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The Nutritional Prevention of Cancer: 400 Mcg Per Day Selenium Treatment

Mary E. Reid
Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, New York, USA

Anna J. Duffield-Lillico
Memorial Sloan Kettering, New York, New York, USA

Elizabeth Slate
Medical University of South Carolina, Charleston, South Carolina, USA

Nachimuthu Natarajan
Roswell Park Cancer Institute, Buffalo, New York, USA

Bruce Turnbull
Cornell University, Ithaca, New York, USA

Elizabeth Jacobs
Arizona Cancer Center, Tucson, Arizona, USA

Gerald F. Combs, Jr.
Grand Forks Human Nutrition Research Center, Grand Forks, North Dakota, USA

David S. Alberts
Arizona Cancer Center, Tucson, Arizona, USA

Larry C. Clark†

James R. Marshall
Roswell Park Cancer Institute, Buffalo, New York, USA

Nonexperimental studies suggest that individuals with higher selenium (Se) status are at decreased risk of cancer. The Nutritional Prevention of Cancer (NPC) study randomized 1,312 high-risk dermatology patients to 200-mcg/day of Se in selenized yeast or a matched placebo; selenium supplementation decreased the risk of lung, colon, prostate, and total cancers but increased the risk of nonmelanoma skin cancer. In this article, we report on a small substudy in Macon, GA, which began in 1989 and randomized 424 patients to 400-mcg/day of Se or to matched placebo. The subjects from both arms had similar baseline Se levels to those treated by 200 mcg, and those treated with 400-mcg attained plasma Se levels much higher than subjects treated with 200 mcg. The 200-mcg/day Se treatment decreased total cancer incidence by a statistically significant 25%; however, 400-mcg/day of Se had no effect on total cancer incidence.

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Address correspondence to Mary E. Reid, Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY 14263. Phone: 716-845-8444. FAX: 716-845-8487. E-mail: mary.reid@roswellpark.org.

† Deceased.

INTRODUCTION

An important contribution to Se chemoprevention is an experimental study initiated in 1983 under the leadership of Dr. Larry Clark. The Nutritional Prevention of Cancer (NPC) study (Clark et al., 1996), a double-blind, randomized trial of 200-mcg/day of Se in selenized yeast versus a matched yeast placebo, was conducted among 1,312 high risk skin cancer patients in the eastern and southeastern United States. Low levels of soil Se characterize this region, so the residents were expected to have low Se intake and status. The patients in this trial had been

diagnosed at a community dermatology clinic or VA hospital for nonmelanoma skin cancer (NMSC) within the year prior to randomization: 1 or more squamous cell cancers or 2 or more basal cell cancers. The rationale for using this population was that, with these participants at elevated risk of NMSC, a trial of Se supplementation should have substantial power to document any effect of Se in preventing NMSC.

The trial failed to decrease the recurrence of skin cancer. In fact, follow-up through the entire period of double-blinded treatment showed that Se supplementation increased the risk of NMSC and SCC recurrence by 20–40% (Duffield-Lillico et al., 2003). When cases that occurred during the first 2 yr after randomization were excluded, the risk estimates were attenuated to nonsignificant levels. Early in the trial, however, the 2 treatment groups began to exhibit distinct internal cancer risks. In 1993, the endpoints for the trial were expanded to include total cancer incidence and mortality and lung, colon, and prostate cancer incidence. The blinded phase of patient treatment and follow-up ended in February 1996. The results indicated that with supplementation, total cancer incidence was decreased by about 25%, total cancer mortality was decreased by over 40%, and prostate cancer incidence was decreased by approximately 50%. All of these effects were statistically significant. Although lung and colorectal cancer incidence were decreased by 30% and 54%, respectively, those effects were not statistically significant (Duffield-Lillico et al., 2002).

A surprising result from secondary analysis of the NPC study was an apparent pattern of effect modification: the adverse effect of Se supplementation on skin cancer recurrence was greater among those with higher baseline plasma Se levels. The protective effect of Se supplementation on total cancer incidence and mortality, and on the incidence of prostate, lung, and colon cancer, was greater among those with lower baseline plasma Se. Thus, the NPC results suggested that, among those with higher baseline plasma Se, the risk of NMSC was increased by Se treatment, and that no protection was afforded against solid tumors. This statistically significant effect modification suggests that the baseline level of Se is biologically significant. Alternatively, the pattern could suggest that the effect of Se levels is curvilinear, so that, among those with higher baseline Se, Se supplementation increased Se concentration to a level that increased the risk of skin cancer or eliminated protection against solid tumors.

One of the major NPC study sites was Macon, GA. In 1989, Dr. Clark opened a small adjunct study in Macon. Subjects were randomized to a dose of 400-mcg/day of Se as selenized yeast or a matched yeast placebo. Only 424 individuals were randomized to this arm and the study ended on February 1, 1996, when the 200-mcg arm of the trial was unblinded. This report will summarize the characteristics of these participants and evaluate the effect of this dose of Se supplementation on NMSC and total cancer incidence. Although the small sample and limited duration of this study limit statistical power, the incidences of NMSC and total cancer can be analyzed. There are too few cancers for site-specific analyses of internal cancer. Nonetheless, the NMSC and total cancer incidence results may be important because they may shed light on the possibility that there is a threshold to the protective effects of Se. It is also important to evaluate the likelihood that Se supplementation increases the risk of NMSC.

