Selenium, Apoptosis, and Colorectal Adenomas

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Abstract

Background: **Selenium is an essential trace element found in cereals, wheat, dairy products, meat, and fish. This micronutrient may prevent carcinogenesis through several biochemical pathways; one suggested pathway is enhanced apoptosis.**

Objectives: **The relation between selenium and colorectal adenomas was evaluated because the colorectal adenoma is the established precursor lesion of most colorectal cancers. Apoptosis was a pathway of interest because decreased apoptosis has been associated with an increased prevalence of adenomas. Our objectives were as follows: to investigate the association between (*a*) selenium and colorectal adenomas and (*b*) selenium and apoptosis.**

Methods: **The study population was assembled for the Diet and Health Study III (*n* = 803), a cross-sectional study conducted at the University of North Carolina Hospital (Chapel Hill, NC). There were 451 participants in the analysis of selenium and adenoma prevalence and 351 participants in the analysis of selenium and apoptosis. Selenium was measured from serum collected at the time of colonoscopy. Apoptosis was measured in biopsies from normal rectal epithelium obtained during the colonoscopy procedure.**

Results: **Participants in the highest fifth of serum selenium were less likely to have adenomas in comparison with those in the lowest fifth (prevalence ratio, 0.6; 95% confidence interval, 0.4-1.1). Selenium and apoptosis (>2.76 cells per crypt) were not strongly related, but results collectively suggested a roughly inverse association.**

Conclusions: **High selenium was associated with a reduced prevalence of colorectal adenomas. Apoptosis, however, did not seem to be the mechanism by which selenium was related to adenoma prevalence in our data.** (Cancer Epidemiol Biomarkers Prev 2006;15(3):486–93)

Introduction

Colorectal cancer ranks third in cancer incidence among both men and women in the United States, with an estimated lifetime risk of 6% (1) and an estimated 147,000 new cases in 2004 (2). There is a 25-fold variation in colorectal cancer incidence worldwide; the highest rates are in North America, Australia, New Zealand, western Europe, and select areas of eastern Europe (3). Variations in incidence of colorectal cancer with respect to geography and migration suggest that diet may play an important role in colorectal cancer risk (4-6).

One possible dietary risk factor for colorectal cancer is selenium (7). Selenium, which may prevent carcinogenesis through several biochemical pathways, is an essential trace element found in cereals, wheat, dairy products, meat, and fish (8-10). Results from observational studies of the association between selenium and colon cancer are inconclusive (11-22); however, the Nutritional Prevention of Cancer Trial (23) suggests a strong inverse association between selenium and colon cancer risk. Possible biological mechanisms for this association, hypothesized from laboratory studies, include repair and prevention of oxidative damage, intracellular signaling, activation of thyroid hormone, regulation of immune response, and enhanced apoptosis (7, 24, 25).

Apoptosis, or programmed cell death, has evolved in multicellular organisms to remodel tissue during development, maintain tissue homeostasis (proliferation-apoptosis balance), remove senescent cells, and delete cells with irreparable genetic damage. It is a highly regulated process with distinct morphologic and biochemical features (26-29). In nontechnical terms, the cell uses a genetically controlled program to cause its own death in little more than a few hours (29).

It has been suggested that the incidence of certain diseases, such as cancer, is increased by an inhibition of normal apoptosis (30-32). Martin et al. (33) reported that a low rate of apoptosis was strongly associated with a higher prevalence of colorectal adenomas in a previous analysis of data from the Diet and Health Study III [DHS III; odds ratios (OR), 0.12; 95% confidence interval (95% CI), 0.07-0.20]; therefore, the apoptotic mechanism is a promising pathway to investigate for colorectal cancer.

The possibility that selenium may increase anticancer apoptotic activity has been suggested by several carcinogenesis studies (34-42). It is unclear how selenium might induce apoptosis; however, several selenium metabolites, such as hydrogen selenide, methylselenol, and selenodiglutathione, are being researched (43-45). Selenide and methyselenol are metabolized from organic sources of selenium (naturally obtained through diet), such as selenomethionine, selenocysteine, and methylselenocysteine. Selenodiglutathione is metabolized from inorganic sources of selenium, such as selenate and selenite (46).

It has been suggested that methylselenol may directly activate caspases, which are thought to be downstream executors of the apoptosis (47). It has been observed that selenodiglutathione is associated with an increased expression of Fas ligand, a well-known mediator of apoptosis (43, 47). Fas ligand from tumor cells signals the immune system to induce apoptosis.

Received 9/28/05; revised 12/9/05; accepted 1/23/06.

Grant support: NIH grants R01 CA44684, P30 DK34987, and R03 CA101132; Lineberger Comprehensive Cancer Center; Cancer Research and Prevention Foundation; and National Institute of Environmental Health Sciences grant P30ES10126.

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

Note: This work was completed while Alexandra Connelly-Frost was a predoctoral trainee (NIH grant P30 DK34987) in the Department of Epidemiology at the University of North Carolina at Chapel Hill under the direction of Robert S. Sandler.

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doi:10.1158/1055-9965.EPI-05-0759

Pathways to apoptosis for methylselenocysteine, a lower homologue of selenomethionine, have also been suggested (45, 47). Ip et al. (45) propose that methylselenocysteine increases the expression of cyclin D_1 and cdk5, two proteins associated with increased apoptosis. They also report that methylselenocysteine up-regulates c-jun. In addition to these promoting factors, selenium down-regulates AKT2, which transmits survival signals. The combination of these signals increases the probability of cell death through apoptosis.

Because most colorectal cancers arise from benign adenomas (48), an opportunity for early detection and intervention exists if modifiable risk factors for the adenoma can be identified. Few studies, however, have investigated the relation between selenium and colorectal adenomas (11, 18, 49-53).

The purpose of this study was to examine the cross-sectional association between serum selenium and colorectal adenomas in an effort to further elucidate whether selenium might be a viable chemopreventive agent for colorectal cancer. A potential mechanism of anticarcinogenic action, through increased apoptotic activity, was also investigated.

Materials and Methods

Study Population. The study population consisted of the participants in the DHS III, a cross-sectional study conducted at the University of North Carolina Hospital (Chapel Hill, NC). University of North Carolina Hospital is a 750-bed university hospital serving as a major health care facility for residents of adjacent counties and as a referral center for much of the state. Patients are diverse with respect to socioeconomic status, race, and ethnicity.

