

Serum Selenium Levels in Relation to Markers of Neoplastic Progression Among Persons With Barrett's Esophagus

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Background: Persons with Barrett's esophagus have a substantially greater risk of esophageal adenocarcinoma than the general population. Higher serum selenium levels have been associated with a reduced risk of several cancers; however, their association with the risk of esophageal adenocarcinoma is unknown. We used a cross-sectional study to investigate the relationship between serum selenium levels and markers of neoplastic progression among persons with Barrett's esophagus. **Methods:** Medical history, blood, and esophageal tissue specimens were collected from 399 members of a cohort study of Barrett's esophagus patients undergoing endoscopic surveillance. Serum selenium levels were measured by flameless atomic absorption spectrophotometry. DNA content of tissue samples was measured by flow cytometry. Loss of heterozygosity (LOH) at 9p and 17p, chromosomal regions which include the p16 and p53 tumor suppressors, respectively, was detected by automated fluorescent genotyping. Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs). All statistical tests were two-sided. **Results:** Persons with serum selenium levels in the upper three quartiles (i.e., $>1.5 \mu\text{M}$) were less likely to have high-grade dysplasia (OR = 0.5, 95% CI = 0.3 to 0.9) or aneuploidy (OR = 0.4, 95% CI = 0.2 to 0.8) than those with levels in the lowest quartile. Serum selenium levels in the upper three quartiles were associated with similar reductions in risk of 17p (p53) LOH (OR = 0.5, 95% CI = 0.2 to 0.9) and increased 4N fraction (OR = 0.6, 95% CI = 0.3 to 1.2). By contrast, serum selenium levels were not associated with 9p (p16) LOH (OR = 1.0, 95% CI = 0.5 to 1.7), a marker that appears early in neoplastic progression. **Conclusion:** Our preliminary results, from a cross-sectional analysis with biologic markers, suggest that higher serum selenium levels may be associated with a reduced risk of esophageal adenocarcinoma among persons with Barrett's esophagus. Because serum selenium was not associated with 9p (p16) LOH, we speculate that selenium may act primarily at later stages of progression toward adenocarcinoma. [J Natl Cancer Inst 2003;95:750-7]

The incidence of esophageal adenocarcinoma has risen dramatically over the past three decades in the United States and in many other Western countries for reasons that are not well understood (1,2). Most esophageal adenocarcinomas arise in a metaplastic epithelium termed Barrett's esophagus (3), in which the normal stratified squamous epithelium of the esophagus is replaced by specialized intestinal metaplasia as a complication of chronic gastroesophageal reflux (4). In the United States, adults who have been diagnosed with Barrett's esophagus have

a 30- to 75-fold greater risk of invasive esophageal adenocarcinoma than other adults (5-7). Because of this increased cancer risk, many physicians recommend that persons with Barrett's esophagus undergo regular endoscopic surveillance for the purpose of detecting cancer at the earliest possible stage (8-14). However, endoscopic surveillance for cancer is expensive (15), and no medical therapy or anti-reflux procedure has been shown to reduce the risk of esophageal adenocarcinoma. Given the large number of persons affected by Barrett's esophagus—estimated to be more than 1 million in the United States (16-19)—further investigations into preventive measures are warranted.

Evidence from laboratory and population-based studies indicates that some selenium-containing compounds (e.g., selenomethionine) have anticarcinogenic effects (20-24). Selenium may interfere with the development of cancer by inhibiting cellular proliferation, promoting apoptosis, and protecting DNA from oxidative damage (20). In a randomized trial, persons assigned to take 200 μg of selenium daily (in the form of high-selenium Brewer's yeast) had a lower incidence of all cancers, prostate and colorectal cancers, and death from cancer than persons assigned to take the placebo (21). In observational studies, higher levels of selenium in blood or toenail samples have been associated with a decreased risk of squamous esophageal, gastric, lung, liver, pancreatic, bladder, and thyroid cancers (22-24). However, the effect of selenium on the risk of esophageal adenocarcinoma is largely unknown.

