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Selenium, Folate, and Colon Cancer

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Abstract

Background—Selenium is an essential trace element which has been implicated in cancer risk; however, study results have been inconsistent with regard to colon cancer. Our objectives were to 1) investigate the association between selenium and colon cancer 2) evaluate possible effect measure modifiers and 3) evaluate potential biases associated with the use of post-diagnostic serum selenium measures

Methods—The North Carolina Colon Cancer Study is a large population-based, case-control study of colon cancer in North Carolina between 1996 and 2000 (n=1,691). Nurses interviewed patients about diet and lifestyle and drew blood specimens which were used to measure serum selenium.

Results—Individuals who had both high serum selenium (>140 mcg/L) and high reported folate (>354 mcg/day), had a reduced relative risk of colon cancer (OR=0.5, 95% CI=0.4,0.8). The risk of colon cancer for those with high selenium and low folate was approximately equal to the risk among those with low selenium and low folate (OR=1.1, 95% CI=0.7,1.5) as was the risk for those with low selenium and high folate (OR=0.9, 95% CI=0.7–1.2). We did not find evidence of bias due to weight loss, stage at diagnosis, or time from diagnosis to selenium measurement.

Conclusion—High levels of serum selenium and reported folate jointly were associated with a substantially reduced risk of colon cancer. Folate status should be taken into account when evaluating the relation between selenium and colon cancer in future studies. Importantly, weight loss, stage at diagnosis, or time from diagnosis to blood draw did not appear to produce strong bias in our study.

Keywords

selenium; folate; colon cancer; chemoprevention

Introduction

Worldwide, there is a 25-fold variation in colorectal cancer incidence. The highest rates are seen in North America, Australia, New Zealand, Western Europe, and select areas of Eastern Europe.¹ Variations in incidence of colorectal cancer with respect to geography and migration suggest that diet may play an important role in colon cancer etiology.^{2–4} Selenium is one dietary factor that could impact colon cancer risk.

Selenium is an essential trace element found primarily in cereals, wheat, dairy products, meat and fish. The recommended daily allowance (RDA) of selenium is 55 mcg/day for women and 70 mcg/day for men.⁵ The tolerable upper limit is 400 mcg/day and deficiency is defined as less than 30 mcg/day.⁵ In the United States, plasma selenium levels generally range from 80–250 mcg/L,⁶ with an average consumption of 125 mcg/day.⁷ Selenium interacts with a number of nutrients and micronutrients in the body. Antioxidant nutrients, such as vitamins A, C, E and zinc enhance selenium absorption.⁵ Lead, iron, arsenic, copper and methionine also affect the bioavailability of selenium.^{5;8–10} More recently, a potential relationship between selenium and folate has also been suggested.^{11;12}

Selenium is involved in many biochemical pathways, can exist in multiple forms, and can create a number of different metabolites. Proposed anti-carcinogenic pathways of selenium, include the repair and prevention of oxidative damage, alteration of metabolism of carcinogenic agents, regulation of immune response, and P53-independent apoptosis, and repair of DNA damage.^{13–16} It is likely that selenium acts as an anti-carcinogen through several mechanisms, which vary in importance based on disease status of the individual.

Perhaps the most compelling evidence of a relationship between selenium and colon cancer was provided by the Nutritional Prevention of Cancer Trial (NPC Trial).¹⁷ The NPC Trial (n=1,312) recruited patients with a history of skin cancer from seven dermatology clinics in the eastern US and randomized them to 200 mcg of selenium (selenized yeast) or placebo per day. Although there was an increase in incidence of the primary outcome (basal or squamous cell carcinomas of the skin) over 5 years of follow-up, there was a substantial reduction in incidence of several other cancers, including colorectal cancer (HR=0.42, 95% CI=0.18–0.95). After 3 additional years of follow-up, the association between selenium and colorectal cancer was only slightly attenuated (HR=0.46, 95% CI 0.21–1.02).¹⁸

Unlike consumption of most other nutrients, selenium intake is not reliably assessed using self-report tools, such as food frequency questionnaires, because of the wide variations in soil selenium. Specifically, the selenium content of plants varies not only with selenium content of the soil, but with soil pH and moisture and with the type of plant accumulating it.^{19–21} Meats are similarly affected because animals grazing on plants in low selenium areas, for instance, have lower selenium in their meat than animals feeding on plants grown in selenium adequate or seleniferous areas. Consequently, researchers use biological markers such as serum, plasma, whole blood, and toenail measures to estimate individual selenium intake in epidemiologic studies.

A number of studies have reported lower mean selenium levels in colorectal cancer cases than in controls^{22–27}; however, observational studies providing estimates of the association between selenium and colon cancer generated inconclusive and inconsistent results. Results are equally inconsistent in studies using pre- or peri-diagnostic serum (OR range: 0.5–1.7)^{23;28–30} compared to those utilizing toenails (OR range: 0.4–2.0).^{24;31–33} Reasons for these inconsistent findings are unclear; however, many had small sample sizes, narrow ranges of exposure, and different ranges of exposure.

Rare diseases such as cancers are often studied using case-control designs. Colon cancer studies that have biological samples are likely to have post-diagnostic specimens. Factors that may contribute to bias in the study of post-diagnostic serum selenium and colon cancer are stage at diagnosis, recent weight loss, time from symptoms to diagnosis, and time from diagnosis to blood draw. Although these factors have been reported as reasons against using post-diagnostic serum, there is little empirical support for these concerns.

