

Serum Selenium, Genetic Variation in Selenoenzymes, and Risk of Colorectal Cancer: Primary Analysis from the Women's Health Initiative Observational Study and Meta-analysis

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Abstract

Background: Selenium may prevent colorectal cancer. However, several previous studies are small and few investigated the association between selenium and colorectal cancer among women whose selenium metabolism may differ from men. Furthermore, genetic variants in selenoenzymes may be associated with colorectal cancer risk.

Methods: This nested case-control study investigated whether serum selenium concentration and genetic variants in five selenoenzymes (glutathione peroxidase 1-4 and selenoprotein P) were associated with colorectal cancer risk in 804 colorectal cancer cases and 805 matched controls from the Women's Health Initiative (WHI) Observational Study. A meta-analysis was conducted to compare the WHI result with previous studies including 12 observational studies and two clinical trials on selenium.

Results: Within the WHI, selenium concentrations were relatively high (mean = 135.6 µg/L) and were not associated with colorectal cancer risk ($P_{\text{trend}} = 0.10$); the adjusted OR comparing the fifth with first quintile was 1.26 (95% CI, 0.91-1.73). Moreover, genetic variants in selenoenzymes were not significantly associated with colorectal cancer risk. Consistent with the finding in WHI, our meta-analysis showed no association between selenium and colorectal tumor risk in women (OR = 0.97; 95% CI, 0.79-1.18) comparing the highest quintile with the lowest); however, in men, there was a significant inverse association (OR = 0.68; 95% CI, 0.57-0.82) ($P = 0.01$).

Conclusion: Consistent with previous studies, we observed no protective effect of selenium on colorectal cancer among women.

Impact: Our analyses suggest that a population with relatively high selenium concentrations, especially women, would not benefit from increasing selenium intake. *Cancer Epidemiol Biomarkers Prev*; 20(9); 1822-30. ©2011 AACR.

Introduction

The current evidence from *in vitro* and animal studies strongly supports a protective effect of selenium on colorectal cancer, whereas the evidence from clinical trials and

observational studies is inconclusive (1-15). Secondary analyses of 2 clinical trials provide conflicting findings (16, 17). An early trial for the prevention of the recurrence of nonmelanoma skin cancer [Nutritional Prevention of Cancer Trial (NPCT)] found a statistically significant 61% lower risk of colorectal cancer (95% CI, = 10-83; $P = 0.03$) in the selenium supplementation group (17, 18). In contrast, a recent large clinical trial for prostate cancer prevention [Selenium and Vitamin E Cancer Prevention Trial (SELECT)] found no effect of selenium on colorectal cancer, a prespecified secondary outcome (HR = 1.05; 99% CI, 0.66-1.67) (16). Many observational studies (1-9), but not all (10-15), support a protective effect of selenium on risk of colorectal tumors. Most studies or trials were smaller or restricted to men and, hence, did not provide stratified results by gender. Within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, we reported that serum selenium was inversely associated with colorectal adenoma risk only in men, but not in women (1). Effect modification by gender may be

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plausible given gender differences in selenium metabolism (19–22).

Selenium is hypothesized to reduce cancer risk due to its antioxidant activity, which is mediated through selenoenzymes (23). Selenoenzymes are encoded by a small number of genes (25 in humans) that often contain selenium as selenocysteine at their active center. Genetic variants in selenoenzymes may affect their activity and thus directly or indirectly affect the risk of cancer.

This study investigated whether serum selenium concentrations and single nucleotide polymorphisms (SNP) in 5 selenoenzymes expressed in the colorectal tissue [refs. 24–26; glutathione peroxidase 1–4 (*GPX1–4*) and selenoprotein P (*SEPP1*)] were associated with colorectal cancer risk. Data were from a large case–control study nested within the Women’s Health Initiative (WHI) Observational Study, which recruited postmenopausal women from 40 clinical centers throughout the United States. We also carried out a meta-analysis to summarize the current evidence on selenium and the risk of colorectal tumors.