We hypothesized that higher levels of serum selenium would be associated with a reduced risk of developing esophageal adenocarcinoma among persons with Barrett's esophagus. To investigate this hypothesis, we conducted a cross-sectional analysis in which we related levels of serum selenium to biologic markers of neoplastic progression, including 17p (p53) loss of

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heterozygosity (LOH), increased 4N fraction, aneuploidy, and high-grade dysplasia. Each of these markers has been shown to be predictive of progression to esophageal adenocarcinoma in prospective cohort studies (25,26). Because substantial evidence suggests that inactivation of p16 by chromosomal loss of 9p or other mechanisms is an important early step in progression to esophageal adenocarcinoma (27,28), the relation of serum selenium levels to 9p (p16) LOH was also analyzed. Multivariate analyses were performed to examine the potential confounding effects of other serum micronutrients, as well as established risk and protective factors for esophageal adenocarcinoma (29–32), on the associations between serum selenium levels and each of the biologic markers.

SUBJECTS AND METHODS

Study Population

Study participants were selected from persons undergoing endoscopic surveillance as part of their involvement with the Seattle Barrett's Esophagus Project, a cohort study initiated in 1983 (25). Beginning in 1995, most cohort members agreed to undergo a baseline evaluation, including endoscopy with collection of multiple samples of the esophageal epithelium and a personal interview with dietary assessment, anthropometric measurements, and collection of blood samples for measurement of micronutrient levels. Cohort members then returned regularly for follow-up examinations, which included endoscopy, interview, and blood collection, at intervals selected according to degree of dysplasia in the Barrett's segment. This article focuses on data collected from the cohort members at baseline. Cohort members were eligible for this study if they 1) had completed a baseline evaluation between February 1995 and August 1999 and had specialized intestinal metaplasia in at least one sample of the esophageal epithelium, 2) had a blood sample available for measurement of selenium, 3) had no prior history of esophageal cancer, and 4) were older than 18 years. Of the 399 eligible cohort members, 12 were found to have esophageal adenocarcinoma at their baseline endoscopy. All participants were counseled concerning risks and benefits of endoscopic surveillance for Barrett's esophagus and were advised of potential alternatives, including surgery for high-grade dysplasia. Participants or their guardians gave written informed consent before their involvement in any research activity. The study was approved by the Human Subjects Committee at the University of Washington, with reciprocity from the Fred Hutchinson Cancer Research Center.

Demographic, Lifestyle, and Anthropometric Data

The participants were interviewed in person by trained staff using a standardized questionnaire. Interview data included age, sex, ethnicity, income, education, usual adult body weight, and history of cigarette smoking. The participants also completed a self-administered questionnaire that included items on use of vitamin and mineral supplements. Height, weight, and anthropometric measurements (hip, waist, abdominal, and thigh circumferences) were obtained by using a standardized protocol. Body mass index was calculated as weight in kilograms divided by height in meters squared. Waist-to-hip ratio was calculated as circumference of the waist divided by circumference of the hips at the widest point.

Blood Collection and Storage

Participants fasted overnight before donating a blood sample, which was preserved in three tubes: two with EDTA and one acid-washed tube without this anticoagulant. Blood samples were protected from light at all times. After processing, serum and plasma were collected and divided into several aliquots and stored in cryovials at -70°C . Metaphosphoric acid and dithiothreitol were added to the aliquot designated for ascorbic acid analysis before freezing to prevent degradation of this nutrient.

Nutrient Measures

The concentration of selenium in serum was measured by flameless atomic absorption spectrophotometry with Zeeman background correction (33). Accurate measurement of serum selenium was not possible for three participants because substantial hemolysis had occurred during blood collection; these participants were therefore excluded from all statistical analyses. The concentrations of alpha-tocopherol, alpha-carotene, beta-carotene, lycopene, and ascorbic acid were measured in sera from participants who had baseline evaluations before December 1997 ($n = 211$). The concentrations of carotenoids and alpha-tocopherol in plasma were measured with high-performance liquid chromatography using procedures that have been published previously (34). The concentration of ascorbic acid in plasma was measured with a COBAS MIRA Plus Chemistry Analyzer (Roche, Brandburg, NJ) using a colorimetric procedure described by Lee and colleagues (35). The concentration of total cholesterol in plasma was measured enzymatically with reagents and calibrators from Sigma (Sigma Diagnostics, St. Louis, MO). For all nutrient measures, the interassay coefficients of variation (CV%) were 10% or less. The nutrient levels in the control samples covered the normal reference range for U.S. residents.