It has been hypothesized that selenium drops in response to the disease process, either through changes in nutrition or different metabolic needs of the body.^{30;34} Individuals with more

advanced disease are more likely to have lost weight, changed their eating habits, or been treated with chemotherapy before their blood draw.^{22;35} If these factors decrease serum selenium levels, a spurious association between selenium and cancer would be found in patients with advanced stage disease; results from studies using peri-diagnostic measures would be biased away from the null. There are limited published information on associations of selenium and specific colon cancer stages. Weight loss is also of potential importance because decreased energy intake or possible disease-related metabolic changes resulting in weight loss could lead to lower selenium levels.³⁶ Weight loss is a common symptom of colorectal cancer and, thus, an important factor to take into account.^{37;38} If weight loss does impact selenium levels, use of post-diagnostic selenium close to diagnosis and treatment could bias the association between selenium and colon cancer away from the null.

Time from diagnosis to blood draw and time from symptoms to blood draw could be important in understanding whether selenium responds to disease progression or symptoms of disease progression. Blood taken very close to the time of diagnosis, potentially in the midst of chemotherapy, would contribute the most bias if the disease process, treatment, or symptoms of treatment had an effect on selenium levels. It is possible that symptoms and disease could have further progressed in those who participated in interviews beyond a year after diagnosis, making this group subject to biased selenium measurement, as well. It is not likely, however, that participants who were getting progressively worse over a year or more would have participated in this study.

The objectives of this study were three-fold. First, we aimed to describe the association between serum selenium and colon cancer in an effort to provide evidence for or against the further examination of selenium as a chemopreventive agent for colorectal cancer. As a part of this objective we used the results of the Nutritional Prevention of Cancer selenium supplementation trial to inform our definition of high serum selenium to assess a possible “supranutritional effect” of selenium. Second, we examined possible effect measure modifiers of the association between selenium and colon cancer. The third objective was to evaluate the validity of using post-diagnostic measures of selenium in the study of selenium and colon cancer. As part of this objective, particular attention was given to possible exposure misclassification bias of selenium due to stage at diagnosis, weight loss, time from symptoms to blood draw, and time from diagnosis to blood draw

Methods

The North Carolina Colon Cancer Study (NCCCS) is a population-based, case-control study of colon cancer in North Carolina.^{39–41} The study area included 33 counties in central North Carolina representing a socio-economically diverse group of African Americans and Caucasians. Participants were offered an incentive of \$25 for participation in the study. This study was approved by the Institutional Review Board of the UNC School of Medicine.

Cases were identified through the rapid ascertainment system established in conjunction with the North Carolina Cancer Registry⁴² and were eligible for the study if they received a primary diagnosis of invasive adenocarcinoma of the colon (ICD-9 153) between October 1, 1996 and October 1, 2000. Other eligibility criteria were as follows: age of 40–80 years at the time of diagnosis, residence in one of the 33 counties included in the study area, ability to provide informed consent and to complete an interview, possession of a NC drivers license card or identification card, and permission to contact primary care physician. Written consent to examine tissue and medical records was obtained from participants. The study pathologist confirmed diagnosis and cancer stage through review of pathology slides and pathology reports.

Controls were selected from the same 33-county area in central North Carolina as the cases through a randomized recruitment technique.⁴³ Race-, gender- and age-specific incidence rates between 1991 and 1993 were used to calculate selection probabilities that would result in approximately equal numbers of African-American and Caucasian cases. Using these probabilities, the control group was approximately frequency matched to cases by race, gender, and age (± 5 yr). Controls were selected from two computerized databases: the North Carolina Division of Motor Vehicles for persons younger than 65 years and the Health Care Financing Administration database for those 65 years or older.

There were 1691 completed interviews: 731 African Americans (294 cases; 437 controls) and 957 Caucasians (349 cases; 611 controls). The study cooperation rate [interviewed/(interviewed + refused)] was 84% for cases and 63% for controls. Cooperation rates were slightly higher for Caucasians (cases 89%; controls 64%) in comparison to African Americans (cases 79%; controls 61%).

Data Collection

Data were collected in person by trained nurse interviewers, usually in the participant's home. For cases, the median interview time was 5.5 months after diagnosis (range: 1–18 months). The reference period for the interviews was the year prior to diagnosis (cases) or interview date (controls). Lifestyle questionnaires 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.

Diet—Dietary information was obtained using the 100-item semi-quantitative Block food frequency questionnaire (FFQ) developed at the National Cancer Institute.⁴⁴ The food frequency questionnaire was modified by adding 29 foods commonly consumed in North Carolina in order to better assess regional dietary practices in a sample including African Americans.⁴⁵ Controls estimated their usual frequency and serving size during the past year, whereas cases estimated consumption during the year before diagnosis. A one-year period was chosen to provide a full cycle of seasons, so that responses would be independent of the time of year. In a preliminary analysis, we examined the association between selenium and a number of dietary variables available from the FFQ, including alcohol use, total energy, fat, protein, beta carotene (vitamin A), vitamin C, vitamin E, zinc, folate, iron, fiber, supplement use (selenium and multivitamin), red meat, and daily vegetable intake. Dietary risk factors of interest in multivariate analyses (identified as potential confounders by association with both selenium and colon cancer in preliminary bivariate analyses) were total energy, total fat, total folate, total vitamin E, total calcium, total fiber, and red meat consumption. Dietary and supplemental sources were summed to create total folate, total vitamin E, total calcium and total fiber values.

Selenium—Serum selenium levels were determined using graphite furnace atomic absorption spectrometry (GFAAS) with Zeeman background correction and platform technique. GFAAS 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 first and then injected directly into the graphite furnace with the chemical modifier. The concentrations were calculated using a calibration curve based on aqueous standards. This test can detect levels of selenium from 2–600 mcg/L.⁴⁶

Archived serum samples were thawed and aliquotted (100 mcL) into trace-element free vials for analysis. Samples were mailed in batches of 500 to the laboratory of National Medical Services (Willow Grove, PA) over a period of several months. All batches contained ten percent blind controls for external verification of the lab's coefficient of variation (7%).