The DHS III was designed to investigate dietary and lifestyle factors associated with colorectal adenomas. Study participants were recruited from consecutive patients who underwent outpatient colonoscopy at University of North Carolina Hospital from August 1, 1998 to March 4, 2000. Eligibility requirements were ages 30 to 80 years, ability to understand and complete an interview in English, ability to provide informed consent, no evidence or history of familial polyposis, current colitis, previous colonic resection, or previous colon cancer or adenoma. Patients who did not satisfactorily prepare the colon for the procedure were excluded as were those with incomplete colonoscopic visualization from the colon to the cecum. Eligible patients were asked to provide a 40 mL blood sample, to allow the colonoscopist to obtain rectal biopsy specimens from normal mucosa, and to complete a telephone-administered interview.

During the enrollment period, 2,452 outpatient colonoscopies were done at University of North Carolina Hospital; 1,526 (62%) potential participants were excluded based on eligibility requirements or an unsatisfactory colonoscopic procedure. Reasons for exclusion were not mutually exclusive and included inability to give informed consent (n = 156), previous adenoma(s) (n = 615), current colitis (n = 405), previous colon resection (n = 311), age <30 years (n = 267), previous colon cancer (n = 220), incomplete examination (n = 182), unsatisfactory preparation of colon for procedure (n = 149), polyposis (n = 6), and other factors (e.g., no telephone; procedure was a medical risk for the patient; n = 73). Among the 926 remaining eligible participants, 57 (6%) refused and 66 (7%) were not asked because the research assistant was unavailable, leaving 803 (87%) who consented to participate in the study.

The study pathologist classified each polyp as adenomatous (tubular, villoglandular, or villous pathology) or nonadenomatous (hyperplastic, pseudopolyp, inflammatory, retention, or other). For analysis purposes, patients with one or more adenomatous polyps were classified as prevalent cases.

Data Collection. Dietary information was obtained using a modified version of the Block-National Cancer Institute Food Frequency Questionnaire (54). The version of the Block quantitative food frequency questionnaire used in this study was specially modified for use in North Carolina to include regional foods (55). The Food Frequency Questionnaire interview was conducted by telephone within 12 weeks of colonoscopy and the reference period for dietary intake was the year before colonoscopy. Lifestyle interviews, also administered by telephone within 12 weeks of colonoscopy, were used to gather information on various health-related behaviors, such as smoking, physical activity, and medication use, as well as medical, family, and employment history.

Physical activity [metabolic equivalents (MET/wk)] was evaluated using a modified version of the 7-day activity recall used in the Stanford Five-City Project as described in detail elsewhere (56). The modified version includes five questions on occupational activity. Participants were asked about work and leisure activity as well as weekday and weekend activity. Activities were classified by their energy requirements expressed as METs: very light (1 MET), light (1.5 METs), moderate (4 METs), hard (7 METs), and very hard (10 METs) activity. One MET is the amount of energy expended by a 60 kg person at rest. METs per day were calculated by averaging the METs for 1 week. MET-minutes per day were calculated by multiplying the METs for each activity by the amount of time spent in that activity on a daily basis.

Serum selenium levels were determined using graphite furnace atomic absorption spectrometry with Zeeman background correction and platform technique. Graphite furnace atomic absorption spectrometry uses the characteristic wavelength absorbed from ground-state atoms of an analyte to determine trace metal concentrations. Serum was mixed with 0.1% Triton X-100 and then injected directly into the graphite furnace with the chemical modifier. The concentrations were calculated by a computer using a calibration curve based on aqueous standards. This test is able to detect levels of selenium from 2 to 600 μg/L (57). The coefficient of variation for our DHS III samples was 7%.

Apoptosis was measured from biopsy specimens using strict morphologic criteria (examination of H&E-stained sections under light microscopy). Two biopsies were placed in 10% buffered formalin for routine histology and prepared with H&E stain. Each prepared slide had five levels of tissue sectioned at least 50 μm apart, from which 8 to 12 well-oriented colonic crypts were scored (per biopsy). Apoptosis was observed in isolated single cells, not associated with an inflammatory response, and recognized by cell shrinkage, chromatin condensation, and formation of apoptotic bodies. Cells were not scored as apoptotic if the nucleus did not meet these criteria.

Apoptosis was scored as the number of apoptotic cells per colonic crypt. For each patient, the mean number of apoptotic cells was derived by taking the average number of apoptotic cells from the two biopsy specimens (i.e., a total of 16-24 well-oriented crypts). An experienced technician, blinded to adenoma status, scored all sections. The same technician, blinded of previous apoptosis scores, rescored a random sample (n = 20) of the original biopsy slides. The scoring technique had a reproducibility of 99% (99% agreement between original score and rescore for each crypt).

Covariates, including age (30-39, 40-49, 50-59, 60-69, 70+ years), gender (male, female), race (White, Black), body mass index (BMI) in fifths (kg/m^2), total energy intake (<1,000, 1,000-1,500, >1,500-2,000, >2,000 kcal/d), physical activity in fifths (MET-minutes/d), total folate (fifths), total calcium (fifths), total fiber (fifths), total dietary fat (fifths), red meat consumption (<1, >1 serving per day), regular use of nonsteroidal anti-inflammatory drugs (yes = used more than thrice weekly in the past 5 years; no = used less than

thrice weekly in the past 5 years), smoking history (current, former, never), alcohol use (none, lower 50% of users, upper 50% of users), and first-degree family history of colorectal cancer (yes, no), were obtained from dietary and lifestyle interviews. None of these potential confounders is affected by selenium, apoptosis, or the presence of previously undetected adenomas.

Of the 803 consenting participants, 712 (89%) completed both the diet and the lifestyle interviews. The sample size was further reduced by excluding participants with implausible total energy intakes (n = 20; men, <800 or >5,000 kcal/d; women, <600 or >4,000 kcal/d) and race other than Black or White (n = 40), BMI > 50 (n = 8), leaving 651 participants with complete and plausible covariate information. Implausible BMI and energy intake were excluded because the general validity of the remainder of data collected was questionable, and participants of races other than Black or White were excluded because there were too few to draw reliable conclusions. Because not all participants donated blood and tissue samples, the analysis of selenium and adenoma prevalence included 451 participants and the analysis of selenium and apoptosis included 351 participants.

