Null Results in Brief

Lack of Prospective Associations between Plasma and Urinary Phytoestrogens and Risk of Prostate or Colorectal Cancer in the European Prospective into Cancer-Norfolk Study

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Abstract

Dietary phytoestrogens are suggested to reduce the risk of prostate and colorectal cancer, but the results of epidemiologic studies have not yielded consistent support for this proposed effect, possibly due to inadequate databases of phytoestrogen levels in foods. Biomarkers of phytoestrogen intakes may provide a clearer insight into the relationship between phytoestrogen exposure and the risk of prostate or colorectal cancer risks. From the European Prospective into Cancer-Norfolk cohort (ages 45-75), serum and urine samples were analyzed for seven phytoestrogens [daidzein, enterodiol, enterolactone, genistein, glycitein, *O*-desmethylangolensin (*O*-DMA), and equol] among 193 cases of prostate cancer and 828 controls, and 221 cases of colorectal cancer with 889 controls. Summary variables of total lignans (enterodiol and enterolactone) and total isoflavones (daidzein, genistein, *O*-DMA, equol, and glycitein) were created and analyzed in conjunction with individual phytoestrogens. Logistic regression analyses revealed that there was no significant association between prostate cancer risk and total serum isoflavones [odds ratio (OR), 1.01; 95% confidence interval (CI), 0.93-1.10] or total serum lignans (OR, 0.94; 95% CI, 0.86-1.04) or between colorectal cancer risk and total serum isoflavones (OR, 1.01; 95% CI, 0.94-1.08) or total serum lignans (OR, 1.03; 95% CI, 0.94-1.12). Similarly, null associations were observed for individual serum phytoestrogens and for all urinary phytoestrogen biomarkers. In conclusion, we have found no evidence to support an inverse association between phytoestrogen exposure and prostate or colorectal cancer risk. (Cancer Epidemiol Biomarkers Prev 2008;17(10):2891–4)

Introduction

It has been suggested that the risk of prostate and colorectal cancer may be reduced among those with a greater intake of phytoestrogens (1-6) due to a series of proposed protective mechanisms; primarily, competitive binding to estrogen receptors (7-11). Colorectal cancer has been associated with estrogen status (12, 13), but we are aware of only one published report for colorectal cancer and dietary phytoestrogens in which an inverse association was observed with higher total phytoestrogen intake among Canadians in a case-control study (14). Epidemiologic studies of prostate cancer and dietary phytoestrogen provide evidence of either a decreased risk of prostate cancer with greater intake (15-19) or no association (15-18), depending on the element under examination. The inconsistency of the results of these studies may be due to the varied scope of dietary assessment methods and the accuracy of phytoestrogen nutrient database available for estimating intake.

Serum and urinary biomarkers of phytoestrogen exposure reflect individual phytoestrogen intake (20) and are not reliant on self-reported dietary intakes or nutrient databases. Furthermore, analysis of samples collected prospectively avoids the problems of bias encountered in case-control studies. Using newly developed, highly sensitive and rapid methods for the analysis of phytoestrogens in serum and urine by mass spectrometry, we investigated whether the risk of prostate and colorectal cancer were related to phytoestrogen exposure in a prospective study, the Norfolk arm of the European Prospective Investigation of Cancer (EPIC).

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Materials and Methods

Between 1993 and 1997, 11,607 men and 14,032 women ages 45 to 75 participated in the EPIC-Norfolk study following recruitment from a general practitioners database (21). Details of the health and lifestyle questionnaire, 7-day food diary, anthropometric measurements, and laboratory methods for the quantification of phytoestrogens and circulating sex hormone–binding globulin have been published previously (22). Incident cancer cases that occurred a minimum of 12 months after recruitment were identified through the East Anglia Cancer Registry. The phytoestrogens daidzein, genistein, glycitein, *O*-desmethylangolensin (*O*-DMA), equol, enterodiol, and enterolactone were analyzed by either gas chromatography/mass spectrometry (urinary biomarkers for 89 prostate cancer cases from an earlier analysis; ref. 23) or liquid chromatography/mass spectrometry (serum and urinary biomarkers for colorectal cancer and all other prostate cancer cases). For gas chromatography/mass spectrometry analysis, limits of detection were between 1.2 ng/mL (enterodiol) and 5.3 ng/mL (enterolactone). For liquid chromatography/mass spectrometry analysis, the limit of detection was determined to be between 82 pg/mL (daidzein) and 222 pg/mL (equol). Intrabatch and interbatch coefficient of variation (%) were <4% except for glycitein (5.6%) and equol (5.7%; ref. 24). Details on case-control frequency matching, quality assurance, and methodology have been published elsewhere (22, 24, 25).