Methods

WHI Observational Study

Study population. The WHI Observational Study includes 93,676 postmenopausal women ages 50 to 79 years at the time of recruitment (28). These women were recruited mostly through age-targeted mass mailing and media announcements in areas surrounding the 40 WHI clinical centers across the United States and described in more detail elsewhere (29). Colorectal cancer cases were identified through annual questionnaires or interviews and were adjudicated through local and central reviews of the medical records and pathology reports. When cases and controls were selected for this study (fall 2005), only 4.7% of the initial WHI participants were lost to or had stopped follow-up. As of August 2005, the agreement rate between local and WHI Clinical Coordinating Center adjudications for colorectal cancer was 96%. In the nested case–control study, cases were diagnosed with colorectal cancer between October 1993 and September 2005 ($n = 805$). Central adjudication determined later that 1 case was not a colorectal cancer case, resulting in 804 cases for analysis. Controls were selected from all eligible women in the WHI Observational Study who were alive and had not been diagnosed with colorectal cancer at the time of the case’s diagnosis. They were individually matched to cases by age (± 3 years), enrollment date to the study (± 168 days), race/ethnicity (white, black, Hispanic, or other), and hysterectomy (yes or no; $n = 805$).

Data collection. Participants in the WHI Observational Study had clinic visits at baseline and year 3. Self-administered questionnaires were completed at baseline and annually thereafter. The baseline questionnaire acquired detailed information on demographic and lifestyle characteristics, medical history, reproductive history, and hormone regimen (29). Dietary intake was

assessed at baseline by using a 122-item food frequency questionnaire (30). During the clinic visits at baseline and year 3, interviewers collected data on use of dietary supplements and nonsteroidal anti-inflammatory drugs (NSAID). Overnight fasting blood samples were collected at baseline and year 3 and maintained at 4°C until serum was separated from cells and stored at –70°C within 2 hours after blood draw (29).

Laboratory methods. Serum selenium concentration was measured by using atomic absorption spectrometry (Perkin Elmer) according to the standard protocol (31–33). Each sample was run in duplicate and considered acceptable if the coefficient of variation (CV) was less than 10%. Internal quality control (QC) samples were run before and after each batch to ensure the quality of the assay and the mean CV of these samples was 7.3%. In addition, blinded duplicates from 96 women who were recruited, but not enrolled in WHI, were included in batches with study samples. Three batches had CVs greater than 21%. All 3 batches were originally run on 2 dates. When we reanalyzed all the batches that were originally run on these 2 dates (3 batches with CV > 21% and another with CV < 10%), all yielded acceptable CVs. After these reruns, the mean CV for the blinded QCs was 5.8%. The Spearman correlation between blood samples collected at baseline and year 3 visits from 100 control women was 0.68. Of the selected 1,609, a total of 1,600 (799 cases and 801 controls) had sufficient serum samples available for selenium assay.

Five selenoenzymes (*GPX1*, *GPX2*, *GPX3*, *GPX4*, and *SEPP1*) have been reported to be both purportedly associated with oxidative stress and expressed in the colorectal tissue (24–26). Each set of putative functional/nonsynonymous SNPs and tagging SNPs (tagSNP) were genotyped to efficiently capture the overall variation of the gene. To select tagSNPs, we first sequenced the genes in European American subjects in HapMap population (34). Using this sequencing data, we selected all SNPs in the selenocysteine insertion sequence, which facilitates selenoenzyme synthesis by a unique stem-loop structure (23), and all nonsynonymous SNPs in exons (35). We then selected additional tagSNPs from our sequencing data (35), using criteria of linkage disequilibrium (LD) of $r^2 \geq 0.8$, and minor allele frequency of 5% or more (36). A total of 42 SNPs were genotyped using Matrix-assisted Laser Desorption/Ionization Time-of-Flight on the Sequenom MassARRAY 7K platform (Sequenom, Inc.). Each plate included 5% blinded duplicates from the study samples as QC. We excluded 2 SNPs (rs75404373 and rs3763011) due to less than 90% call rate, 3 SNPs (rs6888691, rs376301 and rs757229) due to $P < 0.01$ for Hardy–Weinberg equilibrium, and 2 SNPs (rs2074452 and rs7579) due to low concordance of blinded duplicates (<90%). For the remaining SNPs, the concordance rate of the blinded duplicates was greater than 95% (average 99.9%). Thus, the final analysis included 34 SNPs.