Statistical Analysis. Generalized linear models (binomial distribution) were used to conduct multiple logistic (logit link function), log prevalence (log link function), and prevalence regression models (identity link function) to estimate prevalence ORs (POR), prevalence ratios (PR), and prevalence differences (PD), respectively, relating serum selenium with colorectal adenomas. For the logistic and log prevalence regressions, a series of analyses were conducted to determine the adequacy of the assumption of a linear trend. This was done by examining unadjusted ln(PR)s for 10ths of the serum selenium distribution using the lowest 10th as the reference level. Selenium was ultimately categorized into fifths, based on the distribution in the total study population, to facilitate tabular display of these results. We did not include all 10 categories used to test the adequacy of the linear trend assumption because the shape of the relationship was adequately described with five. Flexible modeling by linear and quadratic splines was employed to visualize a potential relationship more complex than a simple linear intake-response association and was also valuable in assessing the linearity assumption. Four-knot quadratic and linear splines were used, with knots equal to quintiles of selenium distribution.

Selenium was also specified dichotomously, with ''high selenium'' defined as ≥140 μg/L. This value is 1 SD below the mean in a group whose selenium intakes were supplemented by 200 μg/d in the Nutritional Prevention of Cancer Trial (23). This specification was used to estimate the possible beneficial effect of selenium supplementation.

Initially, candidates for inclusion in the modeling step as effect measure modifiers or confounders were identified (58). Potential effect measure modifiers were evaluated on both multiplicative (likelihood ratio test) and additive (interaction contrast ratio) scales, one covariate at a time without other covariates in the model. Potential confounders were evaluated by assessing confounder-disease and confounder-exposure associations (POR, PR, and PD), selecting only those that were associated with both adenomas and selenium for inclusion in the modeling stage. Modeling (including all effect modifiers and confounders identified in the initial step) was done using backward elimination based on likelihood ratio homogeneity test for effect measure modifiers (α = 0.20) and change-in-estimate for confounders [>15% change required in ln(POR), ln(PR), or PD to be a considered a confounder and retained in model]. Effect measure modification was evaluated before confounding.

The relation between selenium and apoptosis was investigated in several ways. Multiple linear regression was employed to estimate a linear relation between apoptosis and selenium level. Apoptosis was also flexibly modeled to assess a nonlinear relationship between selenium (4 knots) and apoptosis (>2.76 cells per crypt). The median value for apoptosis in the total population was 2.76 cells per crypt. Finally, generalized linear models (binomial distribution) were used to conduct multiple logistic, log prevalence, and prevalence regression models to produce estimates relating serum selenium with apoptosis. Modeling procedures described in the previous paragraph were used.

Both the PR and the POR were computed because results from these two models are often discrepant for common diseases, which are frequently the subject of cross-sectional studies, such as ours. The distributions of participant characteristics in the total study population (data not shown) were compared with those of the subpopulation with measured selenium and with those of the subpopulation with both measured selenium and apoptosis to evaluate possible selection bias in these subsamples. All analyses were conducted using SAS version 8.1 (SAS Institute, Cary, NC).

Results

Characteristics of the study population used in our analyses are presented in Table 1. There were substantially more White participants than Black participants. The majority was also ages >50 years and female. Selenium levels ranged from 43 to 250 μg/L serum, with a mean of 125 μg/L, a median of 122 μg/L, and an approximately normal distribution. The proportion of participants with selenium >140 μg/L was higher among White participants, educational attainment of college degree or more, low or normal BMI, nonsteroidal anti-inflammatory drug nonregular users, former or never smokers, those with average energy intakes (1,500-2,000 kcal/d), high folate (>695 μg/d), and who consumed <1 serving of red meat per day. Mean selenium did not differ for those with or without adenomas (122 versus 126 μg/L, respectively) nor did selenium differ for advanced versus nonadvanced adenomas (129 versus 121 μg/L, respectively).

Table 2 describes the relationship between selenium and colorectal adenomas using ORs, PRs, and PDs. Selenium was categorized for these analyses because it did not have a simple linear association with adenoma prevalence. No covariates were identified as potential effect modifiers or confounders in the stratified analysis by our criterion. Serum selenium >140 μg/L was associated with a slightly reduced prevalence of adenomas. When selenium was categorized in fifths, the presence of adenomas was less likely in the highest fifth in comparison with the lowest fifth. Described in absolute terms, the prevalence of adenomas was lower in the highest fifth in comparison with the lowest fifth of selenium.

Figure 1 displays the results of the flexible model. The predicted probability of adenomas did not seem to follow a strictly monotonic intake-response curve in which prevalence of adenomas would decrease with each increasing unit of selenium but rather suggested a concave-up or saturation-like relationship. The predicted prevalence (Pe) of adenomas decreased from ~50 to ~120 μg/L selenium (Pe ≃ 0.5-0.3). Between 120 and 160 μg/L selenium, the predicted prevalence of adenomas increased substantially (Pe ≃ 0.4) and then returned to its previous level (Pe ≃ 0.3). Above 160 μg/L, the predicted prevalence of adenomas consistently decreased and was lower than predicted prevalence at all lower selenium levels (<160 μg/L; Pe ≃ 0.3-0.1).

Among the 460 participants with measured apoptosis, 351 (76%) also had measured selenium (Table 1). Energy intake was the only factor that qualified as a potential confounder of the relation between selenium and apoptosis. (Adenoma status was not assessed as a confounder because it could be affected

Table 1. Distribution adenomas, mean selenium, and mean apoptosis by participant characteristics, DHS III