MATERIALS AND METHODS

The protocol for the overall NPC Study is described in detail by Clark (et al., 1996) and by Duffield-Lillico (et al., 2002). Briefly, participants were required to have a life expectancy of at least 5 yr and to have had no internal malignancies treated within the previous 5 yr. Exclusion criteria included a history of significant liver or kidney disorders. Although the use of VA hospitals meant that males comprised the majority of NPC participants, recruitment was gender-neutral.

The 400-mcg substudy recruitment began at the Macon GA dermatology site on September 12, 1989 and continued through April 3, 1992. Participants were randomized, double-blinded, to 400-mcg/day of Se in yeast or to an identical-appearing low Se yeast placebo. The high Se baker's yeast was provided by Nutrition 21 (La Jolla, CA) and by Cypress Systems (Fresno, CA). The total Se content of each batch of pills throughout the treatment period was verified in the laboratories of Dr. Gerald Combs and Dr. Ivan Palmer (South Dakota State University, Brookings, SD)(Olson et al., 1975). A speciation analysis of the selenized yeast used in the 200 mcg study was completed in 2004 (Larsen et al., 2004.) Selenomethioine was in the highest concentration, ranging from 54–62%; selenite concentrations did not exceed 1%; with the remainder of selenium in unidentified forms. Plasma Se concentrations were determined in Dr. Combs' laboratory by automated electrothermal atomic absorption spectrophotometry (Perkin Elmer 3030; Perkin Elmer Corp., Norwalk, CT). Quality control included multiple aliquots of human plasma as external control samples; a coefficient of variation of less than 7% was required for acceptance of results.

The baseline interview collected important sociodemographic and behavioral variables, including education, occupation, years farming, vitamin supplement use, use of sunscreen, cancer screening, alcoholic beverage consumption, smoking status, and years of smoking. In addition, medical and medication history was obtained at baseline and updated at each biannual visit. Patient medical records from each clinic were reviewed periodically to ensure the complete ascertainment and accuracy of the NMSC endpoint information.

At the end of the blinded treatment period on February 2, 1996, 46% of the 424 participants were still on treatment, 16% were off treatment but were still having routine dermatologic examinations, 28% had been censored for dermatologic but not other endpoints, and 10% had died. After a total of 2202 PY of follow-up, no participants had been lost to vital follow-up, although 2 subjects (1 in the Se group and 1 in the placebo group) declined to provide additional illness information. Participant-reported compliance indicated that 81% of placebo and 78% of

Se participants missed taking a pill less than twice a month.

One of the 400-mcg participants whose initial blood samples were drawn more than 4 days after randomization was excluded from analysis. Thus, all statistical analyses were based on data from the 423 participants with initial blood draws within 4 days of randomization. As in the 200-mcg arm of the NPC study, standard statistical significance tests were used to evaluate differences in baseline variables among individuals in the 2 treatment groups. For subjects who did not develop cancer, person-years (PY) of follow-up were computed from the date of randomization to that of death or February 1, 1996, which ever came first. PY of follow-up for cancer cases (internal or NMSC) were calculated through the date of the first category-specific post randomization primary cancer diagnosis documented in dermatologic or surgical pathology, operative or medical reports. Participants with multiple internal cancers at different sites were counted only once in the analysis of total cancer incidence.

Risk ratios (RR) and 95% confidence intervals (CI) were calculated using the ratio of the incidence density for the treatment groups for basal cell carcinoma (BCC), squamous cell carcinoma (SCC), total NMSC and for total internal cancer incidence. *P* values were derived from log rank tests. Supporting analyses included the calculation of Cox Proportional Hazards ratios (HR) and 95% CI, which allowed for adjustment by age at baseline (continuous), gender, and smoking status as covariates as appropriate.

The statistical association between NMSC incidence and baseline plasma Se concentration was also evaluated. Based on the distribution among the 423 subjects with valid values, baseline plasma Se concentrations were divided by the median and by tertiles. The effects of Se supplementation on BCC, SCC, and non-NMSC, were assessed within these subgroups of baseline plasma Se with the same techniques. We wanted to examine modification of the effect of Se treatment on internal cancers by baseline treatment, but the numbers of subjects and outcomes were too small for reliable analysis.

Unadjusted incidence rate ratios (RR) and 95% confidence intervals (CI) were calculated to determine the association between Se treatment and cancer incidence. In addition, adjusted Cox proportional hazard models (HR) were generated, adjusting for age, gender, and smoking at baseline. Stratified analyses were performed for gender and baseline selenium levels. Effect modification by baseline Se status was evaluated using the Mantel Haenszel test for heterogeneity in unadjusted models.

RESULTS

Table 1 describes the baseline characteristics of subjects randomized to the 400-mcg and 200-mcg doses from the Macon, GA clinic. Also included are the characteristics for the remaining NPC participants from all of the other sites combined. At the Macon GA clinic, 423 subjects were randomized in the 400-mcg experiment; 315 were randomized in the 200-mcg arm. These can be contrasted to the 935 randomized at the other research sites. The ages of Se and placebo patients in the 400-mcg groups were similar, and they were similar to those in the two 200-mcg groups. A significant difference ($P < .001$) distinguishing the Macon subjects from those in the other sites is that approximately 33% of the 400-mcg subjects and 40% of the 200-mcg subjects in Macon were female. At the other sites, only 20% were female. The experimental and control subjects in all 3 study groups were well matched in terms of body mass index (BMI). At the Macon site, the percentages of never smokers were larger and the percentages of smokers smaller than at the other sites. However, these differences were not statistically significant. The experimental and control groups were well balanced on plasma Se levels, between the 400 and 200-mcg groups in Macon and within the study overall. In general, any distributional differences were well within the bounds of what might be expected by chance.