Demographic and lifestyle characteristics—Demographic and lifestyle characteristics were obtained from the main study questionnaire. A number of demographic and lifestyle factors were evaluated including age, gender, race, education, smoking, BMI one year before interview, physical activity, non steroidal inflammatory use (NSAID use) during the past five years, and first degree family history of colon cancer.

Physical activity (METs/week) was evaluated using a modified version of the seven-day activity recall used in the Stanford Five-City Project as described in detail elsewhere.⁴⁷ 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 metabolic equivalents (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. MET-minutes per day were calculated by multiplying the METs for each activity by the amount of time spent in that activity daily.

Weight change was not directly measured in the main questionnaire; it was defined as usual weight one year prior to interview (self-report) minus weight at time of interview (measured by interviewer). Time from symptoms to diagnosis and time from diagnosis to blood draw were also obtained from the main interview questionnaire.

Statistical Analysis

Participants were excluded from the analyses if they had out of range or missing energy information (n=47), had BMI greater than 50 kg/m² (n=13), or were of races other than African-American or Caucasian (n=8). Additionally, eligible subjects who did not give blood or for whom blood was not available were also excluded from analyses (n=283). One participant with a selenium value above 300 mcg/L was also deleted from the analysis. Analyses of selenium and colon cancer included 1,364 participants.

Multivariate logistic regression models were used to describe the relation between selenium and the presence of colorectal cancer. Selenium was categorized in two ways: dichotomously (with “high selenium” defined as ≥ 140 mcg/L) and in fifths (70–105, 106–116, 117–128, 129–146, 147–290 mcg/L). The value of 140 mcg/L is one standard deviation below the mean in a group whose selenium intakes were supplemented by 200 mcg/day in the NPC Trial.¹⁷ This specification was used to estimate the possible beneficial effect of selenium supplementation.

Multivariate logistic regression models assessed the relation between high selenium (≥ 140 mcg/L; highest fifth of selenium) and colon cancer. Known and suspected risk factors for colon cancer that were measured in the NCCCS were evaluated as potential confounders. Potential confounders assessed were education (less than high school, high school graduate or some college, college graduate or more), smoking (current, former, never), alcohol use (none, upper 50%, lower 50%) body mass index (BMI) one year before interview (<18.5 , 18.5–25, >25 kg/m²), physical activity (fifths of metabolic equivalents per day (METs)), non-steroidal inflammatory (NSAID) use during the past five years (regular, occasional, never), and first degree family history of colon cancer (yes, no), total energy (<1000 , 1000–1500, >1500 –2000, >2000 kilocalories per day), total fat (fifths), total folate (fifths), total vitamin E (fifths), total calcium (fifths), total fiber (fifths), and red meat (none, <1 , >1 serving/day).

Modeling was done by backwards elimination, based on the likelihood ratio test for effect measure modifiers ($\alpha = 0.20$) and absolute change in lnOR for confounders ($>15\%$).⁴⁸ All potential confounders were assessed as potential effect measure modifiers on both multiplicative (likelihood ratio test, ratio of relative risks) and additive (interaction contrast ratio) scales.⁴⁹ Joint effects of dietary risk factors for colon cancer and selenium were of

particular interest because they have not been reported in previous studies of selenium and colon cancer. Multiple logistic models were similarly used to assess the relation between serum selenium and 1) local colon cancer compared to controls and 2) regional or distant cancer compared to controls. These analyses were designed to examine whether the relationship between selenium and colon cancer was restricted to later stage diagnoses. All models were adjusted for recruitment factors (age, race, and gender) and the offset terms used to identify eligible participants. The offset term is the value for each age-gender-race stratum calculated from the selection probabilities used to identify eligible control participants [offset=ln [probability of case selection/probability of control selection]].⁵⁰

Mean selenium was compared among cases according to several factors thought to affect selenium levels: stage at diagnosis, time from symptoms to blood draw, time from diagnosis to blood draw, and weight change in the year before interview. We conducted a simple sensitivity analysis of the impact of weight loss on the relationship between selenium and local and regional/distant colon cancer by limiting the study population to cases and controls who did not experience any weight loss in the year before interview.

We used time from diagnosis to blood draw in order to evaluate whether post diagnosis selenium levels might result in bias. We reasoned that selenium levels drawn near the time of diagnosis could be influenced by disease factors (such as weight loss) or treatment factors (such as surgery or chemotherapy). Therefore, we evaluated whether the same results would have been achieved if we excluded cases who had their blood drawn shortly after diagnosis (<3 months).

Results

Approximately 85 percent of the total study population had serum selenium measurements available. Demographic, lifestyle, and dietary characteristics of the study subpopulation with measured selenium (n=1364) were very similar to the total study population (n=1627) (Table 1), suggesting minimal concern for selection bias from included covariates. Serum selenium among controls had an approximately normal distribution with a mean of 130 and a range of 71–272 mcg/L of serum. As shown in Table 1, mean selenium was slightly higher among Caucasian controls ($\mu=133$ mcg/L) in comparison to the African-American controls ($\mu=125$ mcg/L). A large number of dietary factors were also related to selenium status among controls. Selenium increased with higher reported intakes of vitamin C, vitamin E, zinc, folate, iron, calcium, fiber, and red meat. As expected, selenium was higher among participants taking selenium supplements ($\mu=150$ mcg/L) compared to those who were not ($\mu=130$ mcg/L). Interestingly, selenium was higher in controls who consumed alcohol ($\mu=138$ mcg/L) in comparison to those who did not ($\mu=127$ mcg/L).