Characteristic	Population with measured selenium (n = 451)			Population with measured selenium and apoptosis (n = 351)			
	n*	Adenoma prevalence (%)	Mean (SD) selenium μg/L	Selenium >140 μg/L prevalence (%)	n†	Mean apoptosis (cells per crypt)	Apoptosis above the median (row %)
Adenoma							
Yes	133	NA	122	24	108	2.47	23
No	318	NA	126	29	243	2.90	63
Age							
30-39	30	10	135 (33)	37	26	2.86	50
40-49	111	22	121 (36)	32	86	2.79	54
50-59	162	31	122 (34)	27	129	2.78	55
60-69	85	32	126 (35)	28	64	2.82	48
70+	63	46	132 (37)	38	46	2.59	35
Race							
Black, non-Hispanic	86	24	119 (35)	20	71	2.84	56
White, non-Hispanic	365	31	126 (36)	33	280	2.76	49
Gender							
Male	187	38	125 (35)	29	144	2.73	47
Female	264	24	125 (36)	31	207	2.81	53
Education level							
Less than high school	81	28	123 (35)	25	64	2.81	56
High school or some college	185	30	122 (36)	29	148	2.79	49
College or more	184	30	129 (35)	35	138	2.74	50
First-degree family history							
Yes	117	26	130 (36)	37	94	2.79	49
No	334	31	123 (35)	28	257	2.77	55
BMI‡							
12-22	88	23	129 (6)	33	63	2.95	58
22-25	89	31	128 (36)	37	67	2.67	43
25-28	96	35	127 (39)	34	78	2.74	47
28-32	93	30	122 (28)	28	76	2.66	49
32-49	85	27	118 (43)	20	67	2.86	57
Physical activity§							
1,020-1,916	76	32	126 (37)	25	60	2.61	43
1,917-2,070	86	36	126 (30)	31	65	2.68	40
2,071-2,320	87	29	128 (38)	40	66	2.71	47
2,321-2,802	99	25	122 (39)	28	76	2.92	63
>2,082	90	29	124 (33)	27	72	2.87	54
Regular NSAID use‖							
Yes	224	25	121 (34)	27	172	2.83	56
No	227	34	128 (37)	34	179	2.72	45
Smoking							
Never	206	26	128 (36)	34	161	2.75	49
Former	160	34	127 (37)	35	118	2.76	49
Current	83	31	114 (32)	12	71	2.86	58
Alcohol (kcal/d)							
None	331	26	124 (36)	28	260	2.79	52
Lower half (<20)	57	33	129 (34)	40	48	2.75	44
Upper half (>20)	63	43	126 (37)	33	43	2.70	48
Total energy (kcal/d)							
<1,000	68	27	122 (37)	22	52	2.70	44
1,000-1,500	173	28	128 (37)	35	124	2.73	44
1,500-2,000	114	30	130 (35)	39	96	2.80	55
>2,000	93	36	117 (33)	20	76	2.85	59
Fat (g/d)§							
9-36	91	24	122 (39)	24	69	2.76	46
37-48	89	24	126 (35)	37	68	2.85	50
48-61	95	31	125 (35)	34	72	2.68	46
61-81	87	36	131 (36)	37	66	2.75	56
>81	89	34	120 (32)	21	77	2.83	55
Folate (μg/d)§							
32-189	84	20	122 (35)	26	66	2.83	55
190-304	92	32	119 (31)	18	73	2.76	53
308-551	87	27	124 (39)	29	72	2.68	46
554-694	89	26	128 (38)	37	67	2.82	51
>695	90	30	131 (35)	42	66	2.79	48
Calcium (mg/d)§							
76-424	91	25	120 (33)	24	70	2.77	50
425-620	83	30	135 (37)	34	69	2.70	42
621-912	92	30	122 (34)	28	72	2.92	61
913-1,507	96	34	121 (35)	29	74	2.71	54
>1,507	89	27	127 (38)	38	66	2.76	45
Selenium supplement use							
Yes	17	29	124 (49)	35	11	2.77	51
No	433	29	125 (35)	31	339	2.78	45

(Continued on the following page)

Table 1. Distribution adenomas, mean selenium, and mean apoptosis by participant characteristics, DHS III (Cont'd)

Characteristic	Population with measured selenium (n = 451)			Population with measured selenium and apoptosis (n = 351)			
	n*	Adenoma prevalence (%)	Mean (SD) selenium μg/L	Selenium >140 μg/L prevalence (%)	n†	Mean apoptosis (cells per crypt)	Apoptosis above the median (row %)
Vitamin supplement use							
Yes	272	30	126 (35)	33	201	2.75	53
No	174	29	120 (36)	25	146	2.79	49
Red meat (serving per day)							
0 or <1	400	29	127 (36)	33	308	2.77	51
>1	51	35	116 (27)	14	43	2.83	51

*Population with serum selenium had the following missing values: education (n = 1), physical activity (n = 13), smoking (n = 2), total energy (n = 3), folate (n = 9), selenium supplement use (n = 1), and vitamin supplement use (n = 5).
†Population with serum selenium and apoptosis had the following missing values: education (n = 1), physical activity (n = 12), smoking (n = 1), total energy (n = 3), folate (n = 8), selenium supplement use (n = 1), and vitamin supplement use (n = 4).
‡BMI (kg/m^2) at time of colonoscopy.
§Quintile cut points based on the distribution among total study population.
‖Nonsteroidal anti-inflammatory use in past 5 years: Regular use is more than thrice weekly; nonregular use is less than times weekly.

by selenium and/or apoptosis.) Multivariate linear regression results showed an inverse relationship between selenium and apoptosis (regression coefficient = −6.56; P = 0.02). Total energy did not confound the association between apoptosis and selenium.

Table 3 describes the relationship between selenium and high apoptosis (>2.76 cells per crypt) using ORs, PRs, and PDs. Total energy, again, was not a confounder of the logistic, log linear, or linear prevalence association between selenium and apoptosis. High selenium (>140 μg/L) was associated with a decreased prevalence of high apoptosis in comparison with low or average selenium levels (<140 μg/L). In other words, those with higher selenium were less likely to have high apoptosis. Described in absolute terms, the prevalence of high apoptosis was lower in those with high selenium.

Four-knot linear and quadratic splines were used to assess a flexible relation between apoptosis and selenium. Figure 2 shows the predicted prevalence of high apoptosis by selenium level. The largest predicted prevalence of high apoptosis was seen at low levels of selenium. There is a general downward trend in predicted prevalence of high apoptosis with increasing selenium values; however, this decrease was not monotonic. All of these analyses of selenium and apoptosis collectively suggest that selenium and apoptosis have a roughly inverse association (i.e., when selenium is high, apoptosis is more likely to be low).

Discussion

Serum selenium in the highest fifth (>153 μg/L) was associated with a lower prevalence of adenomas in comparison with low selenium (<92 μg/L). In a previous analysis of these data (33), high apoptosis (>3.0 cells per crypt) was strongly associated with a decreased prevalence of colorectal adenomas. Unexpectedly, our results suggested that apoptosis was an unlikely mechanism linking selenium and adenoma prevalence. Because high apoptosis and high selenium were both associated with a reduced relative prevalence of adenomas, in order for apoptosis to be the mechanism by which selenium exerted its effect, it would need to be positively associated with selenium (i.e., when selenium increased, apoptosis should also have increased.) Selenium, however, seemed to have a roughly inverse association with apoptosis.