Endoscopy

The methods used to document Barrett's segment length and to obtain tissue samples for histologic and flow cytometric analyses have been described previously (25,29). Briefly, Barrett's segment length was defined as the distance between the esophagogastric and squamocolumnar junctions. Samples of the esophageal epithelium were obtained with jumbo biopsy forceps by the "turn and suction" technique (36,37). In participants without a history of high-grade dysplasia, four biopsy specimens, one per quadrant of the esophagus, were obtained from every other centimeter of the Barrett's segment. In participants with a history of high-grade dysplasia, four biopsy specimens, one per quadrant, were obtained from every centimeter of the Barrett's segment. One biopsy specimen from each level of the Barrett's segment where samples had been obtained was divided into two. Half was frozen in 10% dimethyl sulfoxide and stored at -70°C for subsequent flow cytometric analysis. The other half and the remaining three tissue samples from each level were placed in Hollande's solution for subsequent histologic examination. Additional biopsy specimens were obtained from the Barrett's segment at 2-cm intervals, frozen in 10% dimethyl sulfoxide, and stored at -70°C for subsequent LOH analyses. LOH analyses were performed on one biopsy specimen from each 2-cm interval.

Histology

Tissue samples were cut serially into 4- μm sections, mounted onto slides, and stained with either hematoxylin and eosin alone,

or hematoxylin and eosin, saffron, and Alcian blue at pH 2.5, as recommended for diagnosing Barrett's esophagus (38,39). One pathologist, who was blinded to the results of the flow cytometric analyses, assigned a diagnosis of negative for dysplasia, indefinite for dysplasia, low-grade dysplasia, high-grade dysplasia, or intramucosal carcinoma to each slide using established criteria (40).

DNA Content Flow Cytometry

The methods used to prepare biopsy specimens for cell sorting, to perform flow cytometry, and to analyze the resulting data have been described previously (25,41). If more than 6% of the cells in at least one biopsy specimen from the Barrett's epithelium had a DNA content in the range of 3.85N–4.1N, the participant was given a diagnosis of increased 4N fraction (25,37,42). The cut point of 6% was selected because it yields the optimal sensitivity and specificity for distinguishing persons with an increased risk of esophageal adenocarcinoma from those without (42). The term "increased" 4N fraction is used when the cut point of 6% is exceeded because in the normal columnar epithelium of the upper gastrointestinal tract, a small percentage of cells (<6%) have a DNA content of 4N (37); these cells have doubled their DNA content in preparation for cell division.

Aneuploidy was diagnosed if the following two criteria were met in at least one tissue sample from the Barrett's epithelium: 1) two discrete peaks were observed on the histogram—one reflecting the presence of an aneuploid population and the other reflecting the presence of a diploid (2N) population, and 2) the aneuploid peak represented at least 2.5% of the cells in the biopsy specimen (43). For the analyses described in this article, a diagnosis of aneuploidy was assigned only if the DNA content of the nondiploid population was outside the range of 3.85N–4.1N. Because the cancer incidence among persons with Barrett's esophagus who meet the diagnostic criteria for aneuploidy but have a DNA content ranging from greater than 2.0N to 2.7N has recently been found to be similar to that of persons with normal DNA content (42), we defined a second aneuploidy variable in which aneuploidy was diagnosed only if the DNA content of the nondiploid population was greater than 2.7N and the other criteria for aneuploidy were met. Measurements of the 4N fraction and aneuploidy were available for 343 of 384 (89%) participants without cancer.

9p (p16) and 17p (p53) LOH

After purification of cell populations from tissue biopsy specimens by Ki67/DNA content multiparameter flow sorting (44), DNA was extracted and subjected to whole genome amplification by primer extension preamplification (41). Locus-specific polymerase chain reaction (PCR) and LOH analysis were performed with fluorescent-tagged PCR primers (27) on Applied Biosystems (Foster City, CA) 373 or 377 automated DNA sequencing instruments. A study participant was considered to have 9p (p16) or 17p (p53) LOH if at least one sample of the Barrett's epithelium had clear LOH on the basis of objective measures of allele intensity ratios (27). 9p (p16) LOH and 17p (p53) LOH were defined as LOH including the p16 and p53 loci (27). Measurements of 9p (p16) LOH and 17p (p53) LOH were available for 255 (66.4%) and 252 (65.6%) of the 384 participants without cancer, respectively.