Statistical analyses

We used unconditional logistic regression to estimate ORs and 95% CI for colorectal cancer risk associated with increasing serum selenium concentrations given that results were similar when we used conditional logistic regression (data not shown) and unconditional analysis may be beneficial with larger sample size (37). A linear trend of the association was tested by assigning each observation the quintile median serum selenium, based on the distribution in controls and treating this as a continuous variable. All analyses were adjusted for the following 4 matching factors: age at screening, enrollment date to the study (days), race/ethnicity, and hysterectomy. In addition, the following known and potential risk factors for colorectal cancer were evaluated as confounders: physical activity (MET-hours per week), body mass index (BMI, kg/m²), smoking (pack-years; never, former, or current), alcohol consumption (6 categories ranging from nondrinker to 7 or more drinks per week), NSAID use [longest duration (days) of any NSAID use; yes or no], postmenopausal hormone use (years of use; never, former, or current), family history of colorectal cancer (yes or no), history of colorectal cancer screening (yes, no, or missing), history of adenomas and polyps removals (yes or no), education (4 categories ranged from less than high school to college degree or higher), total energy intake (kcal/d), red meat intake (serving/day), vegetable and fruit intake (serving/day), dietary and supplemental folate intake (µg/d), dietary fiber intake (g/d), and dietary and supplemental calcium intake (mg/d). For all variables with the numbers of missing values less than 5% and family history of colorectal cancer, which had 8.8% missing values, the mean value or the most common category were substituted for missing values. The final multivariate model included education and postmenopausal hormone use, because each changed the β -coefficients of the risk estimate for serum selenium by 10% or more.

We also used unconditional logistic regression to examine the association between genetic variations in 5 selenoenzymes and colorectal cancer. Analyses were adjusted for the 4 matching factors. Genetic variants were coded on the basis of log-additive model by assigning the number of copies of the minor allele (i.e., 0 for homozygote common allele, 1 for heterozygotes, and 2 for homozygote rare allele). The OR of the regression model describes the change in the risk of colorectal cancer per minor allele and the *P* value describes the significance of the linear trend. To account for multiple comparisons, a global gene test was used to test whether the overall variation within each gene was associated with the risk of colorectal cancer. This test was conducted by comparing the log likelihood ratio statistics of the model with and without all SNPs in one gene (each SNP coded as 0/1/2; ref. 38).

Interactions of serum selenium concentrations (continuous) with age at screening (continuous), smoking (never, former, or current), postmenopausal hormone use (never, former, or current), and genetic variants in selenoenzymes (log-additive model) were also evaluated.

Interaction effects were tested by including cross-product terms in the regression models and comparing the log likelihood ratio statistics of the main effect model with the joint effects model.

Meta-analysis

A meta-analysis was conducted to compare our results from the WHI with previous studies on selenium and risk of colorectal cancer (the number of studies conducted on genetic variants in selenoenzymes were too small to conduct a meta-analysis). Studies were identified through searches of PubMed using the search term "selenium" and "colorectal cancer" or "colon cancer" or "colorectal adenoma." The last search was conducted on September 27, 2010. All articles were screened by 2 investigators (Y. Takata and U. Peters) to assess whether they provided sufficient information. We restricted analysis to observational studies that assessed selenium as blood or toenail concentrations and randomized clinical trials that used selenium supplements. Because questionnaire data do not provide reliable estimates on selenium intake due to large variation in the selenium content of the same food (39–41) and the fact that selenium supplements only present a relative small fraction of the overall selenium intake (27), we did not include studies using questionnaires to assess selenium intake. As colorectal cancer and adenoma tend to have a similar risk profile (42), both outcomes were included in the meta-analysis. To include as many studies as possible for overall and gender-specific analyses, if the risk estimate and/or the corresponding 95% CI were missing in a given article, attempts were made to contact the author(s) to obtain this information. Three studies were excluded due to insufficient information (4, 8, 12). No other additional screening or eligibility criteria were used, leaving 15 studies (including the current study) in the meta-analysis. For each article, the following information was extracted: first author name, year of publication, mean or median blood or toenail concentration, sample size, OR/HR, and 95% CI. For observational studies, the meta-analysis included the risk estimate comparing the highest quantile (quintile, quartile, or tertile) of selenium concentrations with the lowest, except for 2 studies which reported only the risk estimate on the basis of a given cutoff [median (6) or 75th percentile (7)]. For clinical trials (16, 17), the risk estimate comparing the selenium supplemented with the placebo groups was used. Risk estimates from individual studies were combined and the corresponding summary 95% CI and *P* values were obtained under a random effects meta-analysis model (43, 44). Forest plots were used to display the results from individual studies, as well as the summary results. To investigate heterogeneity among studies, we calculated *I*² statistics, which is a measure of the percentage of total variation across studies due to heterogeneity beyond chance, and obtained the heterogeneity *P* values based on Cochran's *Q* statistic (45). Funnel plot was used to assess potential selection or publication bias. Subgroup analyses by gender, biospecimen type