Figure 1 illustrates the trajectories of plasma Se over the treatment period by treatment group and treatment site. The 400-mcg subjects were studied for approximately 6 yr. There was little difference among the 3 placebo groups in baseline levels or over time. In both Macon and in the other study sites, the 200-mcg treatment increased plasma Se from approximately 115 to nearly 200 ng/ml of Se; this took place within approximately 1 yr of treatment. The 400-mcg/day dose increased levels to a new equilibrium of approximately 250 ng/ml, also stabilizing after approximately 1 yr.

Table 2a describes total NMSC incidence by treatment group and by site. It can be seen that the Macon 200-mcg group experienced a substantial increase in the risk of NMSC (HR = 1.5, CI = 1.13–2.04, $P < .006$), compared to the smaller but significant increase in risk at the other sites (HR = 1.18, CI = 1.02–1.37, $P < .02$). The 400-mcg group, however, showed no evidence that the risk of NMSC was increased by treatment. A gender stratified analysis was performed. For females there was a significant inverse association between selenium supplementation in the 400-mcg arm and NMSC (41 cases, RR = .40, 95% CI = .20–.80) and a significant positive association in the other sites with the 200-mcg dose and NMSC (128 cases, RR = 1.46, 95% CI = 1.01–2.10). For men, supplementation consistently increased the incidence of NMSC. However, only in the 200-mcg arm were these risk estimates significant. The gender stratified analysis for total cancer incidence showed that only in men at the other sites (besides Macon) was Se supplementation associated with a significant reduction (RR = .68, 95% CI = .49–.92) (data not shown).

Table 2b summarizes the impact of treatment on SCC. In the Macon 200-mcg group, treatment was associated with nearly a doubling of risk (HR = 1.88, CI = 1.28–2.79); at the other sites, it appears Se supplementation nonsignificantly increased risk by 20% (HR = 1.18, CI = .95–1.46). This suggests that the previously reported effect of Se treatment on increasing NMSC incidence may be driven by the subjects from the Macon clinic. On the other hand, however, there is little evidence that the 400-mcg treatment dose had any impact on the risk of SCC. The

TABLE 1
Baseline Characteristics of Participants by Treatment Group and Location (Macon vs. Others)

	Macon (400 mcg)		Macon (200 mcg)		Other Sites (200 mcg)	
Variable	Se	Pla	Se	Pla	Se	Pla
Patients Randomized	210	213	154	161	467	468
Age in Years, mean (SD)	63.8 (10.6)	63.8 (10.1)	62.7 (10.9)	63.3 (10.9)	63.6 (9.9)	62.9 (9.6)
Gender, % male	66.2	68.1	59.1	61.5	79.2	79.9[1]
BMI, kg/m^2, mean (SD)	25.7 (3.8)	26.1 (3.9)	25.2 (3.6)	25.2 (4.1)	25.8 (4.0)	25.6 (4.2)
Smoking status, %						
Never	39.5	37.1	40.2	36.7	31.5	27.8
Former	36.7	40.8	36.4	38.5	40.7	40.4
Current	23.8	22.1	23.4	24.8	27.8	31.8
Plasma Selenium, ng/ml						
Mean (SD)	119.0 (24.3)	114.0 (18.1)	113.0 (20.9)	115.1 (18.2)	114.8 (23.2)	113.7 (22.5)
33rd.Centile	107.2	106.0	105.6	108.8	105.6	102.7
50th.Centile	113.8	113.2	114.8	116.0	113.2	111.6
67th. Centile	124.8	121.6	120.3	122.4	123.2	121.3

[1] Proportion of males in Macon-200 mcg versus Other Sites- 200 mcg was significantly different, X_2 p-value $<.001$.

point estimate of the relative risk was 1.05 and the confidence intervals span a wide range around 1.0.

Table 2c summarizes the impact of Se treatment on BCC. It can be seen that, for both the Macon 200-mcg group and for the other sites in the NPC Study, treatment was associated with nonsignificant increases in risk. On the other hand, the 400-mcg dose apparently imparted no increased risk. Again, the statistical boundaries about the point relative risk estimate of .95 could readily be expected on the basis of chance with no true effect.

Table 3 describes the effect of Se treatment on total cancer incidence. Total cancer incidence was lower in Macon than in the other 200-mcg sites (including the placebo groups). The results for subjects treated with 400-mcg are based on small numbers and must be carefully evaluated. Although they appear divergent from the results for the 200-mcg dose in Macon, the difference is not statistically significant. The 200-mcg dose effects in Macon are, however, relatively consistent with the results observed at the other clinical sites. At those other sites, total cancer incidence was decreased by statistically significant

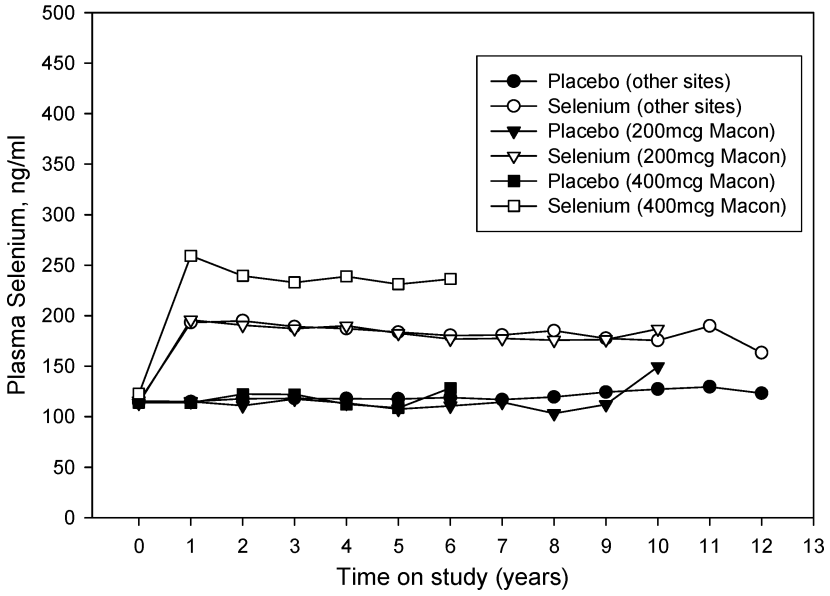

FIG. 1. Plasma Selenium by treatment group and by clinic site.