Statistical Analysis

Logistic regression was used to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the associations between levels of serum selenium and the biologic markers of neoplastic progression. All tests of statistical significance were two-sided. Participants diagnosed with cancer at the baseline endoscopy were excluded from statistical analyses unless otherwise specified. Variables evaluated as potential confounders were age (categorized as 30–44 years, 45–54 years, 55–64 years, 65–74 years, 75 years or older, or as a continuous variable), sex, cigarette use (never, tertiles based on pack-years; or current smoker, non-smoker [including former smokers]; or current, former, never), usual body mass index during adulthood (<25 kg/m², 25–27.4 kg/m², 27.5–30 kg/m², >30 kg/m²), waist-to-hip ratio (quartiles), annual income (<\$15 000, \$15 000–30 000, \$30 000–45 000, >\$45 000), education (less than 12th grade, high school graduate, some college, college graduate), use of vitamin or mineral supplements during the last year (yes, no), use of nonsteroidal anti-inflammatory drugs (never, former, current), length of Barrett's segment (<3 cm, 3–6 cm, 7–10 cm, >10 cm, or as a continuous variable), alpha-tocopherol (quartiles), ascorbic acid (quartiles), alpha-carotene (quartiles), beta-carotene (quartiles), and lycopene (quartiles) (29–32,45).

Before alpha-tocopherol, ascorbic acid, alpha-carotene, beta-carotene, and lycopene were included in analyses, the variables were transformed by taking the natural logarithm of the values of each variable. To adjust plasma alpha-tocopherol levels for total cholesterol levels, a linear regression was performed in which the natural logarithm of alpha-tocopherol was the dependent variable and the natural logarithm of cholesterol was the independent variable; the residuals from this regression represented alpha-tocopherol levels that had been adjusted for cholesterol. Alpha-carotene, beta-carotene, and lycopene were also adjusted for cholesterol by the same method. Measurements of alpha-tocopherol, ascorbic acid, alpha-carotene, beta-carotene, and lycopene were not available for approximately half the participants. The potential confounding effects of each of these variables was assessed only among participants for whom complete data were available.

None of the variables evaluated as a potential confounder changed the risk estimates for selenium to an important degree; therefore, the unadjusted risk estimates are presented.

Variables evaluated as potential effect modifiers were age (<60 years, ≥60 years), sex, usual body mass index during adulthood (<25 kg/m², 25–27.4 kg/m², 27.5–30 kg/m², >30 kg/m²; or <30 kg/m², ≥30 kg/m²), cigarette use (never, tertiles based on pack-years), education (less than 12th grade, high school graduate, some college, college graduate), use of any vitamin or mineral supplements during the last year (yes, no), use of supplements containing selenium during the last year (yes, no; or 0, 1–19, 20 or more micrograms/day), use of nonsteroidal anti-inflammatory drugs (never, former, current), and length of Barrett's segment (<3 cm, ≥3 cm). The statistical significance of any interaction was determined by performing a likelihood ratio test comparing models with and without the interaction term(s). In the interaction term, levels of serum selenium were modeled either as a binary variable (upper three quartiles versus lowest quartile) or as a categorical variable (quartiles). All analyses were performed with Stata statistical software, version 6.0 (46).

RESULTS

The mean age of the 396 participants for whom serum selenium data were available was 61.5 years (standard deviation [SD] = 11.9 years) and nearly 80% were men (Table 1). More than 95% of participants were Caucasian, and most had attended or graduated from college.

The mean serum selenium concentration of the participants was 1.68 μM (SD = 0.26 μM , range = 0.85–2.7 μM). The associations between serum selenium levels, categorized into quartiles, and each of the biologic markers of neoplastic progression are presented in Table 2. Compared with participants with serum selenium levels in the lowest quartile, those with levels in the upper three quartiles had a statistically significantly lower risk of 17p (p53) LOH (OR = 0.5, 95% CI = 0.2 to 0.9), aneuploidy (OR = 0.4, 95% CI = 0.2 to 0.8), and high-grade dysplasia (OR = 0.5, 95% CI = 0.3 to 0.9). Among participants with serum selenium levels in the upper three quartiles, however, there was little evidence of a further decrease in risk in association with higher serum selenium levels. When aneuploidy