Our results for the association between selenium and adenoma prevalence are similar to three of four studies providing risk estimates in the literature, which also reported a strong inverse association between serum selenium and colorectal adenomas (11, 49, 50). Adjusted for age, gender, and race, Russo et al. (50) found that persons with plasma selenium >130 μg/L were five times less likely to have a colorectal adenoma (OR, 0.2; 95% CI, 0.01-1.0). After controlling for age and smoking, Clark et al. (ref. 49) found that those with selenium levels >128 μg/L (n = 48) were more than thrice less likely to have an adenoma (OR, 0.3; 95% CI, 0.1-1.0). In a Spanish population (n = 139), Fernandez-Banares et al. (11) found a similar relation as these two American studies; high selenium (>82 μg/L) was associated with a substantially reduced risk of large colorectal adenomas after adjusting for age, gender, smoking, and alcohol (OR, 0.2; 95% CI, 0.1-0.8). We did not observe any confounding in our model of selenium and adenomas; however, all but one (49) of the above-mentioned studies controlled for common adjustment factors (e.g., age, gender, and race), without evidence of confounding in their data.

It is interesting that these authors used different cut points yet achieved similar results. For example, Fernandez-Banares et al. found a reduction in risk of large colorectal adenomas for

Table 2. Association between serum selenium and presence of colorectal adenomas (n = 451)

Serum selenium (μg/L)	n (%)	POR (95% CI)*	PR (95% CI)*	PD (95% CI)*
<140	313 (69)	1.0 (Reference)	1.0 (Reference)	0.00 (Reference)
>140	138 (31)	0.8 (0.5-1.2)	0.8 (0.6-1.2)	−0.05 (−0.13 to 0.04)†
Fifths				
Low (43-92)	90 (20)	1.0 (Reference)	1.0 (Reference)	0.00 (Reference)
Low (93-115)	91 (21)	0.8 (0.4-1.5)	0.8 (0.5-1.3)	−0.05 (−0.18 to 0.08)
Middle (116-131)	92 (20)	1.1 (0.6-2.0)	1.0 (0.7-1.6)	0.02 (−0.12 to 0.15)
Middle (132-152)	90 (20)	1.4 (0.7-2.5)	1.2 (0.8-1.8)	0.07 (−0.07 to 0.21)
High (153-250)	89 (20)	0.5 (0.3-1.1)	0.6 (0.4-1.1)	−0.12 (−0.24 to 0.00)‡

*Results were unchanged after adjustment for age, race, and gender.
†Prevalence of adenomas is 5% lower in those with selenium >140 μg/L in comparison with <140 μg/L.
‡Prevalence of adenomas is 12% lower in those with selenium >153 μg/L in comparison with <93 μg/L.

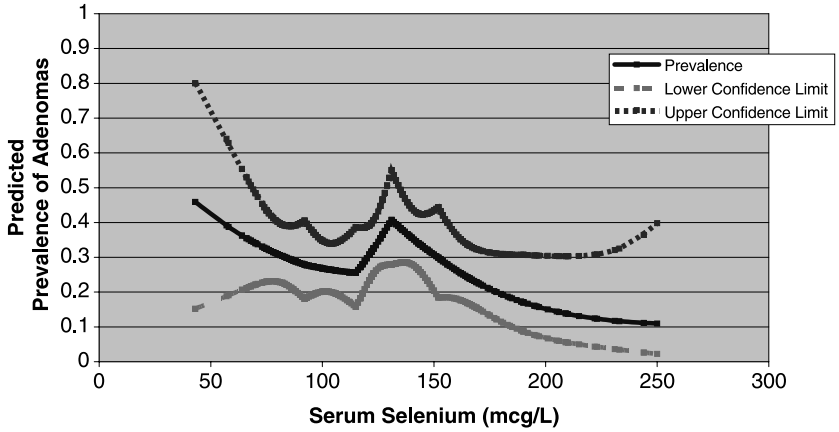

Figure 1. Predicted prevalence of adenomas by selenium level: unadjusted four-knot quadratic spline.

high selenium defined as >82 μg/L. This result is similar to that found in U.S. studies with high selenium defined as >130 μg/L. It is possible that selenium works through different mechanisms depending on the range of selenium in the population being studied. Typical intakes range from 20 μg/d in China, 35 μg/d in Finland, and 50 to 200 μg/d in North America. In the United States, plasma selenium levels generally range from 80 to 250 μg/L (59), with an average consumption of 125 μg/d (60).

Jacobs et al. recently published a pooled analysis of three randomized trials (the Wheat Bran Fiber Trial, the Polyp Prevention Trial, and the Polyp Prevention Study) studying the effect of nutritional interventions on recurrent colorectal adenoma (61). When data from these three trials were combined, the aggregate risk estimate for adenoma recurrence in the highest fourth in comparison with the lowest fourth of serum selenium (OR, 0.7; 95% CI, 0.5-0.9) was similar to our results. Also consistent with our results, Jacobs et al. reported no difference in serum selenium level by adenoma number, size, or location. The Jacobs study, however, reported a linear trend for odds of polyp recurrence by increasing selenium level (61). We did not see a similar trend in our study. It is plausible that the shape of the relationship between selenium and primary adenomas is different from the association between selenium and recurrent adenomas because individuals with recurrent adenomas are a high-risk subset of the population.

Because the DHS III is a cross-sectional study, temporal sequence must be addressed. If we assume negligible confounding, selection bias, and information bias, the main bias of concern is the possibility that adenomas reduce serum selenium. There is no evidence, to our knowledge, that adenomas sequester selenium; however, this possibility cannot be entirely dismissed.

If we assume that serum selenium reflects dietary selenium and that both are stable enough over time for the cross-sectional association to reflect a longitudinal one, our results suggest that high dietary selenium reduces the subsequent prevalence of detectable adenomas. This association could result from several possible scenarios: (*a*) dietary selenium reduces the incidence of adenomas, (*b*) dietary selenium retards the growth of adenomas to colonoscopically undetectable size, and (*c*) dietary selenium reduces the duration of adenomas.