TABLE 2A
Total non-melanoma skin cancer cases by treatment group and by treatment site

	Cases					Adjusted[1]		
Treatment site	Se	Pla	RR	(95% CI)	P	HR	(95% CI)	P
Macon (400 mcg)	98	108	0.88	(0.66–1.16)	0.35	0.91	(0.69–1.20)	0.51
Macon (200 mcg)	99	80	1.49	(1.10–2.03)	.008	1.5	(1.13–2.04)	.006
Other sites (200mcg)	367	336	1.24	(1.07–1.45)	.004	1.18	(1.02–1.37)	.024

[1]Adjusted for age (continuous), smoking status (never, former, current) and gender.

margins. Figure 2 graphically presents the risk estimates and 95% confidence intervals for the 3 analysis groups for SCC and total cancer incidence.

Table 4 describes the impact of Se treatment on NMSC incidence by clinic site, stratified by levels of baseline plasma Se. It can be seen that, in the Macon 400-mcg study group, there is little evidence that baseline Se status modified the effect of treatment. On the other hand, in the 200-mcg group in Macon and at the other sites, there is evidence of increased risk among those at all levels of baseline plasma Se. Results adjusted for potential confounders are virtually identical to these (data not shown).

DISCUSSION

A number of nonexperimental studies have suggested that individuals with higher selenium (Se) status, measured in blood or toenails, are at decreased risk of a wide range of cancers. The association of Se status with breast cancer risk has been examined in several studies and does not appear to be strong (Willet et al., 1983; Overvad et al., 1991; Ghadirian et al., 2000; Van Noord et al., 1993; Van Noord et al., 1987; Hunter et al., 1990; Van't Veer et al., 1990; VanDen Brandt et al., 1994). There is stronger evidence for an association of Se status with cancer of the lung. Although some studies (Willet et al., 1983; Garland et al., 1995; Ratnasinghe et al., 2000) observed little evidence of an association (Knekt et al., 1998; Kabuto et al., 1994; Van Den Brandt et al., 1993), others observed decreased risk among those with higher status. Overall, there is evidence that higher Se status is associated with a decreased risk of colon cancer. Two studies (Nelson et al., 1995; Garland et al., 1995) found no evidence of protection, but these (Willet et al., 1983; Schober et al., 1987; Ghadirian et al., 2000) observed decreased risk among those with higher status. Van den Brandt (et al., 1993) observed an association between higher blood levels of Se and a decreased risk with higher stores for cancer of the colon, but not for cancer of the rectum.

The evidence for a protective association appears to be relatively strong for cancer of the prostate, with null reports by Knecht (et al., 1998) and Ghadirian (et al., 2000), but 5 prospective studies (Willet et al., 1983; Nomura et al., 2000; Coates et al., 1988; Yoshizawa et al., 1998; Helzlsouer 2000) showing decreased risk among those with higher Se status. The mixed results of other studies (Knekt et al., 1998; Salonen et al., 1984; Salonen et al., 1985; Reinhold et al., 1989; Fex et al., 1987; Kok et al., 1987; Nomura et al., 1987; Comstock et al., 1997; Mark et al., 2000; Burney et al., 1989; Glattre et al., 1989; Vinceti et al., 1998; Breslow et al., 1995; Helzlsouer et al., 1989; Zheng et al., 1993) are based in many instances on relatively small numbers of subjects and deal with a wide range of other relatively uncommon cancers. These nonexperimental data received some support from a large experimental study conducted in a region of China with low micronutrient intake and elevated esophageal and gastric cardia cancer risk (Blot et al., 1993). In that study, an antioxidant cocktail supplement, consisting of beta carotene, vitamin E, and Se, decreased cancer incidence significantly (Ratnasinghe et al., 2000; Mark et al., 2000).

Inasmuch as preventing NMSC recurrence was the primary study endpoint, it is important that these findings offer no evidence that Se supplementation, even at 400-mcg/day, increases

TABLE 2B
Squamous cell carcinoma incidence by treatment group and by treatment site

	Cases					Adjusted[1]		
Treatment site	Se	Pla	RR	(95%CI)	P	HR	(95%CI)	P
Macon (400 mcg)	56	53	1.05	(0.71–1.56)	0.80	1.05	(0.72–1.53)	0.79
Macon (200 mcg)	65	42	1.76	(1.18–2.66)	0.004	1.88	(1.28–2.79)	.001
Other sites (200 mcg)	179	154	1.20	(0.96–1.50)	0.09	1.18	(0.95–1.46)	0.14

[1]Adjusted for age (continuous), smoking status (never, former, current) and gender.