was defined as greater than 2.7N, as with 28 of the 34 persons originally classified as having aneuploidy, the inverse association between aneuploidy and serum selenium levels was strengthened (Table 2). Summary ORs comparing participants in the upper three quartiles with those in the lowest quartile ranged from 0.3 (95% CI = 0.1 to 0.6) for aneuploidy greater than 2.7N to 0.6 (95% CI = 0.3 to 1.2) for increased 4N fraction. By contrast, serum selenium levels were not associated with risk of 9p (p16) LOH (summary OR = 1.0, 95% CI = 0.5 to 1.7).

We postulated that the inverse associations between serum selenium levels and the biologic markers would be relatively weak among participants who consumed supplements containing selenium because fewer of these individuals would have low serum selenium levels. However, the participants who reported use of selenium-containing supplements had only slightly higher levels of serum selenium than participants who reported no use of these supplements (data not shown). Consequently, the ORs relating serum selenium levels to the biologic markers were not consistently different for participants who reported use of selenium-containing supplements than for participants who reported no use of these supplements (data not shown).

The inverse associations between serum selenium levels (categorized as upper three quartiles versus lowest quartile) and 17p (p53) LOH, increased 4N fraction, aneuploidy, and high-grade dysplasia tended to be stronger among participants with a body mass index lower than 30 kg/m² than among participants with a higher body mass index. This interaction was statistically significant ($P = .046$) only for those with high-grade dysplasia, with ORs of 0.4 (95% CI = 0.2 to 0.7) among participants with a body mass index lower than 30 kg/m² and 1.9 (95% CI = 0.4 to 9.6) among participants with a body mass index of 30 kg/m² or higher. The inverse associations between serum selenium levels and reduced risk of 17p (p53) LOH, increased 4N fraction, and high-grade dysplasia also tended to be stronger among participants who were aged 60 years or older, although the interaction with age was not statistically significant. The associations between serum selenium levels and the biologic markers among men were generally similar to those observed among women (data not shown).

We also examined the effects of the duration of participation in the Seattle Barrett's Esophagus Project and adjustment for other serum micronutrients on the associations between serum selenium levels and the biologic markers. Twenty-eight percent (110 of 396) of the participants had been enrolled in a program of endoscopic surveillance with the Seattle Barrett's Esophagus Project for a mean of 5.6 years (SD = 3.3 years) before donating the blood and tissue samples analyzed in this study. After learning that they had Barrett's esophagus, some of the continuing participants may have adopted healthier lifestyles, particularly if they had been diagnosed with high-grade dysplasia. If their intake of selenium increased because of these lifestyle changes, the ORs relating serum selenium levels to the biologic markers would be biased upward (i.e., toward an OR of 1.0). However, we observed that the inverse associations between serum selenium levels and risk of 17p (p53) LOH, increased 4N fraction, aneuploidy, and high-grade dysplasia were stronger among the continuing participants than among the other participants. For example, the OR for the association between serum selenium levels (upper three quartiles versus lowest quartile) and aneuploidy was 0.2 (95% CI = 0.04 to 1.1) for the continuing participants and 0.4 (95% CI = 0.2 to 1.0) for the participants

Table 1. Selected characteristics of 396 study participants enrolled in the Seattle Barrett's Esophagus Project

Characteristics	n (%)
Age, y	
30–44	29 (7.3)
45–54	94 (23.7)
55–64	101 (25.5)
65–74	106 (26.8)
≥75	66 (16.7)
Sex	
Male	311 (78.5)
Female	85 (21.5)
Ethnicity	
Caucasian	378 (95.4)
African American	0 (0)
Asian	5 (1.3)
Native American	2 (0.5)
Other	8 (2.0)
Declined to answer	3 (0.8)
Annual income, \$	
<15 000	29 (7.3)
15 000–30 000	100 (25.2)
30 000–45 000	87 (22.0)
45 000–60 000	56 (14.1)
>60 000	104 (26.3)
Declined to answer	15 (3.8)
Did not know	5 (1.3)
Education	
Less than 12 th grade	28 (7.1)
High school graduate	112 (28.3)
Some college	106 (26.8)
College graduate	150 (37.9)
Histologic diagnosis*	
Metaplasia	128 (32.3)
Indefinite or low-grade dysplasia†	198 (50.0)
High-grade dysplasia	58 (14.6)
Adenocarcinoma	12 (3.0)
Barrett's segment length, cm‡	
<3	118 (29.8)
≥3	278 (70.2)

*Histologic diagnoses were made by a pathologist (blinded to all flow cytometric results) using established criteria (40).