Selenium could reduce the incidence of adenomas through several anticarcinogenic pathways (other than apoptosis), including the repair and prevention of oxidative damage, alteration of metabolism of carcinogenic agents, regulation of immune response, and repair of DNA damage (24, 25, 62, 63). It is also possible that selenium could cause adenomas to regress, shrink, or grow more slowly instead of preventing their initial growth. Any of these circumstances could result in an inverse association between selenium and colorectal adenomas.

Our results concerning the relation between selenium and apoptosis in humans were contrary to results from several laboratory studies, suggesting that selenium increased anticancer apoptotic activity (34-42). There are several reasons that our results may differ from laboratory studies. It is possible that selenium may only affect transformed cells on their way to becoming polyps. In addition, dietary selenium may act differently than pure forms of selenium used in laboratory studies on animals and isolated cell lines. Furthermore, preclinical findings do not always apply to humans; a randomized clinical trial of selenium is merited.

There are several notable strengths of our study. This study is among a few that have evaluated a potential association between selenium and adenomas and the first to examine

Table 3. Association between serum selenium and high apoptosis (>2.76 cells per crypt; *n* = 351)

Selenium (μg/L)	Apoptosis below the median (<2.76), *n* (%)	Apoptosis above the median (>2.76), *n* (%)	Prevalence of high apoptosis (>2.76)		
			POR (95% CI)*	PR (95% CI)*	PD (95% CI)*
Low/average (<140)	119 (68)	137 (77)	1.0 (Reference)	1.0 (Reference)	0.00 (Reference)
High (>140)	55 (32)	40 (23)	0.6 (0.4-1.0)	0.8 (0.6-1.0)	−0.11 (−0.23 to 0.00)
Percentiles†					
20th (43-92)	36 (21)	41 (23)	1.0 (Reference)	1.0 (Reference)	1.00 (Reference)
40th (93-115)	30 (17)	45 (25)	1.3 (0.7-2.5)	1.1 (0.9-1.5)	0.65 (−0.09 to 0.22)
60th (116-131)	34 (20)	36 (20)	0.9 (0.5-1.8)	1.0 (0.7-1.3)	−0.02 (−0.17 to 0.14)
80th (132-152)	39 (22)	26 (15)	0.6 (0.3-1.1)	0.8 (0.5-1.1)	−0.13 (0.30 to 0.03)
100th (153-250)	35 (20)	29 (17)	0.7 (0.4-1.4)	0.9 (0.6-1.2)	−0.08 (−0.25 to 0.09)

*Point estimates were unchanged after adjustment for age, race, gender, and calories.
†Percentiles determined in the total population (*n* = 451).

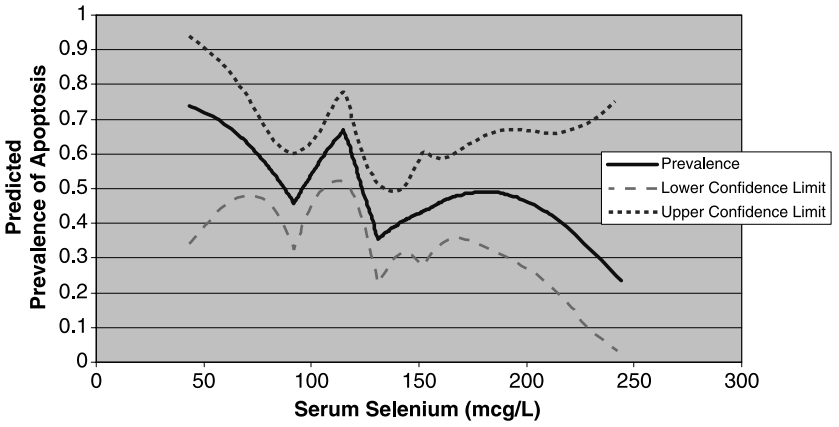

Figure 2. Predicted prevalence of high apoptosis by selenium level: unadjusted four-knot quadratic spline.

whether apoptosis was the mechanism by which selenium may ultimately reduce colorectal cancer risk. The selenium-adenoma relation is important to investigate because it is generally acknowledged that the vast majority of all colorectal cancers arise from adenomatous polyps. Assessment of a nutritional variable, such as selenium, around the time of adenoma diagnosis will help determine whether selenium is truly associated with neoplastic risk. In contrast to cancer patients, individuals with adenomas do not typically experience weight loss due to the presence or treatment of polyps; this is important because weight loss is hypothesized to affect selenium levels and possibly account for lower selenium levels in cancer cases in case-control studies.

The DHS III was well suited for this analysis because the study obtained extensive dietary and lifestyle information on all participants, allowing for proper evaluation of potential confounding factors. Moreover, a validated measure of apoptosis, rarely available in human observational studies, was measured, allowing us to test a potential mechanism by which selenium might affect the prevalence of adenomas. Few studies have examined intermediate end point biomarkers for colon cancer, such as apoptosis. In addition, all of the controls underwent full colonoscopy, thereby eliminating misclassification of adenoma status and patients with previous adenomas were excluded.

Also novel to our approach is our definition of high selenium. High selenium was defined as $\geq$140 μg/L, the serum level anticipated in individuals taking a 200 μg selenium supplement. This is vital to assess the possibility that selenium reduces the risk of cancer at supranutritional levels. Many studies approached their analysis with the presumption that low selenium would put individuals at higher risk for cancer, which may not necessarily be the case, particularly when ''low'' levels in the population are nutritionally adequate (e.g., U.S. population).

We also compared results using the PR and the POR. The PR and POR are often discrepant for common diseases, which are frequently the subject of cross-sectional studies, such as ours. Although PORs are habitually used, we focused on the PRs in Results because the POR can be misleading with regard to confounding and effect measure modification when the outcome is not uniformly rare (64-66). In this case, the PR and POR estimates were consistent, indicating that the POR did a good job of estimating PRs in this population (Tables 2 and 3). The POR estimates were slightly farther from the null, as expected, and less precise than the PR estimates.

Several limitations to our study should be acknowledged. First, because DHS III is a cross-sectional study, temporal sequence cannot be determined. Second, we did not measure selenoproteins, which are likely to provide insight into the mechanism of action of selenium. Third, there are hazards to investigating surrogate outcomes, such as adenomas and apoptosis. Because adenomas are common and biologically heterogeneous and relatively few progress to cancer, it may be false to assume that selenium could reduce colorectal cancer risk because of its inverse association with adenomas (i.e., if selenium is only associated with the adenomas that do not progress to cancer; ref. 67). Selenium could play a role early in the initiation of adenomas or later in the progression of adenomas to cancer or both. Hence, it is important to establish a link not only with the precursor (adenoma) but also with the disease (colon cancer).