TABLE 2C
Basal cell carcinoma incidence by treatment group and by treatment site

	Cases					Adjusted[1]		
Treatment site	Se	Pla	RR	(95% CI)	P	HR	(95% CI)	P
Macon (400 mcg)	76	83	0.90	(0.65–1.24)	0.50	0.95	(0.69–1.29)	0.73
Macon (200 mcg)	75	69	1.20	(0.85–1.68)	0.28	1.22	(0.88–1.70)	0.23
Other sites (200 mcg)	332	305	1.16	(0.99–1.36)	0.06	1.12	(0.96–1.31)	0.16

[1]Adjusted for age (continuous), smoking status (never, former, current) and gender.

or decreases the risk of or protects against NMSC. Although supplementation by 400-mcg/day increased plasma levels substantially above the level that was associated with an increase of NMSC in the 200-mcg NPC study, this 400-mcg supplementation does not appear to have increased NMSC.

The data evidence little effect of Se supplementation at 400-mcg/day in altering total cancer incidence. Our estimate of Se's effect, based on the entire period of blinded supplementation in the entire study cohort, was that 200-mcg/day Se decreased total cancer incidence by a statistically significant 25% (Duffield-Lillico et al., 2002); our estimate based on the Macon cohort is that 400-mcg/day Se had no effect on total cancer incidence.

In the 200-mcg NPC cohort, those with lower baseline levels of Se were more likely than those with a higher baseline to have their risk of NMSC increased by Se supplementation. We observed no such pattern in our analysis of the 400-mcg cohort. The experience of the 200-mcg cohort was that any benefit of Se supplementation was concentrated in those with lower baseline plasma Se levels. We were not able to evaluate any such restriction of the potential benefit of Se supplementation in the 400-mcg study group. These data, therefore, offer no evidence that an intervention that increases selenium status above a fixed threshold is harmful in terms of NMSC risk. On the other hand, these data offer no evidence that an intervention

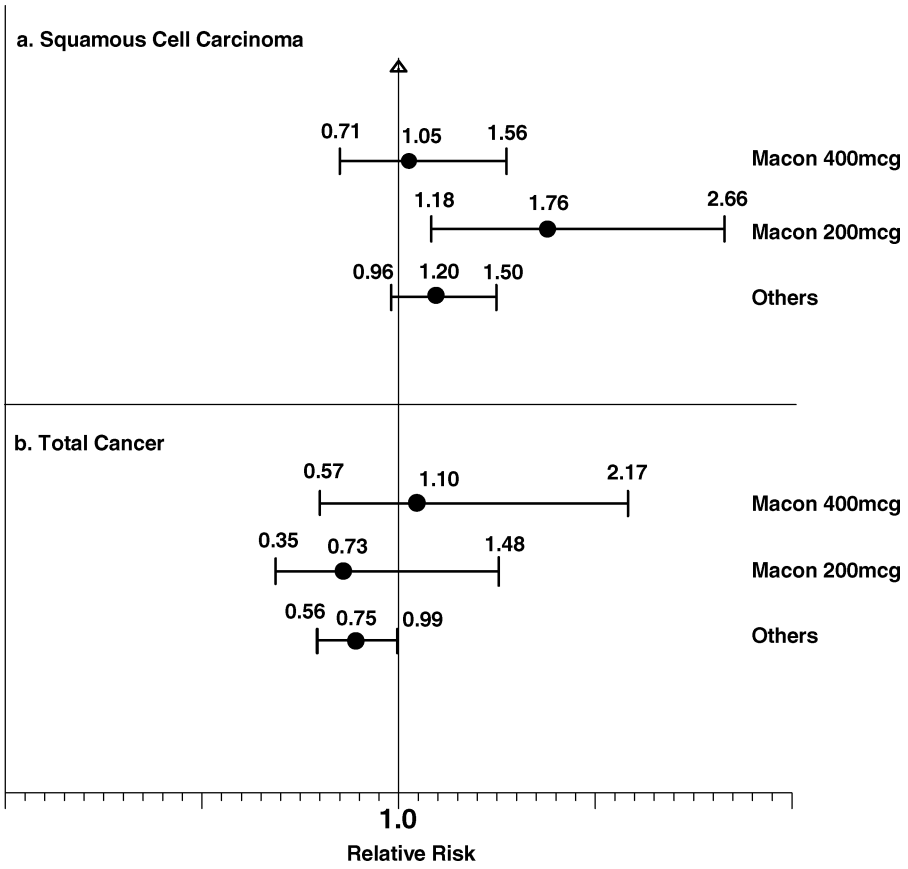

FIG. 2. Relative Risks and 95% confidence intervals for association Between Selenium Treatment and: a. Squamous Cell Carcinoma b. Total Cancer Incidence.

TABLE 3
Total internal cancer cases and incidence by treatment group and by study site

Site	Treatment	Cases (incidence/100PY) Se	Pla	RR (95%CI)
Macon	400 mcg	21 (1.90)	19 (1.72)	1.10 (0.57–2.17)
Macon	200 mcg	15 (1.25)	21 (1.72)	.73 (0.35–1.48)
Other	200 mcg	90 (2.60)	116 (3.40)	.75 (0.56–0.99)

that increases plasma selenium concentration from a baseline of approximately 120 ng/ml to nearly 300 ng/ml decreases internal cancer risk. This is not the first instance when a chemopreventive agent was most efficacious at a lower dose (Baron JA et al, 2003). In these results we not only see a lack of dose response; we see that a doubling of a dose that had significant effects on cancer incidence at several cancer sites had no apparent effect at all. The absence of a dose response in epidemiology raises questions as to the casual interpretations of this association. We understand that at these doses of selenium in patients who are nutritionally adequate at baseline, the effect of selenium on cancer would not be the result of a reduction in oxidative stress. The protection seen is strongest in the patients with the lowest plasma selenium at baseline also suggests that there may be a level at which selenium is most effective and above that level, supplementation has no effect.