Table 1. Characteristics of the WHI Observational Study, stratified by case-control status

	Cases ^a	Controls ^a	<i>P</i> _{difference}
Number	804	805	–
Age, y	66.6 ± 6.9	66.7 ± 6.9	0.96
Race/ethnicity			
White	675 (84.0)	676 (84.0)	
Black	82 (10.2)	82 (10.2)	
Hispanic	20 (2.5)	20 (2.5)	
Other/unknown	27 (3.3)	27 (3.3)	0.99
Education			
Less than high school	40 (5.0)	47 (5.9)	
High school diploma/GED	122 (15.3)	137 (17.2)	
Some school after high school	355 (44.4)	291 (36.5)	
College degree or higher	282 (35.3)	322 (40.4)	0.01
BMI, kg/m ²	28.3 ± 6.1	27.5 ± 5.7	0.004
Underweight/Normal (BMI < 25 kg/m ²)	267 (33.7)	291 (36.5)	
Overweight (25 ≤ BMI < 30 kg/m ²)	278 (35.0)	302 (37.9)	
Obese (BMI ≥ 30 kg/m ²)	248 (31.3)	204 (25.6)	0.05
Physical activity, MET-hours	11.6 ± 12.3	13.2 ± 14.4	0.02
Smoking, pack-years	13.8 ± 22.4	11.1 ± 19.8	0.01
Never	366 (46.2)	404 (50.8)	
Former	366 (46.2)	347 (43.7)	
Current	61 (7.6)	44 (5.5)	0.08
NSAID use			
None	564 (70.1)	570 (70.8)	
Any at baseline	240 (29.9)	235 (29.2)	0.84
Postmenopausal hormone use			
Never	407 (50.7)	352 (43.8)	
Former	135 (16.8)	138 (17.2)	
Current	261 (32.5)	314 (39.0)	0.01
Family history of colorectal cancer	163 (22.0)	133 (18.3)	0.06
Selenium supplement use	289 (36.0)	299 (37.1)	0.63
Folate intake, μg/d ^b	663.1 ± 316.7	678.8 ± 336.0	0.29
Calcium intake, mg/d ^b	1,144.6 ± 679.8	1,213.0 ± 760.4	0.05
Dietary fiber intake, g/d	16.0 ± 6.9	16.7 ± 7.0	0.05
Red meat intake, serving per day	0.62 ± 0.55	0.60 ± 0.49	0.38
Alcohol consumption, servings per week	2.41 ± 4.81	2.52 ± 4.76	0.69
Serum selenium concentration, μg/L	137.8 ± 24.3	135.6 ± 21.1	0.05

^aThe mean ± SD or *n* (%) is provided. The number of missing values among 1,600 participants with serum selenium values was 13 for education, 19 for BMI, 17 for physical activity, 21 for smoking, 2 for postmenopausal hormone use, 141 for family history of colorectal cancer, 71 for nutrient and red meat intakes, and 13 for alcohol consumption.

^bFrom diet and supplement.

(blood vs. toenail selenium), colorectal outcome (colorectal cancer vs. adenoma), and time of selenium assessment (before or after diagnosis of colorectal tumors; clinical trials were included in the before diagnosis group because the effect of selenium supplementation was assessed prospectively) were carried out.

Results

WHI Observational Study

Cases and controls did not differ by age and race/ethnicity because they were matched on these criteria

(Table 1). Cases were less educated, had slightly higher BMI, were less physically active, smoked more, and used postmenopausal hormone less often than controls. Use of NSAIDs and selenium supplements was similar between cases and controls. Intakes of calcium and dietary fiber were lower in cases than controls. Most cases were diagnosed with cancer in the colon (81.1%). Given that our participants were recruited from 40 different WHI clinical centers across the United States, the range of serum selenium concentrations was wide (81.0–398.7 μg/L); however, the mean concentrations were relatively high (135.6 ± 21.1 μg/L in controls).

