.

Serum enterolactone concentration was negatively correlated with number of cigarettes smoked per day in both cases (*r* = -0.19) and controls (*r* = -0.14) and with BMI (*r* = -0.20) in cases; it was positively correlated with age (*r* = 0.30) and number of smoking years (*r* = 0.20) in control subjects.

Table 1 Selected baseline characteristics of prostate cancer cases and controls: the ATBC Study

	Cases (n = 214)	Controls (n = 214) ^a	<i>P</i> for case-control difference ^b
Age (yrs)	61.5 ± 5.1 ^c	61.5 ± 5.1	0.13
BMI (kg/m ²)	26.5 ± 3.6	26.2 ± 3.6	0.50
Cigarettes/day	19 ± 8	19 ± 9	0.52
Smoking (n yrs)	39 ± 9	40 ± 8	0.40
Serum enterolactone (nmol/liter)	15.9 ± 15.2	16.9 ± 14.9	0.42
Serum PSA > 4 ng/ml	67	10	<0.0001
History of prostatomegaly	8	7	0.73
Urban residence	67	66	0.92
Education			0.88
Elementary school	67	67	
Some high school	19	21	
High school graduate	14	13	
Consumption of fruit and berries ^d (g/day)	153 ± 108	153 ± 114	0.92
Consumption of vegetables ^e (g/day)	280 ± 102	289 ± 108	0.62
Consumption of cereals ^f (g/day)	213 ± 92	214 ± 76	0.81

^a Controls were matched to cases by age, date of baseline blood collection, intervention group, and local study area.

^b Matched sets; paired *t* test for normally distributed variables, the Wilcoxon signed-rank test for serum enterolactone concentration, age, education, consumption of fruit and berries, and years of smoking, and the McNemar test for dichotomized variables.

^c Mean ± SD or percent.

^d Including fruit, berries, and fruit juices.

^e Including legumes, potatoes, roots, and vegetables.

^f Including rye, wheat, and other cereals.

The distribution of serum enterolactone concentration did not differ between cases and controls [15.9 nmol/liter (SD, 15.2 nmol/liter) *versus* 16.9 nmol/liter (SD, 14.9 nmol/liter); *P* for difference = 0.42]. No obvious relationship was observed between serum enterolactone concentration and risk of prostate cancer (Table 2); the ORs from the lowest to the highest quartile of enterolactone were 1.00, 0.72 (95% CI, 0.43–1.23), 0.98 (95% CI, 0.58–1.68), and 0.71 (95% CI, 0.42–1.21), and *P* for trend was 0.37. Adjustments for age, area of residence, BMI, consumption of lignan-containing foods, education, history of prostatomegaly, or smoking habits did not substantially change the results (<6% change in ORs; data not shown). Moreover, the trial supplementation did not significantly modify the enterolactone-prostate cancer association (*P* for the interaction = 0.50).

At the time of prostate cancer diagnosis, the men were a mean age of 65.4 years (SD, 5.2 years). The mean time from blood collection to diagnosis was 3.9 years, with a range from 36 days to 7.2 years. The serum enterolactone-prostate cancer association was not altered when the analysis was restricted to men (169 case-control pairs) diagnosed at least 2 years after blood collection (OR for the highest quartile, 0.77; 95% CI, 0.43–1.41).

Because an occult malignancy in the prostate may influence serum enterolactone concentration, we excluded from the analysis 144 case-control pairs where either case, control, or both had elevated PSA (PSA > 4 ng/ml) or had failed to provide a blood sample for PSA analyses (2 cases and 1 control). This exclusion did not produce substantially different results from those including the whole study population (OR for the highest tertile, 0.71; 95% CI, 0.33–1.55).

Half of the cases had localized (stage 1 or 2) disease confined to the prostate. Serum enterolactone was not associated with localized cancers (OR, 1.10; 95% CI, 0.57–2.12), but it has a modest inverse association with advanced (stage 3 or 4) cancer (OR for the highest tertile, 0.72; 95% CI, 0.36–1.42).