An important limitation of this study is the uncertainty created by the small number of subjects who took part in the adjunct, 400-mcg study. On the other hand, the intense present interest in the chemopreventive potential of Se increases the im-

TABLE 4
Non-melanoma skin cancer incidence by clinic site and treatment group and by baseline plasma selenium

	Cases (incidence)* Se	Pla	RR	(95% CI)	P
Macon 400 mcg					
By median					
<113.2 ng/ml	50 (15.41)	55 (15.78)	0.98	(0.65–1.46)	0.91
>=113.2 ng/ml	48 (11.04)	53 (13.76)	0.80	(0.53–1.21)	0.27
By tertile					
<106.8 ng/ml	32 (16.33)	39 (17.23)	0.95	(0.57–1.55)	0.82
106.8–122.4 ng/ml	35 (13.00)	37 (13.82)	0.94	(0.57–1.53)	0.79
>122.4 ng/ml	31 (10.57)	32 (13.34)	0.79	(0.46–1.34)	0.36
Macon 200 mcg					
By median					
<114.8 ng/ml	51 (15.44)	42 (11.10)	1.39	(0.91–2.14)	0.11
>=114.8 ng/ml	48 (14.45)	38 (9.04)	1.60	(1.02–2.51)	0.03
By tertile					
<108.0 ng/ml	34 (16.16)	27 (12.60)	1.28	(0.75–2.21)	0.34
108.0–120.9 ng/ml	33 (13.5)	25 (8.78)	1.54	(0.89–2.70)	0.10
>120.9 ng/ml	32 (15.38)	28 (9.35)	1.64	(0.96–2.84)	0.06
Other sites 200 mcg					
By median					
<=112.4 ng/ml	182 (24.20)	165 (18.73)	1.29	(1.04–1.60)	0.02
>112.4 ng/ml	185 (26.04)	171 (21.79)	1.19	(0.97–1.48)	0.09
By tertile					
104.0 ng/ml	115 (25.17)	117 (19.75)	1.27	(0.98–1.66)	0.06
104.0–122.0 ng/ml	122 (23.89)	106 (18.32)	1.30	(1.00–1.71)	0.05
122.0 ng/ml	130 (26.26)	113 (22.85)	1.15	(0.89–1.49)	0.28

*Incidence calculated as case/100 years of follow-up.

portance of these results; they represent the largest study to date of relatively normal subjects treated with a Se dose as high as 400-mcg/day. We completed a much smaller study evaluating toxicity in Watchful Waiting (WW) patients treated with 1600 or 3200-mcg/day of selenized yeast; those subjects were studied for a much shorter period of 12 to 24 mo (Reid et al., 2004). This study of 400-mcg treatment, as in the shorter 1600 and 3200-mcg dose studies, involved supplementation at either the upper limit (400-mcg) or for greater than the limit recommended by the IOM (IN: Dietary Reference Intakes 2000). A complete toxicological evaluation of this dose is under review.

The data confirm that the 400-mcg doses affected plasma levels of Se. The subjects had similar baseline levels, and those treated with 400-mcg/day attained plasma Se levels much higher than the subjects in Macon and the other sites who were treated with 200-mcg/day.

In summary, recognizing the limitations of these findings because of the small sample size and the short study duration, we interpret these data as suggesting that Se supplementation at the 400-mcg level does not increase the risk of NMSC. On the other hand, we see little evidence in these data that a 400-mcg/day Se supplement imparts greater or even equal benefit to that seen with a 200-mcg/day supplement.