†Indefinite for dysplasia or low-grade dysplasia.

‡Barrett's segment length was defined as the distance between the esophago-gastric and squamocolumnar junctions.

Table 2. Frequency distribution (%) of serum selenium levels in relation to biologic markers of neoplastic progression among persons with Barrett's esophagus

Markers of neoplastic progression†	Serum selenium in quartiles*				Upper three quartiles vs. lowest quartile
	I (lowest)	II	III	IV (highest)	
9p (p16) LOH‡					
No (n = 111)	20.7	22.5	27.0	29.7	
Yes (n = 144)	21.5	24.3	28.5	25.7	
Odds ratio (95% CI)	Referent	1.0 (0.5 to 2.2)	1.0 (0.5 to 2.1)	0.8 (0.4 to 1.7)	1.0 (0.5 to 1.7)
17p (p53) LOH					
No (n = 207)	18.8	24.2	30.0	27.0	
Yes (n = 45)	33.3	20.0	15.6	31.1	
Odds ratio (95% CI)	Referent	0.5 (0.2 to 1.2)	0.3 (0.1 to 0.8)	0.6 (0.3 to 1.5)	0.5 (0.2 to 0.9)
Increased 4N fraction§					
No (n = 311)	23.2	24.4	26.0	26.4	
Yes (n = 32)	34.4	15.6	28.1	21.9	
Odds ratio (95% CI)	Referent	0.4 (0.1 to 1.3)	0.7 (0.3 to 1.8)	0.6 (0.2 to 1.5)	0.6 (0.3 to 1.2)
Aneuploidy					
No (n = 309)	22.3	24.3	26.5	26.9	
Yes (n = 34)	41.2	17.6	23.5	17.6	
Odds ratio (95% CI)	Referent	0.4 (0.1 to 1.1)	0.5 (0.2 to 1.2)	0.4 (0.1 to 1.0)	0.4 (0.2 to 0.8)
Aneuploidy with ploidy >2.7N¶					
No (n = 315)	21.9	24.4	27.0	26.7	
Yes (n = 28)	50.0	14.3	17.9	17.9	
Odds ratio (95% CI)	Referent	0.2 (0.1 to 0.8)	0.3 (0.1 to 0.8)	0.3 (0.1 to 0.8)	0.3 (0.1 to 0.6)
High-grade dysplasia					
No (n = 326)	22.7	26.1	26.4	24.8	
Yes (n = 58)	37.9	13.8	24.1	24.1	
Odds ratio (95% CI)	Referent	0.3 (0.1 to 0.8)	0.5 (0.3 to 1.1)	0.6 (0.3 to 1.2)	0.5 (0.3 to 0.9)
High-grade dysplasia or cancer					
No (n = 326)	22.7	26.1	26.4	24.8	
Yes (n = 70)	37.1	18.6	24.3	20.0	
Odds ratio (95% CI)	Referent	0.4 (0.2 to 0.9)	0.6 (0.3 to 1.1)	0.5 (0.2 to 1.0)	0.5 (0.3 to 0.9)

*Cut points for quartiles were $\leq 1.50 \mu\text{M}$, first quartile, 1.51–1.67 μM , second quartile; 1.68–1.84 μM , third quartile; and $\geq 1.85 \mu\text{M}$, fourth quartile. CI = confidence interval.

†Persons with cancer were excluded from analyses unless otherwise noted.

‡Loss of heterozygosity (LOH) was determined as previously described (27,41).

§Increased 4N fraction was defined as more than 6% of cells in at least one biopsy specimen from the Barrett's epithelium having a DNA content in the range of 3.85–4.1N (25,37,42).

||Aneuploidy was diagnosed if the following two criteria were met in at least one tissue sample from the Barrett's epithelium: 1) two discrete peaks were observed on the histogram—one reflecting the presence of an aneuploid population and the other reflecting the presence of a diploid (2N) population, and 2) the aneuploid peak represented at least 2.5% of the cells in the biopsy specimen (43). For the analyses described in this table, a diagnosis of aneuploidy was assigned only if the DNA content of the non-diploid population was outside the range of 3.85–4.1N.