Table 2. Risk of colorectal cancer by serum selenium concentrations in the WHI Observational Study

	Cases/ controls	OR (95% CI) ^a by quintiles of selenium concentrations ($\mu\text{g/L}$)					P_{trend}	
		≤ 117.5	117.6–128.5	128.5–138.7	138.7–152.6	> 152.6		
		Range Median	110.5	123.2	133.5	144.8	162.2	
Adjusted for matching factors only ^a	799/801	Reference	1.09 (0.79–1.49)	1.11 (0.81–1.52)	1.27 (0.93–1.73)	1.17 (0.85–1.60)	0.22	
Fully adjusted ^b	799/801	Reference	1.13 (0.82–1.56)	1.16 (0.84–1.59)	1.34 (0.98–1.84)	1.26 (0.91–1.73)	0.10	
Site-specific analysis ^c								
Colon	648/801	Reference	1.01 (0.72–1.42)	1.14 (0.81–1.60)	1.34 (0.96–1.87)	1.28 (0.91–1.79)	0.05	
Rectum	149/801	Reference	1.75 (0.99–3.07)	1.21 (0.66–2.22)	1.48 (0.83–2.65)	1.25 (0.68–2.31)	0.76	

^aAnalysis was adjusted for matching factors (age, enrollment date, race/ethnicity, and hysterectomy).

^bAnalysis was adjusted for matching factors (age, enrollment date, race/ethnicity, hysterectomy) and education and postmenopausal hormone use.

^cTwo cases who had cancer in both colon and rectum were excluded; $P_{\text{difference}} = 0.18$, obtained by polytomous logistic regression. Rectal cancer includes cancer in rectosigmoid junction and rectum.

Serum selenium concentration was not associated with the risk of colorectal cancer ($P_{\text{trend}} = 0.10$); the adjusted ORs (95% CIs) for colorectal cancer risk comparing the second, third, fourth, and fifth quintiles of serum selenium concentrations with the first quintile were 1.13 (0.82–1.56), 1.16 (0.84–1.59), 1.34 (0.98–1.84), and 1.26 (0.91–1.73), respectively (Table 2). Although results of site-specific analyses did not differ between colon and rectum ($P = 0.18$), the positive association between serum selenium and risk of colon cancer was marginally significant ($P = 0.05$). There were no statistically significant interactions of serum selenium with age ($P_{\text{interaction}} = 0.41$), smoking ($P_{\text{interaction}} = 0.12$), and postmenopausal hormone use ($P_{\text{interaction}} = 0.29$).

Only one SNP in *GPX4* gene (rs8178974) was statistically significantly associated with colorectal cancer risk ($P_{\text{trend}} = 0.02$); however, the overall variation in *GPX4* gene was not associated with the risk of colorectal cancer (global $P = 0.20$; Supplementary Table S1; Supplementary Fig. S1). All other SNPs, either tested individually or combined within a gene, were not associated with colorectal cancer risk. When stratified by White and African Americans, results generally did not differ from the overall analyses. Genetic variants in selenoenzymes did not modify the association between serum selenium and colorectal cancer (global $P_{\text{interaction}} = 0.09$ –0.87; Supplementary Fig. S2).

Meta-analysis

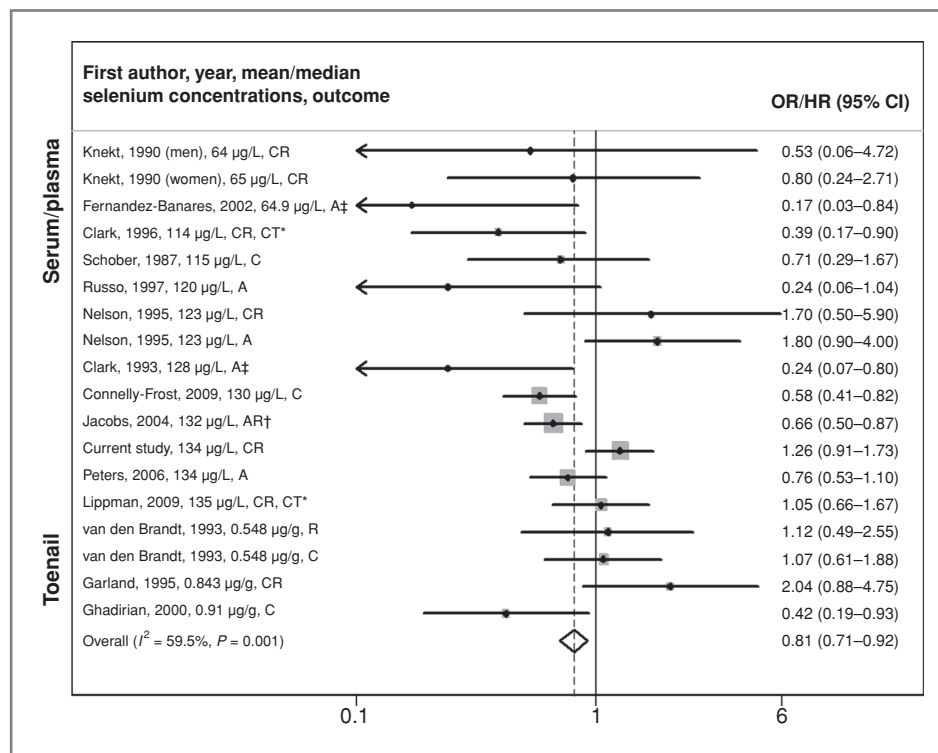
The meta-analysis showed a statistically significant inverse association between selenium and colorectal tumor risk (OR = 0.81; 95% CI, 0.71–0.92; Fig. 1); however, results were heterogeneous ($I^2 = 59.5\%$; $P = 0.001$). Similar results were obtained after excluding the 2 clinical trials (OR = 0.80; 95% CI, 0.70–0.92; $I^2 = 60.4\%$; $P = 0.001$). When stratified by gender, selenium was significantly and inversely associated with colorectal tumor risk in