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REFERENCES

1. Baron JA, Cole BF, Sandler RS, Haile RW, Ahnen D, Bresalier R, McKeown-Eyssen G, Summers RW, Rothstein R, Burke CA, Snover DC, Church TR, Allen JI, Beach M, Beck GJ, Bond JH, Byers T, Greenberg ER, Mandel JS, Marcon N, Mott LA, Pearson L, Phil M, Saibil F, van Stolk RU: A randomized trial of aspirin to prevent colorectal adenomas. *NEJM* **348**(10), 891–9, 2003.
2. Blot WJ, Li JY, Taylor PR, Guo W, Dawsey S, Wang GQ, Yang CS, Zheng SF, Gail M, Li, GY, Yu Y, Liu BQ, Tangrea J, Sun YH, Liu F, Fraumeni JF, Zhang YH, and Li B: Nutrition Intervention Trials in Linxian, China: Supplementation with Specific Vitamin/Mineral Combinations, Cancer Incidence, and Disease-Specific Mortality in the General Population. *Journal of the National Cancer Institute* **85,** 1483–1492, 1993.
3. Breslow RA, Alberg AJ, Helzlsouer KJ, Bush TL, Norkus EP, Morris JS, Spate VE, and Comstock GW: Serological Precursors of Cancer: Malignant Melanoma, Basal and Squamous Cell Skin Cancer, and Prediagnostic Levels of Retinol, B-Carotene, Lycopene, a-Tocopherol, and Selenium. *Cancer Epidemiology Biomarkers & Prevention* **4,** 837–842, 1995.
4. Burney PG, Comstock GW, and Morris JS. Serologic precursors of cancer: serum micronutrients and the subsequent risk of pancreatic cancer. *Am J Clin Nutr* **49,** 895–900, 1989.
5. Clark LC. and et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. *JAMA* **24,** 1957–63, 1996.
6. Coates RJ, Weiss NS, Daling JR, Morris JS, and Labbe RF. Serum Levels of Selenium and Retinol and the Subsequent Risk of Cancer. *American Journal of Epidemiology* **128,** 515–523, 1988.
7. Comstock GW, Alberg AJ, Huang HY, Wu K, Burke AE, Hoffman SC, Norkus EP, Gross M, Cutler RG, Morris JS, Spate VL, and Helzlsouer KJ: The Risk of Developing Lung Cancer Associated with Antioxidants in the Blood: Ascorbic Acid, Carotenoids, a-Tocopherol, Selenium, and Total Peroxyl Radical Absorbing Capacity. *Cancer Epidemiology Biomarkers & Prevention* **6,** 907–916, 1997.
8. Duffield-Lillico AJ, Reid ME, Turnbull BW, Combs GF, Slate EH, Fischbach LA, Marshall JR, Clark LC, and For the Nutritional Prevention of Cancer Study Group: Baseline Characteristics and the Effect of Selenium Supplementation on Cancer Incidence in a Randomized Clinical Trial: A Summary Report of the Nutritonal Prevention of Cancer Trial. *Cancer Epidemiology Biomarkers & Prevention* **11,** 630–639, 2002.
9. Dietary reference intakes, vitamin C, vitamin E, selenium, and b-carotene and other carotenoids: overview, antioxidant definition, and relationship to chronic disease. 2000. Washington, DC, National Academy Press.
10. Duffield-Lillico AJ, Slate EH, Reid ME, Turnbull BW, Wilkins PA, Combs GF, Park HK, Gross EG, Graham GF, Stratton MS, Marshall JR, Clark LC, and For the Nutritional Prevention of Cancer Study Group Selenium. Supplementation and Secondary Prevention of Nonmelanoma Skin Cancer in a Randomized Trial. *Journal of the National Cancer Institute* **95,** 1477–1481, 2003.
11. Fex G, Pettersson B, and Akesson B. Low Plasma Selenium as a Risk Factor for Cancer Death in Middle-Aged Men. *Nutrition and Cancer* **10,** 221–229, 1987.
12. Garland, M, Morris JS, Stampfer MJ, Colditz GA, Spate VL, Baskett CK, Rosner B, Speizer FE, Willett WC, and Hunter DJ. Prospective Study of Toenail Selenium Levels and Cancer Among Women. *Journal of the National Cancer Institute* **87,** 497–505, 1995.
13. Ghadirian P, Maisonneuve P, Perret C, Kennedy G, Boyle P, Krewski D, and Lacroix A.: A Case-Control Study of Toenail Selenium and Cancer of the Breast, Colon, and Prostate.*Cancer Detection & Prevention* **24,** 305–313, 2000.
14. Glattre E, Thomassen Y, Thoresen SO, and et al.: Prediagnostic serum selenium in a case-control study of thyroid cancer. *International Journal of Epidemiology* **18,** 45–49, 1989.
15. Helzlsouer KJ, Comstock GW, and Morris JS: Selenium, Lycopene, a-Tocopherol, B-Carotene, Retinol, and Subsequent Bladder Cancer. *Cancer Research***49,** 6144–6148, 1989.
16. Helzlsouer KJ, Huang HY, Alberg AJ, Hoffman S, Burke A, Norkus EP, Morris JS, and Comstock GW: Association Between a-Tocopherol, y-Tocopherol, Selenium, and Subsequent Prostate Cancer.*Journal of the National Cancer Institute* **92,** 2018–2023, 2000.
17. Hunter DJ, Morris JS, Stampfer MJ, Colditz GA, Speizer FE, and Willett WC: A Prospective Study of Selenium Status and Breast Cancer Risk. *Journal of the American Medical Association* **264,** 1128–1131, 1990.
18. Kabuto M, Imai H, Yonezawa C, Neriishi K, Akiba S, Kato H, Suzuki T, Land CE, and Blot WJ: Prediagnostic Serum Selenium and Zinc Levels and Subsequent Risk of Lung and Stomach Cancer in Japan. *Cancer Epidemiology Biomarkers & Prevention* **3,** 465–469, 1994.
19. Knekt P, Marniemi J, Teppo L, Heliovaara M, and Aromaa, A: Is Low Selenium Status a Risk Ractor for Lung Cancer? American Journal of Epidemiology, **148,** 975–982, 1998.
20. Kok FJ, De Bruijn AM, Hofman A, Vermeeren R, and Valkenburg HA. Is Serum Selenium a Risk Factor for Cancer in Men Only? American Journal of Epidemiology, **125,** 12-16, 1987.
21. Larsen EH, Hansen M, Paulin H, Moesgaard S, Reid ME, Rayman M. Speciation and bioavailability of selenium in yeast-based intervention agents used in cancer chemoprevention studies. *JAOAC* **87**(1), 225–231, 2004.
22. Mark SD, Qiao YL, Dawsey SM, Wu, YP, Katki H, Gunter EW, Fraumeni JF, Blot WJ, Dong ZW, and Taylor PR: Prospective Study of Serum Selenium Levels and Incident Esophageal and Gastric Cancers. *Journal of the National Cancer Institute* **92,** 1753–1763, 2000.
23. Nelson RL, Davis FG, Sutter E, Kikendall JW, Sobin LH, Milner JA, and Bowen PE. Serum Selenium and Colonic Neoplastic Risk. *Diseases of the Colon & Rectum* **38,** 1306–1310, 1995.