The relation between selenium and characteristics associated with cancer diagnosis and prognosis among cases was also evaluated (data not shown). Among all colon cancer cases combined, stage and amount of weight loss were inversely related to selenium (multiple linear regression coefficient: $b = -4.9$ ($p=0.02$) and $b = -0.2$ ($p=0.02$), respectively). As the amount of weight loss increased, selenium decreased; the lowest selenium value by weight change was in the group that lost over 25 pounds in the year before the interview. Selenium decreased with advanced disease; the lowest selenium value by stage was seen in distant stage cases.

Time from diagnosis to blood draw did not have a linear relation with selenium ($b=0.01$ ($p=0.14$)); however, selenium was lower in those who had their blood taken shortly after diagnosis (within 3 months). Selenium values were relatively similar amongst those who had their blood taken ≥ 3 months after diagnosis. When cases were categorized by stage at diagnosis, weight change was much more strongly associated with selenium in those with regional or

distant cancer. Also of interest is that mean weight loss differed by stage (local=-5 lbs., regional=-9 lbs., distant=-17 lbs.) and by time from diagnosis to blood draw (<3 months=-15 lbs., 3-6 months=-11 lbs., 6-12 months=-7 lbs., >12 months=0.1 lbs.).

Table 2a shows results from logistic regression models examining associations of all colon cancers, local colon cancers, and regional or distant colon cancers. All models were adjusted for age, race, gender and the offset term. Gender, often suspected as an effect measure modifier, did not modify the association between selenium and colon cancer on the multiplicative or additive scales in any of the three models (data not shown). Among the multiple dietary factors considered, folate (reported through the FFQ) was the only effect measure modifier. In fact, folate was an effect measure modifier on both scales. To ease interpretation and presentation of effect measure modification estimates, folate was dichotomized (below and above the median value among controls; low= ≤ 354 mcg/day, high= ≥ 354 mcg/day) in all logistic regression result tables.

Participants who had high selenium (≥ 140 mcg/L), without taking reported folate status into account, were less likely to have colon cancer than those who had low or average selenium (OR=0.8, 95% CI=0.6,1.0) (data not shown). The joint effects of serum selenium and reported folate, however, appeared synergistic. A single referent group was used to describe the joint effects of selenium and folate. Individuals who had both high selenium (> 140 mcg/L) and high folate (≥ 354 mcg/day) were half as likely to have colon cancer in comparison to individuals who had low folate and low selenium. The relative risk of colon cancer for those with high selenium and low folate was essentially null as was the relative risk of colon cancer for those with low selenium and high folate. Results were similar regardless of stage at diagnosis; however, evidence of interaction was not as strong in regional/distant cases in comparison to local cases, as evidenced by the ICR and RRR ranges.

Analyses of selenium in fifths illustrated that among participants with high folate, the strongest estimated effect was in the upper three fifths of selenium in comparison to the lowest fifth (Table 2a). There was not a strict monotonic intake-response relationship between selenium and colon cancer risk. Of note, folate status was not as influential in the association between selenium and regional or distant colon cancer cases as it was for selenium and local colon cancer cases.

To assess misclassification of selenium exposure by weight loss, we restricted the analysis to participants who did not lose weight in the year before their interview (Table 2b). Exclusion of participants who lost weight in the year before the interview did not appreciably alter our results. Individuals with high selenium and high folate were less than half as likely to have colon cancer as those who had low selenium and low folate. This association was similar in local and regional/distant cancers when selenium and folate were evaluated as dichotomous variables.

To assess misclassification of selenium exposure by time from diagnosis to blood draw, we excluded participants who had their blood taken less than 3 months after diagnosis (Table 2c). Among participants who had their blood taken 3 months or more after diagnosis and treatment, those with high selenium and high folate were less likely to have colon cancer. The magnitude of the effect was similar but slightly greater in those with local cancer in comparison to those with regional or distant cancer.

Discussion

In this study we investigated the relation between selenium and colon cancer while evaluating several potential sources of bias. The risk of colon cancer for those with high serum selenium and low reported folate was approximately equal to the risk among those with low selenium

and low folate as was the risk of colon cancer for those with low selenium and high folate. However, individuals who had both high selenium and high folate were half as likely to have colon cancer in comparison to individuals who had low folate and low selenium. When cases were categorized by stage, results were similar; however, evidence of a synergistic relation between selenium and folate was slightly stronger in local cases than among regional/distant cases. We did not find evidence of bias in the association between post diagnostic serum selenium and colon cancer due to weight loss, stage at diagnosis, or time from diagnosis to blood draw (selenium measurement).

Our relative risk estimate for high selenium and colon cancer, without taking folate status into consideration, was similar to results of several other studies using pre- or peri-diagnostic serum^{23;29;30} and toenails^{24;51} (OR=0.8). No other epidemiologic studies, to our knowledge, have reported an interaction between selenium and folate in colon cancer risk. Many studies of selenium and colon cancer did not discuss results regarding a potential interaction between selenium and other dietary variables. Likewise, many studies of folate and colon cancer did not report a possible interaction with selenium, perhaps because of the challenges of assessing selenium intake through self-report dietary instruments.