¶Because the cancer incidence among persons with Barrett's esophagus who meet the diagnostic criteria for aneuploidy but have a DNA content in the range of >2.0N to 2.7N has recently been found to be similar to that of persons with normal DNA content (42), a second definition of aneuploidy was used in which aneuploidy was diagnosed only if the DNA content of the non-diploid population was greater than 2.7N and the other criteria for aneuploidy were met.

whose entry into the cohort coincided with the collection of blood and tissue samples.

Among those with measured serologic levels of other micronutrients (alpha-tocopherol, ascorbic acid, alpha-carotene, beta-carotene, and lycopene), adjustment for those levels had little effect on the associations between serum selenium levels and the biologic markers. For example, the OR for the association between serum selenium levels (upper three quartiles versus lowest quartile) and high-grade dysplasia was 0.3, regardless of whether alpha-tocopherol (95% CI = 0.1 to 0.8), ascorbic acid (95% CI = 0.1 to 1.0), or beta-carotene (95% CI = 0.1 to 0.9) was also included in the statistical model.

DISCUSSION

Participants in the Seattle Barrett's Esophagus Project with serum selenium levels in the upper three quartiles ($>1.5 \mu\text{M}$) had a statistically significantly lower risk of 17p (p53) LOH, aneu-

ploidy, and high-grade dysplasia than participants with levels in the lowest quartile. By contrast, serum selenium levels were not associated with reduced risk of 9p (p16) LOH. The distribution of serum selenium levels among the participants in our study closely resembled that in a sample of the general population of the United States (47). Thus, serum selenium levels in the intermediate or high-normal range for U.S. residents (1.5–2.1 μM) may be associated with a reduced risk of esophageal adenocarcinoma among persons with Barrett's esophagus.

Our results are consistent with previous studies of the relationship between selenium and risk of esophageal cancer and gastric cardia adenocarcinoma (21,22,48). In the only randomized controlled trial of selenium as a single supplement published to date, persons who took supplements for a mean of 4.5 years had a lower risk of esophageal cancer of any histologic type than those who did not (relative risk [RR] = 0.3, 95% CI = 0.1 to 1.5) (21). Separate risk estimates for the effect of selenium on risk of esophageal adenocarcinoma were not re-

ported. In another randomized trial, residents of Linxian, China, who received a combination of selenium, alpha-tocopherol, and beta-carotene for 5 years had lower rates of gastric cardia adenocarcinoma than those who received the placebo (RR = 0.8, 95% CI = 0.7 to 1.0) (48). In a case-cohort study of the same Chinese population, higher levels of serum selenium were associated with a reduced risk of both esophageal squamous cell carcinoma and gastric cardia adenocarcinoma (22).

In the Linxian case-cohort study (22), the investigators observed an inverse relationship between serum selenium levels and cumulative incidence of esophageal squamous cell carcinoma and gastric cardia adenocarcinoma across the entire distribution of serum selenium levels. By contrast, our results suggest the existence of a threshold, at approximately 1.5 μM , beyond which further increases in serum selenium levels are not associated with further decreases in risk of progression toward esophageal adenocarcinoma. Our results do not conflict with those of the Linxian study (22), however, because if this threshold exists, it would not have been identified in their study because the participants' serum selenium levels were too low. (The 90th percentile of serum selenium levels among the Linxian case-cohort study participants was 1.18 μM .)

In a previous cross-sectional analysis of data collected from participants in the Seattle Barrett's Esophagus Project, a diet low in selenium was associated with a higher risk of increased 4N fraction, but low serum selenium levels were not (49). However, because only 45 participants were included in the analyses with serum selenium, the lack of association could be explained by insufficient statistical power.