Finally, potential bias is an issue in all epidemiologic studies, particularly when subsamples of the data are analyzed. The three subpopulations (with measured selenium, measured apoptosis, and measured selenium and apoptosis) were very similar to the total study population in distribution of all covariates, suggesting that selection bias based on these covariates was minimal. In relation to the selenium-adenoma analysis, two differences were noted. Black participants with adenomas and less than high school educated participants with adenomas were less likely to have provided blood to the DHS III. In other words, Black participants and those with less than high school education were disproportionately underrepresented in the case group for the subpopulation. This type of selection bias would likely bias results toward the null because Black participants and those with lower education tended to have lower selenium levels in our study (i.e., if cases were dropped from low selenium level group, the results will be biased toward the null).

Conclusion

High selenium was associated with a reduced prevalence of colorectal adenomas. Apoptosis, however, did not seem to be the mechanism by which selenium was related to adenoma prevalence. Because high levels of selenium, most likely achieved through a supplemented diet, were associated with the greatest reduction in prevalence of adenomas, our analysis provides support for a future clinical trial investigation of selenium as a potential chemopreventive agent for colon adenomas and colon cancer.

References

1. American Cancer Society. Cancer facts and figures, 2004. American Cancer Society, Inc.; 2004.
2. Reis LG, Eisner MP, Kosary CL, et al. SEER cancer statistics review, 1975-2001. Bethesda (MD): National Cancer Institute.
3. Parkin DM. International variation. Oncogene 2004;23:6329–40.
4. Jansson B. Geographic mappings of colorectal cancer rates: a retrospect of studies, 1974-1984. Cancer Detect Prev 1985;8:341–8.
5. Boyle P, Zaridze DG. Colorectal cancer as a disease of the environment. Ecol Dis 1983;2:241–8.