The effect modification of selenium by folate in our study is interesting because most epidemiologic studies report an inverse association between folate and colon cancer risk.^{52–54} According to Lamprecht et al., there are three main mechanisms by which low folate might increase the risk of colon cancer: changes in normal DNA-methylation process, imbalance of steady state level of DNA precursors, and alterations in chromosomes and chromatin.⁵⁵ Recent in vivo and in vitro evidence, however, suggest that the relationship between folate and colon cancer may be more complicated; it is hypothesized that folate is a dual modulator. In normal epithelial cells, folate deficiency causes chromosomal and genomic instability, uracil misincorporation, impaired DNA repair, increased mutations, and induces DNA strand breaks. However, in preneoplastic or neoplastic colorectal epithelial cells, folate deficiency prevents effective DNA synthesis, and as a result, inhibits tumor growth and progression.^{53;54;56–58}

There is limited information about how selenium and folate interact and new information about timing of folate consumption (before or after the neoplastic process has begun) has made this relationship more challenging to decipher. These nutrients might interact biologically or work independently to produce a reduced risk of colon cancer. Both are reported to improve DNA repair mechanisms^{16;59}, play a role in DNA methylation^{11;53}, and support immune function⁶⁰.

Selenium and folate are both involved in DNA methylation: selenium as a methyl consumer and folate as a methyl donor.⁶¹ Deficiency of both selenium and folate results in global DNA hypomethylation and increased cancer susceptibility. Davis and Uthas sought to elucidate the relationship between selenium and folate with regard to DNA methylation and found that selenium and folate in rats had opposite effects on homocysteine concentrations.¹² They also reported that selenium modulated the detrimental effects of folate deficiency (elevated homocysteine levels) by shunting the accumulation of homocysteine through the transsulfuration pathway.^{11;12} This inter-relationship, however, does not explain how adequate/high levels of both nutrients might jointly reduce cancer risk.

Adequate intake of folate and selenium are reported to support a Th1 cytokine-mediated immune response, thus, enhanced immune function is another possible mechanism by which selenium and folate might jointly reduce the risk of colon cancer.⁶⁰ Genetic variation could also be key to understanding a potential synergistic association between these two nutrients. Future research on the interaction of these two nutrients is warranted.

The third aim of our study was to evaluate possible misclassification of selenium exposure due to stage at diagnosis, recent weight loss, and time from diagnosis to serum selenium measure. This was done by performing analyses restricted to groups least likely to be influenced by these biases. We did not find evidence of misclassification by any of these three characteristics.

To our knowledge, only two small studies ($n < 150$) have investigated selenium status in relation to colorectal cancer stage.^{22,35} A decrease in selenium with more advanced disease might suggest that circulating selenium levels change in response to disease, indicating that selenium is more likely to be a disease marker than a risk factor. Dworkin et al. found that selenium was lower in advanced stage cancers in comparison to controls.³⁵ Fernandez-Banares found a dose-response relation between selenium and stage in those less than 60 years of age.²² Unfortunately, neither of these studies reported risk estimates. Our modeled risk estimates by stage at diagnosis, indicated that high selenium, in conjunction with high folate, was associated with decreased risk of all stages of colon cancer. Weight loss has only been quantitatively addressed in one previous study³² and time from diagnosis to selenium measurement has not been evaluated in previous studies.

There are several advantages to our study. First, the NCCCS had an adequate range of selenium levels to detect a difference between high and low selenium and we used cut points established in the NPC Trial to assess the impact of selenium levels analogous to that achieved using a 200 mcg selenium supplement. Second, the relatively large sample afforded the opportunity to study nutrient interactions, risk estimates by stage, and evaluate several potential biases. Third, in the evaluation of the bias by time from diagnosis to blood draw, the wide range of time periods (1–18 months) permitted the investigation of this potential bias in selenium and cancer associations from case-control studies. Fourth, detailed information was available on various diet and lifestyle factors that are important potential confounders of the association between selenium and colon cancer. Fifth, sera from cases and controls were handled in the same manner and assayed at the same lab. Finally, selection bias was not likely because characteristics of the total study population were very similar to the study subpopulation with measured selenium.

Certain limitations should also be acknowledged. Folate food fortification occurred during data collection and the extent to which this secular change may have affected the dietary intakes of study participants is uncertain. Furthermore, we analyzed multiple dietary risk factors for colon cancer as potential effect measure modifiers, increasing the likelihood of a subgroup finding. Although we did not find evidence of bias by weight change, time from diagnosis to blood draw, or stage at diagnosis, there might be other concerns with regards to the use of post-diagnostic serum. There has also been debate over the most appropriate measure of selenium status (toenails, serum/plasma, erythrocytes); however, a recent article suggests that both toenail and plasma selenium levels similarly reflect selenium intake.⁶² Finally, as in all case-control studies, recall bias could be a problem if case status influenced report of diet or lifestyle factors. Selenium was not prone to recall bias because it was measured in serum, however, folate intake was self-reported and, thus, subject to this form of bias.

In conclusion, high levels of serum selenium and reported folate jointly produced a substantially reduced risk of local and regional/distant colon cancers. Our findings suggest that it is important to take folate status into account when evaluating the relation between selenium and colon cancer in future studies. Stage at diagnosis, weight loss, and time from diagnosis to blood draw were not sources of bias in our study.

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Table 1
Characteristics of Cases and Controls by Serum Selenium Status, NCCCS (n=1627)

Participant Characteristic	Total Population		Participants with Measured Selenium		Mean Selenium		Mean Selenium ≥140 mcg/L	
	Case (n=620) %	Control (n=1007) %	Case (n=532) %	Control (n=832) %	Case (n=532) mcg/L	Control (n=832) mcg/L	Case (n=532) %	Control (n=832) %
Age								
40–49	10	6	9	6	123	142	18	37
50–59	22	18	22	19	123	135	21	33
60–69	34	33	34	33	125	132	27	30
70+	34	43	35	43	128	126	22	23
Race								
African-American	45	40	43	37	122	125	25	30
Caucasian	55	60	57	63	128	133	21	24
Gender								
Male	53	50	54	53	126	132	23	27
Female	47	50	46	47	125	129	23	29
Education Level								
Less than high school (HS)	35	30	36	29	124	123	20	23
HS graduate or some college	48	48	48	50	125	131	26	29
College graduate or more	17	22	16	22	129	140	22	32
BMI 1 year ago ^d								
15.6–24.1	17	19	17	19	126	134	25	34
24.2–26.3	15	19	16	20	125	131	18	25
26.4–28.8	20	19	21	19	126	129	22	24
28.9–32.2	21	19	20	19	124	130	24	25
>32.2	24	19	22	19	126	128	26	28
Physical Activity ^b								