In our study, serum selenium levels in the intermediate or high-normal range were not associated with a decreased risk of 9p (p16) LOH, a genetic abnormality commonly observed early in neoplastic progression in Barrett's esophagus (28) and which may be important in the expansion of clones with a proliferative advantage (27,50). By contrast, higher serum selenium levels were associated with a decreased risk of 17p (p53) LOH, increased 4N fraction, aneuploidy, and high-grade dysplasia; the latter three abnormalities occur at approximately the same time in neoplastic progression as, or subsequent to, 17p (p53) LOH (14,26,28). Thus, selenium might reduce cancer risk among persons with Barrett's esophagus by preventing the inactivation of p53 or by preventing further neoplastic progression after p53 has been inactivated. Our findings are consistent with laboratory investigations showing that selenium can eliminate preneoplastic abnormalities in animals (51) and induce apoptosis in p53-deficient cancer cells (52).

Our study has several strengths. Because selenium in the food sources generally consumed by U.S. residents has a whole-body half-life of more than 200 days (53), information about selenium exposure over a period of several months can be gathered by measuring the concentration of selenium in a single sample of serum. We examined the effect of selenium on several validated and novel markers of esophageal adenocarcinoma risk, including increased 4N fraction, 17p (p53) LOH, aneuploidy, aneuploidy greater than 2.7N, and high-grade dysplasia, all of which have been shown to be predictive of progression to esophageal adenocarcinoma (25,26,42). Although 9p (p16) LOH has not been validated as a marker of esophageal adenocarcinoma risk in prospective studies, substantial evidence suggests that inactivation of p16 is a key event in the progression from Barrett's metaplasia to esophageal adenocarcinoma (27,28,50). Finally,

we examined whether any of a large number of potential confounders could explain the observed associations between serum selenium levels and the biologic markers of esophageal adenocarcinoma risk and found that they could not.

In interpreting our study, some limitations related to its design should be considered. First, because this was a cross-sectional analysis with biologic markers, our results do not establish a causal relationship between low serum selenium levels and increased risk of esophageal adenocarcinoma, but instead, pose an important hypothesis that merits further investigation. Second, by evaluating the relationship between serum selenium levels and several biologic markers, we were testing multiple hypotheses simultaneously, which increases the probability that some of the statistically significant associations occurred by chance. Third, information on several potential confounders (ascorbic acid, alpha-tocopherol, alpha-carotene, beta-carotene, lycopene) was available for only half the participants. However, within this subset of the study population, none of the variables was observed to confound the associations between levels of serum selenium and the biologic markers. Fourth, because current serum selenium levels are likely to be correlated with past levels, it is not possible to determine from our data whether higher levels over the past few months or over the past several years are associated with a reduced risk of markers associated with progression toward adenocarcinoma.

If the results of our study are confirmed in longitudinal studies and randomized controlled trials, the public health impact could be substantial. The incidence of esophageal adenocarcinoma has risen rapidly over the last three decades (1); by the 1990s, the incidence of esophageal adenocarcinoma had exceeded 10 cases per 100 000 person-years among Caucasian men aged 60 years or older (7). The increasing incidence of esophageal adenocarcinoma is of great concern because more than 90% of those affected will die of the disease (2,54). Because most esophageal adenocarcinomas arise in Barrett's epithelium (3), any therapy that reduces cancer risk among persons with Barrett's esophagus should decrease the incidence of esophageal adenocarcinoma. The potential health benefits of selenium-containing compounds merit further investigation not only because our results suggest that they could reduce the risk of esophageal adenocarcinoma but also because higher levels of selenium in blood or tissue have been associated with a reduced risk of several other cancers (22-24). Additionally, selenium has been demonstrated to inhibit the growth of neoplastic cells (52) and tissue (51).

In summary, we found that levels of serum selenium in the intermediate or high-normal range were associated with a reduced risk of several validated biologic markers of progression toward adenocarcinoma among persons with Barrett's esophagus. Persons with serum selenium levels above 1.5 μM generally had a similar risk of these biologic markers. Thus, a threshold may exist beyond which further increases in serum selenium levels have no additional benefit. Because serum selenium levels were not associated with 9p (p16) LOH, we speculate that selenium may act primarily at later stages of progression toward adenocarcinoma. Our results should be considered preliminary because they are based on cross-sectional analyses with biologic markers. Prospective studies are needed to define more clearly the relationship between selenium and risk of esophageal adenocarcinoma among persons with Barrett's esophagus and the stage, or stages, at which it may act.

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NOTES

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