24. Nomura A, Heilbrun LK, Morris JS, and Stemmermann GN: Serum Selenium and the Risk of Cancer, by Specific Sites: Case-Control Analysis of Prospective Data. *Journal of the National Cancer Institute* **79,** 103–108, 1987.
25. Nomura AM, Lee J, Stemmermann GN, and Combs GF. Serum Selenium and Subsequent Risk of Prostate Cancer. *Cancer Epidemiology Biomarkers & Prevention* **9,** 883–887, 2000.
26. Overvad K, Gron P, Langhoff O, Tarp U, Foldspang A, and Thorling EB. Selenium in Human Mammary Carcinogenesis: A Case-Referent Study. *European Journal of Cancer* **1,** 27–30, 1991.
27. Olson OE, Palmer IS, and Cary EE. Modification of the official fluorometric method for selenium in plants. *Journal Assoc Off Anal Chem* **58,** 117–126, 1975.
28. Ratnasinghe D, Tangrea JA, Forman MR, Hartman T, Gunter EW, Qiao YL, Yao SX, Barett MJ, Giffen CA, Erozan Y, Tockman MS, and Taylor PR: Serum Tocopherols, Selenium and Lung Cancer Risk Among Tin Miners in China.*Cancer Causes and Control* **11,** 129–135, 2000.
29. Reid ME, Marshall JR, Duffield-Lillico AJ, Stratton S, Natarajan N, and Fakih M. A Report of High Dose Selenium Supplementation Response and Toxicities. *Journal of Trace Elements in Medicine & Biology* **18,** 69–74, 2004.
30. Reinhold U, Biltz H, Bayer W, and Schmidt KH. Serum selenium levels in patient with malignant melanoma. *Acta Dermatol-Venerologica* **69,** 132–136, 1989.
31. Salonen JT, Alfthan G, Huttunen JK, and Puska P. Association Between Serum Selenium and the Risk of Cancer. *American Journal of Epidemiology* **120** 342–349, 1984.
32. Salonen JT, Salonen R, Lappetelainen R, Maenpaa PH, Alfthan G, and Puska P. Risk of Cancer in Relation to Serum Concentrations of Selenium and Vitamins A and E: Matched Case-Control Analysis of Prospective Data. *British Medical Journal* **290,** 417–420 1985.
33. Schober SE, Comstock GW, Helsing KJ, Salkeld RM, Morris JS, Rider AA, and Brookmeyer R. Serologic Precursors of Cancer I. Prediagnostic Serum Nutrients and Colon Cancer Risk. *American Journal of Epidemiology* **126,** 1033–1041, 1987.
34. Van Den Brandt PA, Goldbohm RA, Van't Veer P, Bode P, Dorant E, Hermus RJJ, and Sturmans F. A Prospective Cohort Study on Selenium Status and the Risk of Lung Cancer. *Cancer Research* **53,** 4860–4865, 1993.
35. Van Den Brandt PA, Goldbohm RA, Van't Veer P, Bode P, Dorant E, Hermus RJJ, and Sturmans F. A Prospective Cohort Study on Toenail Selenium Levels and Risk of Gastrointestinal Cancer. *Journal of the National Cancer Institute* **85,** 224–229, 1993.
36. Van Den Brandt PA, Goldbohm, RA, Van't Veer P, Bode P, Dorant E, Hermus RJ, and Sturmans F. Toenail Selenium Levels and the risk of Breast Cancer. *American Journal of Epidemiology* **140,** 20–26, 1994.
37. Van Noord PA, Collette HJ, Maas MJ, and De Waard F. Selenium Levels in Nails of Premenopausal Breast Cancer Patients Assessed Prediagnostically in a Cohort-Nested Case-Referent Study Among Women Screened in the DOM Project. *International Journal of Epidemiology* **16,** 318–322, 1987.
38. Van Noord PA, Maas MJ, Van Der Tweel I, and Collette C. Selenium and the Risk of Postmenopausal Breast Cancer in the DOM Cohort. *Breast Cancer Research & Treatment* **25,** 11–19. 1993.
39. Van't Veer P, Van der Wielen RP, Kok FJ, Hermus RJ, and Sturmans F. Selenium in Diet, Blood, and Toenails in Relation to Breast Cancer: A Case-Control Study. *American Journal of Epidemiology* **131,** 987–994, 1990.
40. Vinceti M, Rovesti S, Gabrielli C, Marchesi C, Bergomi M, Martini M, and Vivoli G. Cancer Mortality in a Residential Cohort Exposed to Environmental Selenium Through Drinking Water. *Journal of Clinical Epidemiology* **48,** 1091–1097, 1995.
41. Vinceti M, Rothman KJ, Bergomi M, Borciani N, Serra L, and Vivoli G. Excess Melanoma Incidence in a Cohort Exposed to High Levels of Environmental Selenium. *Cancer Epidemiology Biomarkers & Prevention* **7,** 353–357, 1998.
42. Willett WC, Morris JS, Pressel S, Taylor JO, Polk BF, Stampfer MJ, Rosner B, Schneider K, and Hames CG. Prediagnostic Serum Selenium and Risk of Cancer. *Lancet* **2,** 130–134, 1983.
43. Yoshizawa K, Willett WC, Morris SJ, Stampfer MJ, Spiegelman D, Rimm EB, and Giovannucci E. Study of Prediagnostic Selenium Level in Toenails and the Risk of Advanced Prostate Cancer. *Journal of the National Cancer Institute* **90,** 1219–1224, 1998.
44. Zheng W, Blot WJ, Diamond EL, Norkus EP, Spate V, Morris JS, and Comstock GW. Serum Micronutrients and the Subsequent Risk of Oral and Pharyngeal Cancer. *Cancer Research* **53,** 795–798, 1993.