Discussion

The results of this case-control study nested within a trial-based cohort of more than 29,000 Finnish men do not support the hypothesis that high levels of circulating enterolactone are protective against prostate cancer. Our findings are in agreement with the only published epidemiological study of circulating enterolactone and prostate cancer (18). In that nested case-control study based on population cohorts from Finland, Sweden, and Norway, no statistically significant association between serum enterolactone and risk of prostate cancer was found (OR for the highest quartile, 1.08; 95% CI, 0.83–1.39). Moreover, no relationship between dietary intake of lignans and

prostate cancer was observed in a case-control study conducted in the United States (17).

One of the important strengths of our investigation was its prospective design. Blood samples used were obtained up to 7 years before diagnosis of prostate cancer, whereas in traditional case-control studies, blood concentrations of many metabolites, including enterolactone, could be affected by the presence of cancer or by changes in dietary habits induced by the disease. Furthermore, our study design ensured identical collection and handling of blood samples from case and control subjects. Cases and controls were also closely matched by date of blood collection to control for possible bias due to degradation of enterolactone during storage.

Men in our study were aged 50–69 years at blood collection. Among men in this age bracket, an occult malignancy of the prostate occurs at high frequencies (25). In fact, 67% of our cases and 10% of controls had a baseline PSA level > 4 ng/ml, which is associated with a >20% risk of prostate cancer (24). However, exclusion of these subjects from the analyses did not produce substantially different results from those including the whole study population, nor did restriction of analyses to subjects with at least 2 years of follow-up alter the enterolactone-prostate cancer association. Therefore, the lack of inverse association between serum enterolactone and prostate cancer in our data cannot be due to a high prevalence of subclinical prostate cancers at baseline.

PSA testing has been increasingly used in prostate cancer screening and diagnosis since the early 1990s. This has led to the early detection of prostate cancer, and men are identified who may have gone undiagnosed before PSA screening became common. It is possible that risk factors of PSA-detected, early-stage disease could differ from those of clinically manifest tumors. Our results suggested that high circulating enterolactone was modestly associated with advanced prostate cancer but not with localized prostate cancer. However, given that no association between serum enterolactone concentration and either localized or advanced prostate cancer risk was found in a larger prospective study (18), we cannot rule out the possibility that our result is due to chance.

Our study also has some limitations. The generalizability of results may be somewhat restricted because the study included only smokers who participated in a clinical trial. Smoking has been associated with a greater risk of fatal prostate cancer (26, 27), and tobacco may increase the virulence of tumors (28). Furthermore, we have previously observed lower circulating enterolactone levels among smokers than nonsmokers (29). Because our study did not include nonsmokers, we cannot directly evaluate whether smoking modified the serum enterolactone-prostate cancer association. Although serum enterolactone concentration correlated negatively with the number of cigarettes smoked per day and positively with the number of smoking years in ATBC Study controls, the mean enterolactone levels in our study were comparable with those in a cross-sectional study of 1168 Finnish men (median enterolactone, 13.8 nmol/liter; Ref. 29) and in a recent study of prostate cancer (median enterolactone, 15.5–15.6 nmol/liter; Ref. 18). Another potential limitation is the lack of information on antibiotic use by study participants. We have previously observed that any kind of antibiotics reduces serum enterolactone and that this effect may be sustained for at least 6 months (30). However, we have no reason to assume that use of antibiotics differed between cases and controls. Finally, it can be questioned whether blood enterolactone concentration at a single point in time reflects only recent exposure rather than long-term exposure. The reliability of a single measurement of serum

Table 2 Unadjusted ORs and 95% CIs for prostate cancer in quartiles of serum enterolactone concentration: the ATBC Study^a

Serum enterolactone quartile ^b (median)	OR	95% CI
Q1 (3.0)	1.00	
Q2 (9.1)	0.72	0.43–1.23
Q3 (17.2)	0.98	0.58–1.68
Q4 (31.8)	0.71	0.42–1.21
<i>P</i> for trend	0.37	

^a Controls were matched to cases by age, date of baseline blood collection, intervention group, and local study area.

^b Cutoff points for serum enterolactone quartiles were 5.9, 12.4, and 24.4 nmol/liter (based on controls only).

enterolactone, however, appears to be moderately high (0.55–0.79; Refs. 31 and 32), suggesting that serum measurements of this compound could be a useful in epidemiological studies.

In conclusion, high serum enterolactone concentration had no significant protective association with the risk of prostate cancer among Finnish male smokers.

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