For case-control comparisons, a χ^2 test was applied to categorical variables and an unpaired Student's *t* test was conducted for continuous variables (log-transformed). Phytoestrogen levels were log 2–transformed and unconditional logistic regression was applied to obtain odds ratios (OR). Summary variables of total lignans (enterodiol and enterolactone) and total isoflavones (daidzein, genistein, *O*-DMA, equol, and glycitein) were created and analyzed in conjunction with individual phytoestrogens. Participants with missing covariate data were deleted from the logistic regression analysis; data were missing for <5% of the sample unless specified

Table 1. Characteristics of prostate and colorectal cancer cases and controls in EPIC-Norfolk

Prostate cancer	Cases (*n* = 193)	Controls (*n* = 828)	*P**
	Mean		
Age (y)	66.1	66.2	0.85
Height (cm)	171.9	173.2	0.02
Weight (kg)	77.4	79.8	0.01
SHBG (nmol/L)†	43.3	45.4	0.22
Energy (kJ/d)	9,180	8,884	0.05
Fat (g/d)	83.3	80.0	0.07
Lycopene (μg/d)	1,029	1,208	0.08
	n (column %)		
Family history of prostate cancer	5 (3%)	15 (2%)	0.48
Colorectal cancer	**Cases (*n* = 221)**	**Controls (*n* = 889)**	***P****
	Mean		
Age (y)	64.6	64.6	0.92
Height (cm)	168.2	167.5	0.32
Weight (kg)	75.7	75.0	0.54
Alcohol (g/d)	11.4	10.2	0.88
Calcium (mg/d)	809.7	830.4	0.32
Fiber (g/d)	14.1	14.61	0.29
Fat (g/d)	72.3	73.7	0.41
Folate (μg/d)	261.3	263.0	0.93
Energy (kJ/d)	7,989.9	8,087.9	0.58
Red meat (g/d)	53.1	51.1	0.94
Fruit and vegetable intake (g/d)	246.3	254.56	0.32
	n (column %)		
Female	96 (43%)	382 (43%)	0.90
Family history colorectal cancer	19 (9%)	76 (9%)	0.99
NSAID use >3 mo‡	9 (6%)	40 (6%)	0.88
Menopausal hormone therapy			
Current	11%	14%	0.33
Former	11%	6%	
Never	78%	80%	
Ever used oral contraceptives	16%	26%	0.09

Abbreviations: SHBG, sex hormone-binding globulin; NSAID, nonsteroidal anti-inflammatory drugs.
*P values obtained with a t test for log-transformed continuous variables and a χ^2 for categorical variables.
†Data available for 135 cases and 582 controls.
‡Data available for 149 cases and 626 controls.

Table 2. Risk of prostate and colorectal cancer in relation to serum and urine phytoestrogen levels in EPIC-Norfolk

	Serum phytoestrogens* (ng/mL)			Urinary phytoestrogens* (μg/mmol creatinine)		
	Median (ng/mL)	OR (95% confidence interval)	*P*	Median (μg/mmol creatine)	OR (95% confidence interval)	*P*
Prostate cancer †						
Total lignans	5.7	0.94 (0.86-1.04)	0.231	91.6	0.95 (0.88-1.03)	0.219
Total isoflavones	10.3	1.01 (0.93-1.10)	0.809	27.7	0.98 (0.90-1.08)	0.727
Genistein	6.90	0.99 (0.93-1.05)	0.78	7.18	1.00 (0.95-1.05)	0.86
Daidzein	2.50	0.99 (0.93-1.05)	0.68	14.73	0.97 (0.92-1.03)	0.31
Equol	0.01	1.02 (0.96-1.08)	0.52	0.26	1.03 (0.97-1.07)	0.20
O-DMA	0.10	0.97 (0.91-1.03)	0.33	1.13	0.98 (0.94-1.03)	0.42
Glycitein	0.01	1.01 (0.95-1.08)	0.67	1.09	1.01 (0.96-1.06)	0.81
Enterodiol	0.20	1.01 (0.95-1.07)	0.85	5.82	0.98 (0.93-1.04)	0.53
Enterolactone	5.40	0.96 (0.88-1.02)	0.35	83.05	0.98 (0.91-1.05)	0.48
Colorectal cancer ‡						
Total lignans	5.7	1.03 (0.94-1.12)	0.534	102.1	1.06 (0.97-1.15)	0.181
Total isoflavones	9.3	1.01 (0.94-1.08)	0.866	27.4	1.03 (0.95-1.11)	0.495
Genistein	6.25	0.98 (0.94-1.03)	0.43	5.84	1.00 (0.96-1.04)	0.90
Daidzein	2.20	1.01 (0.97-1.06)	0.57	15.12	1.03 (0.98-1.09)	0.21
Equol	0.01	1.04 (0.99-1.09)	0.16	0.07	0.98 (0.95-1.02)	0.40
O-DMA	0.10	1.01 (0.96-1.07)	0.67	1.48	1.01 (0.96-1.06)	0.69
Glycitein	0.01	1.00 (0.94-1.06)	0.89	0.50	1.03 (0.99-1.07)	0.15
Enterodiol	0.10	1.04 (0.99-1.09)	0.16	5.99	1.03 (0.98-1.08)	0.22
Enterolactone	5.20	1.02 (0.95-1.10)	0.55	93.72	1.00 (0.95-1.06)	0.94