men (OR = 0.68; 95% CI, 0.57–0.82) with no evidence of heterogeneity ($I^2 = 16.7\%$; $P = 0.29$; Fig. 2). Among women, there was no association (OR = 0.97; 95% CI, 0.79–1.18), but results remained heterogeneous ($I^2 = 49.5\%$; $P = 0.045$). The protective association was stronger for studies that measured selenium after colorectal tumor diagnosis compared with those that measured selenium before diagnosis (OR = 0.61; 95% CI, 0.47–0.80 vs. OR = 0.88; 95% CI, 0.76–1.02); however, this did not explain the heterogeneity between studies (Supplementary Fig. S3A). The inverse association between selenium and colorectal tumors was limited to studies measuring selenium in serum/plasma (OR = 0.78; 95% CI, 0.68–0.90) and there was no association in studies measuring selenium in toenails, although only 3 studies were included (OR = 1.00; 95% CI, 0.70–1.44; Supplementary Fig. S3B). The inverse association was stronger for colorectal adenoma (OR = 0.69; 95% CI, 0.57–0.85) than for cancer (OR = 0.90; 95% CI, 0.76–1.06; Supplementary Fig. S3C). On the basis of visual inspection of funnel plots, there was potential evidence for publication bias, with a slightly higher number of small studies reporting inverse associations (Supplementary Fig. S4A–C), which is in the expected direction.

Discussion

In this large study of postmenopausal women, serum selenium concentrations were not associated with colorectal cancer risk. This finding is consistent with findings from our meta-analysis, showing no association between selenium and colorectal tumors among women and an inverse association among men. Furthermore, there was no evidence for association between genetic variants in selenoenzymes and risk of colorectal cancer, and results for serum selenium did not differ by genetic variants in selenoenzymes.