Participant Characteristic	Total Population		Participants with Measured Selenium		Mean Selenium		Mean Selenium ≥140 mcg/L	
	Case (n=620) %	Control (n=1007) %	Case (n=532) %	Control (n=832) %	Case (n=532) mcg/L	Control (n=832) mcg/L	Case (n=532) %	Control (n=832) %
1440–1870	18	20	18	19	122	124	21	27
1871–1980	18	20	18	21	120	128	18	28
1981–2134	19	20	18	20	128	132	26	27
2135–2465	21	20	22	20	131	137	27	32
>2465	21	19	21	19	126	132	21	26
NSAIDs regularly(past 5 yrs)								
Never	11	7	11	7	118	127	12	21
Occasionally	41	30	40	29	127	133	23	30
Regularly	48	63	48	64	126	130	26	27
Smoking								
Current	16	18	16	17	115	128	15	27
Former	43	40	45	42	128	133	25	31
Never	40	42	38	41	127	129	24	25
Alcohol (kcal/day)								
None	68	68	68	68	124	127	22	23
Lower half	14	18	14	18	125	140	24	40
Upper half	18	14	19	14	131	137	26	33
1 st degree family history								
Yes	20	9	20	9	130	134	26	32
No	80	90	79	90	124	130	22	28
Dietary Factors								
Total Energy (kcal/day)								
<1000	7	8	8	8	124	133	20	29
1000–1500	26	30	25	30	128	131	29	30

Participant Characteristic	Total Population		Participants with Measured Selenium		Mean Selenium		Mean Selenium ≥140 mcg/L	
	Case (n=620) %	Control (n=1007) %	Case (n=532) %	Control (n=832) %	Case (n=532) mcg/L	Control (n=832) mcg/L	Case (n=532) %	Control (n=832) %
1500–2000	25	31	24	32	126	131	25	29
>2000	43	30	43	30	124	129	20	25
Total Fat (g/day) ^a								
6–47	15	20	15	19	126	135	21	31
48–62	19	20	18	21	127	127	28	26
63–77	12	20	12	20	127	135	27	32
78–99	22	20	23	20	125	129	22	27
>99	32	20	32	19	124	127	21	23
Total Folate (mcg/day) ^a								
45–203	20	20	19	20	123	121	22	18
204–298	26	20	26	20	126	127	23	25
298–450	19	20	21	20	123	129	23	23
451–652	19	20	18	20	128	135	24	36
>652	16	20	16	20	129	140	25	37
Total Vitamin E (mg/day) ^a								
1–7	20	20	21	20	124	125	22	23
8–11	24	20	24	20	124	125	26	20
12–25	24	20	24	20	118	132	15	30
26–160	18	20	18	20	130	133	27	32
>160	13	20	13	20	137	137	30	35
Total Calcium (mg/day) ^a								
114–456	24	20	23	20	127	126	26	23
457–623	22	20	22	19	123	127	21	24
624–845	20	20	21	20	122	133	20	30

Participant Characteristic	Total Population		Participants with Measured Selenium		Mean Selenium		Mean Selenium ≥140 mcg/L	
	Case (n=620) %	Control (n=1007) %	Case (n=532) %	Control (n=832) %	Case (n=532) mcg/L	Control (n=832) mcg/L	Case (n=532) %	Control (n=832) %
845–1251	19	20	21	21	127	131	22	26
>1251	14	20	14	20	129	136	28	36
Total Fiber (g/day) ^a								
1.8–9.0	21	20	21	20	123	125	23	21
9.1–11.9	22	20	21	21	123	130	22	29
12.0–14.5	21	20	22	19	127	133	23	33
14.6–18.4	19	20	19	19	125	132	25	30
>18.4	17	20	17	20	129	133	23	27
Red Meat								
None or <1 serving/day	64	71	64	72	127	131	24	28
>1 serving per day	35	26	35	25	123	128	20	27
Selenium Supplementation								
Yes	1	2	1	2	189	150	71	59
No	99	98	99	98	124	130	23	27

^aQuantified in fifths based on the distribution among controls.