*Log 2–transformed for logistic regression analysis therefore the estimates of risk correspond to a doubling in phytoestrogen level.
†Serum, n = 191 cases and 815 controls; urine, n = 152 cases and 665 controls; models adjusted for age, height, weight, and intake of energy, fat, lycopene (g/d), and whether sample had been analyzed in a prior publication.
‡Serum, n = 214 cases and 877 controls; urine, n = 146 cases and 686 controls; models adjusted for age, sex, height, weight, and average intake of fiber and calcium (g/d).

otherwise. The analyses for prostate cancer included a dichotomous variable to indicate whether gas chromatography/mass spectrometry or liquid chromatography/mass spectrometry was used. Potential confounders were evaluated through a likelihood ratio comparison of models with and without the variable under examination, and were retained in the final model if their inclusion resulted in changes to the OR values for the phytoestrogen under study (>10%) or significant likelihood ratio tests. An interaction term was added to the analysis of colorectal cancer in order to determine if the associations differed between the sexes. All analyses were conducted with SAS software (version 8.02, SAS Institute, Inc.). All P values are two-sided; values below 0.05 were considered significant, with marginal significance noted below 0.1.

Results

Between 1993 and 2006, 193 cases of prostate cancer and 221 cases of colorectal cancer were diagnosed in the EPIC-Norfolk cohort, with a mean follow-up length of approximately 9 years. Demographic and lifestyle characteristics of the prostate and colorectal cancer cases and controls are presented in Table 1.

Prostate cancer risk was not associated with any of the serum or urinary phytoestrogens analyzed (Table 2). The inclusion of data on family history of prostate cancer, physical activity, social class, sex hormone–binding globulin, or the average intake of zinc and selenium resulted in a <10% change in any of the ORs and did not yield significant likelihood ratio tests (data not shown). Colorectal cancer risk was not associated with any of the serum or urinary phytoestrogens (Table 2); there was no evidence to suggest that these associations differed by sex (data not shown). These results were not affected by adjustment for family history of colorectal cancer, aspirin use, smoking status, physical activity, the average daily intake of energy and fat, or use of menopausal hormone therapy (women only; data not shown).

Discussion

The absence of an association of either serum or urinary phytoestrogen levels with prostate cancer is in agreement with several previously published studies (12, 16, 23, 26). Similarly, one study of Japanese men found that the ORs for serum daidzein, genistein, and equol were not significant for prostate cancer risk, despite an inverse trend across quartiles of equol (27). Another Japanese study detected higher serum levels of equol, genistein, and daidzein among cancer cases relative to controls, but caution is warranted in the interpretation of this study as the samples were drawn up to 3 years after diagnosis and the t test used to compare mean levels did not adjust for any potentially confounding variables (28). A study of Scottish men reported an inverse association between prostate cancer and serum enterolactone, but also found no association with serum daidzein, genistein, or equol (15). Overall, the absence of an association between prostate cancer and phytoestrogen biomarkers in the present study does not strongly contradict the existing literature.

In a prospective study of Dutch adults, the relationship between plasma enterolignans and colorectal cancer in the full study was null, but there was evidence of increased risk among particular subgroups: current smokers, subjects with a high body mass index, and

women, particularly postmenopausal women (29). To our knowledge, this is the only other study of colorectal cancer and phytoestrogen biomarkers that has been published; its overall conclusion is congruent with the results of the present study. A retrospective study reported a decreased likelihood of colorectal adenomas, the precursors to colorectal cancer, among those with higher serum enterodiol levels, and a near-significant trend of decreasing OR values across enterolactone quartiles upon exclusion of antibiotic users (P = 0.05), but is not appropriate for comparison with the present results due to substantial differences in study design and end points (30).

The strengths of the present study include the breadth of data available for controlling potential confounders, and its prospective design, thus avoiding bias among cases for their biomarker samples and questionnaire on dietary and questions on health behavior. The mass spectrometry quantification method for samples used in EPIC-Norfolk offers specificity and sensitivity that is greater than that provided by time-resolved fluoroimmunoassay, the method commonly applied in prior phytoestrogen biomarker research (24).

In conclusion, there was no evidence of a relationship between phytoestrogen exposure and the risk of prostate or colorectal risk in this European population. Additional studies are required to establish whether the results from the EPIC-Norfolk study can be generalized to larger and different study populations, for example, in the Far East and in vegetarians with higher intakes of phytoestrogens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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