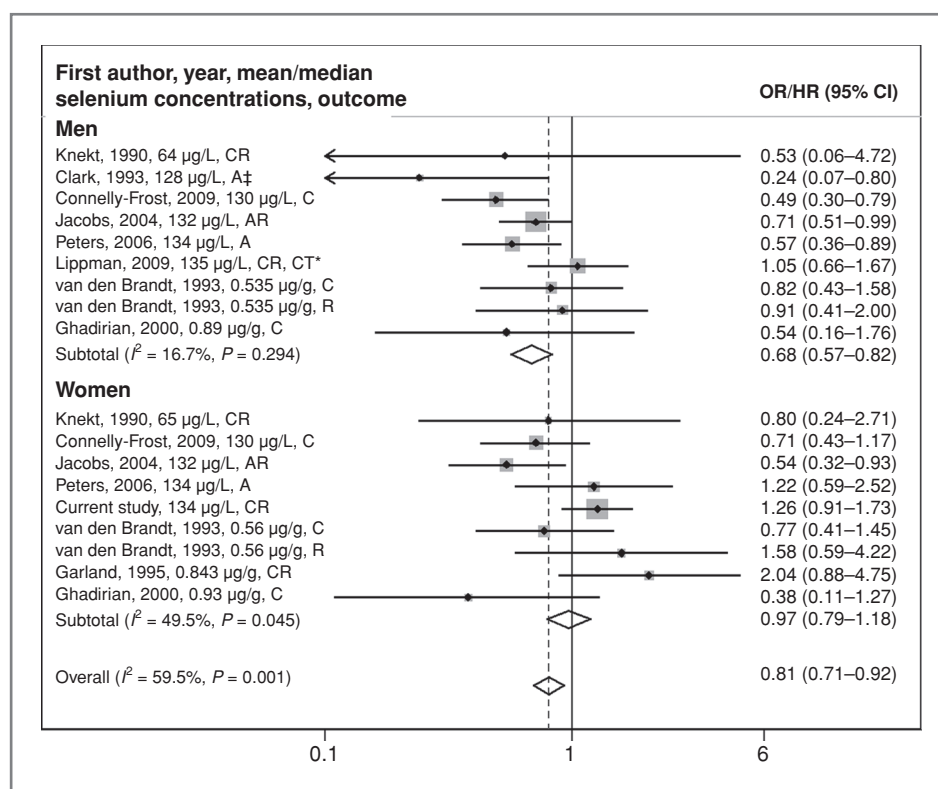
Figure 1. Meta-analysis of selenium and the risk of colorectal tumors. Ordered by biospecimen type (serum/plasma and toenail samples) and by selenium concentrations within each biospecimen type. C, colon cancer; CR, colorectal cancer; R, rectal cancer; A, adenoma; AR, adenoma recurrence; CT, clinical trial. The dot in each study indicates the OR/HR, bars represent the 95% CI and the size of gray square box reflects the study's weight in the meta-analysis. The dashed line indicates the summary OR across all studies. The open diamond on the bottom indicates the 95% CI of the summary OR. †, Risk estimate is based on a given cutoff (the median for Clark 1995 study and the 75th percentile for Fernandez-Banares 2002 study).



In comparison with selenium supplementation trials, our finding is consistent with secondary analysis from SELECT (16), but inconsistent with those from the NPCT (17). The discrepant findings between these clinical trials

could be potentially due to the use of different selenium supplements, selenized yeast (NPCT), which contains a mixture of different selenium forms and purified selenomethionine (SELECT), a major active component of the

Figure 2. Meta-analysis of selenium and the risk of colorectal tumors, stratified by gender. Ordered by biospecimen type (serum/plasma and toenail samples) and by selenium concentrations within each biospecimen type. C, colon cancer; CR, colorectal cancer; R, rectal cancer; A, adenoma; AR, adenoma recurrence; CT, clinical trial. The dot in each study indicates the OR/HR, bars represent the 95% CI and the size of gray square box reflects the study's weight in the meta-analysis. The dashed line indicates the summary OR across all studies. The open diamond on the bottom indicates the 95% CI of the summary OR. †, Risk estimate is based on a given cutoff (the median for Clark 1995 study).



selenized yeast. Furthermore, the discrepant findings could be explained by a proposed threshold effect of selenium on the GPX1 activity, which plateaus at selenium concentration of approximately 80 to 100 $\mu\text{g/L}$ (46, 47). NPCT specifically recruited participants from areas in the United States with relatively low soil selenium content, resulting in a baseline mean plasma selenium concentration of 114 $\mu\text{g/L}$ (17). In contrast, SELECT recruited participants from areas throughout North America including the United States, Canada, and Puerto Rico; a baseline median serum selenium concentration in SELECT was 135 $\mu\text{g/L}$ (16), which was similar to the current WHI study (mean = 136 $\mu\text{g/L}$). Accordingly, baseline selenium concentrations in SELECT and the WHI study may be in a range where further selenium supplementation has no additional cancer preventive effect. Nonetheless, previous observational studies provide conflicting results regarding a threshold effect of selenium. Three small studies with low average selenium concentrations that were similar to those in the NPCT found no association between selenium and colorectal cancer risk (8, 10, 14), whereas 2 studies with selenium concentrations higher than reported in NPCT observed an inverse association (3, 5).