^bMET-minutes per day in fifths based on the distribution among controls

Table 2

Table 2a Selenium and Estimated Risk of Colon Cancer by Stage at Diagnosis										
All Colon Cancers vs. Controls (n=1,362)					Local Colon Cancers vs. Controls (n=1,011)					
Combinations of Selenium and Folate ^a		Case (n=532)	Control (n=830)	Odds Ratios ^b (95% CIs)	Case (n=181)	Control (n=830)	Odds Ratios ^b (95% CIs)	Case (n=304)	Odds Ratios ^b (95% CIs)	
Low Selenium, Low Folate		227	318	1.0 (Ref)	75	318	1.0 (Ref)	136	1.0 (Ref)	
Low Selenium, High Folate		68	90	0.9 (0.7,1.2)	28	90	1.0 (0.7,1.4)	31	0.9 (0.6,1.2)	
High Selenium, Low Folate		182	280	1.1 (0.7,1.5)	63	280	1.3 (0.8,2.1)	104	0.8 (0.5,1.2)	
High Selenium, High Folate		55	142	0.5 (0.4,0.8)	15	142	0.4 (0.2,0.8)	33	0.5 (0.3,0.8)	
			RRR= 0.6 (0.1,1.0) ICR= −0.4 (−0.9,0.0)			RRR= 0.4 (−0.4,1.2) ICR= −0.8 (−1.6,0.0)			RRR= 0.8(0.3,1.2) ICR= −0.1(−0.6,0.4)	
Regional/Distant vs. Controls (n=1,134)										
All Colon Cancers vs. Controls (n=1,362)										
		Case/Control	Odds Ratios ^b (95% CIs)		Case/Control	Odds Ratios ^b (95% CIs)		Case/Control	Odds Ratios ^b (95% CIs)	
Serum Selenium (fifths)			Low Folate ^a	High Folate ^a		Low Folate ^a	High Folate ^a		Low Folate ^a	High Folate ^a
70–105		139/143	1.0 (Ref)	1.0 (Ref)	40/143	1.0 (Ref)	1.0 (Ref)	88/143	1.0 (Ref)	1.0 (Ref)
106–116		106/159	0.8 (0.5,1.2)	0.6 (0.3,1.0)	40/159	1.1 (0.5,2.1)	0.6 (0.3,1.3)	59/159	0.6 (0.4,1.0)	0.6 (0.3,1.0)
117–128		92/187	0.6 (0.4,1.0)	0.4 (0.2,0.6)	31/187	0.9 (0.4,1.8)	0.3 (0.1,0.7)	55/187	0.5 (0.3,0.9)	0.4 (0.2,0.8)
129–146		95/170	0.9 (0.6,1.5)	0.4 (0.2,0.6)	35/170	1.2 (0.7,2.7)	0.4 (0.2,0.9)	51/170	0.8 (0.4,1.4)	0.3 (0.2,0.6)
147–290		100/173	0.9 (0.6,1.5)	0.4 (0.2,0.6)	35/173	1.3 (0.7,2.7)	0.3 (0.1,0.7)	51/173	0.6 (0.3,1.1)	0.3 (0.2,0.6)
<i>p for Trend</i>			<i>p=0.31</i>	<i>p<0.01</i>		<i>p=0.37</i>	<i>p<0.01</i>		<i>p=0.03</i>	<i>p<0.01</i>

Table 2a Selenium and Estimated Risk of Colon Cancer by Stage at Diagnosis

Combinations of Selenium and Folate ^d	All Colon Cancers vs. Controls (n=1,362)			Local Colon Cancers vs. Controls (n=1,011)			Regional/Distant vs. Controls (n=1,134)		
	Case (n=532)	Control (n=830)	Odds Ratios ^b (95% CIs)	Case (n=181)	Control (n=830)	Odds Ratios ^b (95% CIs)	Case (n=304)	Control (n=830)	Odds Ratios ^b (95% CIs)
Low selenium, Low folate	71	163	1.0 (Ref)	31	163	1.0 (Ref)	38	163	1.0 (Ref)
Low selenium, High folate	54	142	0.8 (0.5,1.2)	21	142	0.7 (0.4,1.3)	28	142	0.7 (0.4,1.2)
High selenium, Low folate	24	46	1.2 (0.7,2.1)	11	46	1.2 (0.6,2.7)	9	46	0.8 (0.4,1.9)
High selenium, High folate	19	82	0.4 (0.2,0.8)	6	82	0.4 (0.1,0.9)	13	82	0.5 (0.2,1.0)
			RRR= 0.5 (−0.3,1.3)			RRR= 0.4 (−0.7,1.5)			RRR= 0.9 (0.0,1.7)
			ICR= −0.5 (−1.3,0.3)			ICR= −0.6 (−1.7,0.5)			ICR= −0.0 (−0.9,0.8)

Table 2b Selenium and Estimated Risk of Colon Cancer by Stage at Diagnosis

Combinations of Selenium and Folate ^d	All Colon Cancers vs. Controls (n=601)			Local Colon Cancers vs Controls (n=502)			Regional/Distant vs. Controls (n=521)		
	Case/Control	Odds Ratios ^b (95% CIs) 1	Case/Control	Case/Control	Odds Ratios ^b (95% CIs)	Case/Control	Odds Ratios ^b (95% CIs)	Case/Control	Odds Ratios ^b (95% CIs)
Serum Selenium (fifths)									
		Low Folate ^a	High Folate ^a		Low Folate ^a	High Folate ^a	Low Folate ^a	High Folate ^a	
70–105	30/62	1.0 (Ref)	1.0 (Ref)	13/62	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
106–116	40/78	1.3 (0.6,2.8)	0.7 (0.3,1.9)	18/78	1.9 (0.6,5.7)	0.5 (0.1,1.6)	0.9 (0.3,2.4)	1.3 (0.3,4.8)	
117–128	37/113	0.8 (0.4,1.8)	0.4 (0.2,1.1)	12/113	1.0 (0.3,3.3)	0.2 (0.1,0.7)	0.6 (0.2,1.6)	1.1 (0.3,3.9)	
129–146	25/90	1.1 (0.5,2.5)	0.4 (0.2,1.0)	14/90	1.4 (0.4,4.8)	0.4 (0.1,1.2)	0.7 (0.2,2.0)	0.5 (0.1,2.2)	
147–290	33/92	1.1 (0.5,2.6)	0.4 (0.2,1.0)	12/92	1.3 (0.4,4.3)	0.3 (0.1,1.0)	0.8 (0.3,2.2)	0.7 (0.2,2.8)	
<i>p for Trend</i>		<i>p</i> =0.89	<i>p</i> =0.04		<i>p</i> =0.59	<i>p</i> =0.02	<i>p</i> =0.45	<i>p</i> =0.64	

Table 2c Selenium and Estimated Risk of Colon Cancer Restricted to Cases who had their Blood Drawn >3 Months after Diagnosis