6. Miller AB. Risk factors from geographic epidemiology for gastrointestinal cancer. Cancer 1982;50:2533–40.
7. Combs GF, Jr., Gray WP. Chemopreventive agents: selenium. Pharmacol Ther 1998;79:179–92.
8. Sunde RA. Selenium. In: Stipanuk M, editor. Biochemical and physiological aspects of human nutrition. London: W.B. Saunders Co.; 2000. p. 782–809.
9. Mason AC. Selenium. In: Smith KT, editor. Trace minerals in foods. New York: Basel; 1988. p. 325–55.
10. Alaejos MS, Diaz Romero FJ, Diaz RC. Selenium and cancer: some nutritional aspects. Nutrition 2000;16:376–83.
11. Fernandez-Banares F, Cabre E, Esteve M, et al. Serum selenium and risk of large size colorectal adenomas in a geographical area with a low selenium status. Am J Gastroenterol 2002;97:2103–8.
12. Schober SE, Comstock GW, Helsing KJ, et al. Serologic precursors of cancer. I. Prediagnostic serum nutrients and colon cancer risk. Am J Epidemiol 1987; 126:1033–41.
13. Mikac-Devic M, Vukelic N, Kljaic K. Serum selenium level in patients with colorectal cancer. Biol Trace Elem Res 1992;33:87–94.
14. Salonen JT, Alfthan G, Huttunen JK, Puska P. Association between serum selenium and the risk of cancer. Am J Epidemiol 1984;120:342–9.
15. Willett WC, Polk BF, Morris JS, et al. Prediagnostic serum selenium and risk of cancer. Lancet 1983;2:130–4.
16. Nomura A, Heilbrun LK, Morris JS, Stemmermann GN. Serum selenium and the risk of cancer, by specific sites: case-control analysis of prospective data. J Natl Cancer Inst 1987;79:103–8.
17. Knekt P, Aromaa A, Maatela J, et al. Serum vitamin E, serum selenium and the risk of gastrointestinal cancer. Int J Cancer 1988;42:846–50.
18. Nelson RL, Davis FG, Sutter E, et al. Serum selenium and colonic neoplastic risk. Dis Colon Rectum 1995;38:1306–10.
19. Garland M, Morris JS, Stampfer MJ, et al. Prospective study of toenail selenium levels and cancer among women. J Natl Cancer Inst 1995;87: 497–505.
20. Ghadirian P, Maisonneuve P, Perret C, et al. A case-control study of toenail selenium and cancer of the breast, colon, and prostate. Cancer Detect Prev 2000;24:305–13.
21. Poole C. High dietary selenium intake and incidence of cancers of the lung, breast and large bowel [dissertation]. Boston: Harvard School of Public Health; 1989.
22. van den Brandt PA, Goldbohm RA, van't Veer, et al. A prospective cohort study on toenail selenium levels and risk of gastrointestinal cancer. J Natl Cancer Inst 1993;85:224–9.
23. Clark LC, Combs GF, Jr., Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. JAMA 1996;276:1957–63.
24. Ganther HE. Selenium metabolism and mechanisms of cancer prevention. Adv Exp Med Biol 2000;492:119–30.
25. Ip C. Lessons from basic research in selenium and cancer prevention. J Nutr 1998;128:1845–54.
26. Hengartner MO. The biochemistry of apoptosis. Nature 2000;407:770–6.
27. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.
28. Kaufmann SH, Hengartner MO. Programmed cell death: alive and well in the new millennium. Trends Cell Biol 2001;11:526–34.
29. Que F, Gores GJ. Apoptosis and the gastrointestinal system. Adv Pharmacol 1997;41:409–28.
30. Sjostrom J, Bergh J. How apoptosis is regulated, and what goes wrong in cancer. BMJ 2001;322:1538–9.
31. Vander a, Sherman J, Luciano D. Human physiology: mechanisms of body function. 8th ed. New York: McGraw-Hill; 2001.
32. Wong BC, Zhu GH, Lam SK. Aspirin induced apoptosis in gastric cancer cells. Biomed Pharmacother 1999;53:315–8.
33. Martin C, Connelly A, Keku TO, et al. Nonsteroidal anti-inflammatory drugs, apoptosis, and colorectal adenomas. Gastroenterology 2002;123: 1770–7.
34. Lanfear J, Fleming J, Wu L, Webster G, Harrison PR. The selenium metabolite selenodiglutathione induces p53 and apoptosis: relevance to the chemopreventive effects of selenium? Carcinogenesis 1994;15:1387–92.
35. Lu J, Kaeck M, Jiang C, Wilson AC, Thompson HJ. Selenite induction of DNA strand breaks and apoptosis in mouse leukemic L1210 cells. Biochem Pharmacol 1994;47:1531–5.
36. Redman C, Xu MJ, Peng YM, et al. Involvement of polyamines in selenomethionine induced apoptosis and mitotic alterations in human tumor cells. Carcinogenesis 1997;18:1195–202.
37. Redman C, Scott JA, Baines AT, et al. Inhibitory effect of selenomethionine on the growth of three selected human tumor cell lines. Cancer Lett 1998;125:103–10.
38. Shen HM, Yang CF, Ong CN. Sodium selenite-induced oxidative stress and apoptosis in human hepatoma HepG2 cells. Int J Cancer 1999;81:820–8.
39. Stewart MS, Davis RL, Walsh LP, Pence BC. Induction of differentiation and apoptosis by sodium selenite in human colonic carcinoma cells (HT29). Cancer Lett 1997;117:35–40.
40. Stewart MS, Spallholz JE, Neldner KH, Pence BC. Selenium compounds have disparate abilities to impose oxidative stress and induce apoptosis. Free Radic Biol Med 1999;26:42–8.
41. Waters DJ, Shen S, Cooley DM, et al. Effects of dietary selenium supplementation on DNA damage and apoptosis in canine prostate. J Natl Cancer Inst 2003;95:237–41.
42. Zhong W, Oberley TD. Redox-mediated effects of selenium on apoptosis and cell cycle in the LNCaP human prostate cancer cell line. Cancer Res 2001;61:7071–8.
43. Fleming J, Ghose A, Harrison PR. Molecular mechanisms of cancer prevention by selenium compounds. Nutr Cancer 2001;40:42–9.
44. Ghose A, Fleming J, El Bayoumy K, Harrison PR. Enhanced sensitivity of human oral carcinomas to induction of apoptosis by selenium compounds: involvement of mitogen-activated protein kinase and Fas pathways. Cancer Res 2001;61:7479–87.
45. Ip C, Dong Y, Ganther HE. New concepts in selenium chemoprevention. Cancer Metastasis Rev 2002;21:281–9.
46. Lu J. Apoptosis and angiogenesis in cancer prevention by selenium. Adv Exp Med Biol 2001;492:131–45.
47. Ghose A, Fleming J, Harrison PR. Selenium and signal transduction: roads to cell death and anti-tumour activity. Biofactors 2001;14:127–33.
48. Day DW, Morson BC. The adenoma-carcinoma sequence. Major Probl Pathol 1978;10:58–71.
49. Clark LC, Hixson LJ, Combs GF, Jr., Reid ME, Turnbull BW, Sampliner RE. Plasma selenium concentration predicts the prevalence of colorectal adenomatous polyps. Cancer Epidemiol Biomarkers Prev 1993;2:41–6.
50. Russo MW, Murray SC, Wurzelmann JI, Woosley JT, Sandler RS. Plasma selenium levels and the risk of colorectal adenomas. Nutr Cancer 1997;28: 125–9.
51. Wallace K, Byers T, Morris JS, et al. Prediagnostic serum selenium concentration and the risk of recurrent colorectal adenoma: a nested case-control study. Cancer Epidemiol Biomarkers Prev 2003;12:464–7.
52. Dworkin BM, Rosenthal WS, Mittelman A, Weiss L, Applebee-Brady L, Arlin Z. Selenium status and the polyp-cancer sequence: a colonoscopically controlled study. Am J Gastroenterol 1988;83:748–51.
53. Early DS, Hill K, Burk R, Palmer I. Selenoprotein levels in patients with colorectal adenomas and cancer. Am J Gastroenterol 2002;97: 745–8.
54. Block G, Hartman AM, Dresser CM, Carroll MD, Gannon J, Gardner L. A data-based approach to diet questionnaire design and testing. Am J Epidemiol 1986;124:453–69.
55. Gerber AM, James SA, Ammerman AS, et al. Socioeconomic status and electrolyte intake in Black adults: the Pitt County Study. Am J Public Health 1991;81:1608–12.
56. Blair SN, Haskell WL, Ho P, et al. Assessment of habitual physical activity by a seven-day recall in a community survey and controlled experiments. Am J Epidemiol 1985;122:794–804.
57. Jacobson BE, Lockitch G. Direct determination of selenium in serum by graphite-furnace atomic absorption spectrometry with deuterium background correction and a reduced palladium modifier: age-specific reference ranges. Clin Chem 1988;34:709–14.
58. Kok FJ, de Bruijn AM, Hofman A, Vermeeren R, Valkenburg HA. Is serum selenium a risk factor for cancer in men only? Am J Epidemiol 1987;125: 12–6.
59. Food and Nutrition Board of the Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids: a report of the Panel on Dietary Antioxidants and Related Compounds, Subcommittees on Upper Reference Levels of Nutrients and the Interpretation and Use of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. Washington (DC): National Academy Press; 2000.
60. Niskar AS, Paschal DC, Kieszak SM, et al. Serum selenium levels in the US population: Third National Health and Nutrition Examination Survey, 1988-1994. Biol Trace Elem Res 2003;91:1–10.
61. Jacobs ET, Jiang R, Alberts DS, et al. Selenium and colorectal adenoma: results of a pooled analysis. J Natl Cancer Inst 2004;96:1669–75.
62. Combs GF, Jr. Considering the mechanisms of cancer prevention by selenium. Adv Exp Med Biol 2001;492:107–17.
63. Longtin R. Selenium for prevention: eating your way to better DNA repair? J Natl Cancer Inst 2003;95:98–100.
64. Thompson ML, Myers JE, Kriebel D. Prevalence odds ratio or prevalence ratio in the analysis of cross sectional data: what is to be done? Occup Environ Med 1998;55:272–7.
65. Barros AJ, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. BMC Med Res Methodol 2003;20:21–33.
66. Skov T, Deddens J, Petersen MR, Endahl L. Prevalence proportion ratios: estimation and hypothesis testing. Int J Epidemiol 1998;27:91–5.
67. Schatzkin A, Gail M. The promise and peril of surrogate end points in cancer research. Nat Rev Cancer 2002;2:19–27.