Motivated by previous findings of gender-based differences (1, 13), we conducted a gender-stratified meta-analysis and found statistically significant evidence for association between selenium and colorectal tumors among men only. Note, however, that the largest clinical trial, SELECT, which was only conducted in men, did not find an effect of selenium on colorectal cancer. Results from clinical trials should generally be given more emphasis, because they are not subject to some of the biases in observational studies. The potential gender difference in the association of selenium with colorectal cancer risk may be due to differences in selenium metabolism and selenoenzyme activity (19–22, 48–51). In particular, men had higher serum selenium concentrations than women in populations in the United States ($n = 14,619$; ref. 19) and France ($n = 13,017$; ref. 20). In terms of urinary excretion, women had a higher selenium concentration in urine than men, after adjusting for body weight (21). Hence, these previous findings suggest that men may retain selenium more efficiently than women. GPX1 activity may also be higher in women than in men (50, 51). These findings are intriguing; however, given the limited evidence, the mechanisms behind a potential gender difference on the effect of selenium need to be further elucidated.

We observed a stronger association between selenium concentrations and colorectal tumor risk among studies that measured selenium after diagnosis of colorectal tumors than those measured before the diagnosis. Although this difference did not explain the observed heterogeneity, this finding is potentially relevant given that it may point to reverse causality in studies where selenium was measured after diagnosis.

Given the reported differences in molecular characteristics of tumors and dietary risk factors between colon and rectum tumors (52, 53), we investigated the effect of selenium for both cancer sites, separately. Our site-specific analysis showed a marginally significant positive association between selenium concentrations and risk of colon cancer. However, given that our study was not powered for site-specific analysis this finding may be due to chance.

Our overall null finding for genetic variants in selenoenzymes and colorectal tumors is consistent with most previous studies (54–58), although not all (59). Although our study observed marginal evidence for association between one *GPX4* SNP (rs8178974) and colorectal cancer risk, the overall genetic variation in *GPX4* was not statistically significantly associated with colorectal cancer risk, suggesting that this finding may be due to chance. Previous studies have found that 2 other *GPX4* SNPs (rs713041 and rs2075710) were associated with the risk of colorectal tumors (56, 57, 59) and with selenoenzyme activity and concentrations (49). However, these 2 *GPX4* SNPs were not in LD with the *GPX4* variant (rs8178974) that was associated in our study ($r^2 = 0.03$ and 0.04, respectively). Given that we likely need to expect similar effect sizes as shown in genome-wide association studies for colorectal cancer (OR < 1.15; ref. 60), the power of the current and previous studies is limited. This also suggests that an effect, should one exist, is relatively weak.

A strength of our study includes the large number of cases, making it the largest observational study reported to date on serum selenium and colorectal cancer in women. Blood specimens and exposure information were collected in a prospective cohort setting before colorectal cancer was diagnosed. This reduced the possibility of biases and reverse causality, which may be a potential issue for selenium as discussed earlier. Our QC measures for blinded QCs showed high quality of the measurements. Although our study population had a wide range of serum selenium concentrations (81.0–398.7 $\mu\text{g/L}$), this range did not include individuals with selenium-depleted concentrations (<80 $\mu\text{g/L}$). Our participants who were recruited from areas with similarly low soil selenium content (61) than the NPCT had average selenium concentrations much higher (138 $\mu\text{g/L}$) than those in the NPCT (114 $\mu\text{g/L}$). Nonetheless, our average concentrations were similar to those in other recent studies conducted in the United States (1, 2, 5, 16). Hence, the difference in selenium concentrations between the NPCT and our study might be explained by factors other than the soil selenium content, such as global food distribution, which has increased over time. Accordingly, to test whether selenium has a protective effect in selenium-depleted population, future studies may need to be conducted outside the United States in areas with low selenium concentration and a more local food distribution, such as areas in China.

This study also has some limitations. Like previous studies, our study used biospecimens collected at one time point to assess long-term selenium intake. Nonetheless, the correlation between baseline and the year 3 samples measured in 100 control women was relatively high ($r = 0.68$), suggesting that a single measurement can be used to reflect, although not perfectly, long-term intake, as previously reported (62). Furthermore, selenium concentration in serum may not be reflective of selenium concentration in the target tissue, colorectal tissue in our study, as we previously observed for the prostate (63). Because we selected genetic variants from Caucasians, the genetic coverage of African Americans and other ancestries in our participants might not be sufficient.

In conclusion, our study is among the largest prospective studies in women to comprehensively investigate the association of serum selenium and genetic variants in selenoenzymes with colorectal cancer risk. This study with relatively high selenium concentrations observed no association between serum selenium and colorectal cancer risk, a result consistent with previous studies in women. In contrast, our meta-analysis found an inverse

association in men. Furthermore, we observed no associations between genetic variations in some selenoenzyme genes and colorectal cancer risk, nor did we find evidence of an interaction between genetic variation and serum selenium.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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