Combinations of Selenium and Folate ^d	All Colon Cancers vs. Controls (n=1298)			Local Colon Cancers vs. Controls (n=986)			Regional/Distant vs. Controls (n=1099)		
	Case (n=468)	Control (n=830)	Odds Ratios ^b (95% CIs)	Case (n=156)	Control (n=830)	Odds Ratios ^b (95% CIs)	Case (n=269)	Control (n=830)	Odds Ratios ^b (95% CIs)
Low selenium, Low folate	196	318	1.0 (Ref)	62	318	1.0 (Ref)	119	318	1.0 (Ref)

Table 2a Selenium and Estimated Risk of Colon Cancer by Stage at Diagnosis

Table 2a Selenium and Estimated Risk of Colon Cancer by Stage at Diagnosis											
Combinations of Selenium and Folate ^a	All Colon Cancers vs. Controls (n=1,362)				Local Colon Cancers vs. Controls (n=1,011)				Regional/Distant vs. Controls (n=1,134)		
	Case (n=532)	Control (n=830)	Odds Ratios ^b (95% CIs)		Case (n=181)	Control (n=830)	Odds Ratios ^b (95% CIs)		Case (n=304)	Control (n=830)	Odds Ratios ^b (95% CIs)
Low selenium, High folate	158	90	0.9 (0.7,1.2)		56	90	1.0 (0.7,1.5)		89	90	0.8 (0.6,1.3)
High selenium, Low folate	63	280	1.1 (0.8,1.6)		26	280	1.5 (0.9,2.5)		29	280	0.8 (0.5,1.3)
High selenium, High folate	51	142	0.6 (0.4,0.8)		12	142	0.4 (0.2,0.8)		32	142	0.6 (0.4,0.9)
			RRR= 0.6 (0.1,1.1)				RRR= 0.3 (−0.7,1.2)				RRR= 0.8 (0.3,1.3)
			ICR= −0.5 (−1.0,0.0)				ICR= −1.1 (−2.0, −0.1)				ICR= −0.1 (−0.6,0.4)
Local Colon Cancers v Controls (n=986)											
Regional/Distant vs. Controls (n=1099)											
Serum Selenium (fifths)	Case/Control	Odds Ratios ^b (95% CIs)		Case/Control	Odds Ratios ^b (95% CIs)		Case/Control	Odds Ratios ^b (95% CIs)			
		Low Folate ^d	High Folate ^d		Low Folate ^d	High Folate ^d		Low Folate ^d	High Folate ^d		
	70–105	120/143	1.0 (Ref)	1.0 (Ref)	33/143	1.0 (Ref)	1.0 (Ref)	77/143	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
106–116	94/158	0.8 (0.5,1.3)	0.6 (0.3,1.0)	35/158	1.3 (0.6,2.6)	0.6 (0.3,1.3)	52/158	0.6 (0.3,1.0)	0.6 (0.3,1.1)	0.6 (0.3,1.1)	
117–128	76/187	0.5 (0.3,0.9)	0.4 (0.2,0.7)	26/187	0.9 (0.4,2.0)	0.3 (0.1,0.8)	45/187	0.4 (0.2,0.7)	0.4 (0.3,0.8)	0.4 (0.3,0.8)	
129–146	85/169	0.9 (0.6,1.6)	0.4 (0.2,0.7)	31/169	1.4 (0.6,3.0)	0.4 (0.2,0.9)	47/169	0.8 (0.4,1.4)	0.3 (0.2,0.8)	0.3 (0.2,0.8)	
147–290	93/173	1.0 (0.6,1.6)	0.4 (0.2,0.7)	31/173	1.7 (0.8,3.5)	0.3 (0.1,0.7)	48/173	0.6 (0.3,1.1)	0.4 (0.2,0.8)	0.4 (0.2,0.8)	
<i>p for Trend</i>		<i>p</i> =0.34	<i>p</i> <0.01		<i>p</i> =0.32	<i>p</i> <0.01		<i>p</i> =0.02		<i>p</i> <0.01	

^a Selenium: Low is ≥140 mcg/L of serum; High is >140 mcg/L of serum. Folate: Low is <354 mcg/day; High is ≥354 mcg/day

^b All odds ratios are adjusted for age, race, gender and offset terms.

Note: RRR=Departure from expected multiplicative effect of selenium and folate on colon cancer. Formula: RRR=observed joint effect/expected joint effect. ICR=Departure from expected additive effect of selenium and folate on colon cancer. Formula: ICR=observed joint effect-expected joint effect. In the first column, RRR=0.5/(0.9*1.1). The ICR=0.5−(0.9+1.1−1). Rounding in table may affect the reader’s hand calculated duplication of RRR and ICR.

^a Selenium: Low is <140 mcg/L of serum; High is ≥140 mcg/L of serum. Folate: Low is <354 mcg/day; High is ≥354 mcg/day

^b All odds ratios are adjusted for age, race, gender and offset terms.

Note: RRR=Departure from expected multiplicative effect of selenium and folate on colon cancer. Formula: RRR=observed joint effect/expected joint effect. ICR=Departure from expected additive effect of selenium and folate on colon cancer. Formula: ICR=observed joint effect-expected joint effect.

^a Selenium: Low is <140 mcg/L of serum; High is $\geq$ 140 mcg/L of serum. Folate: Low is <354 mcg/day; High is $\geq$ 354 mcg/day

^b All odds ratios are adjusted for age, race, gender and offset terms.

Note: RRR=Departure from expected multiplicative effect of selenium and folate on colon cancer. Formula: RRR=observed joint effect/expected joint effect. ICR=Departure from expected additive effect of selenium and folate on colon cancer. Formula: ICR=observed joint effect-expected joint effect.