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Original Contribution

Effects of Selenium Supplementation on Cardiovascular Disease Incidence and Mortality: Secondary Analyses in a Randomized Clinical Trial

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Despite the documented antioxidant and chemopreventive properties of selenium, studies of selenium intake and supplementation and cardiovascular disease have yielded inconsistent findings. The authors examined the effect of selenium supplementation (200 μg daily) on cardiovascular disease incidence and mortality through the entire blinded phase of the Nutritional Prevention of Cancer Trial (1983–1996) among participants who were free of cardiovascular disease at baseline (randomized to selenium: $n = 504$; randomized to placebo: $n = 500$). Selenium supplementation was not significantly associated with any of the cardiovascular disease endpoints during 7.6 years of follow-up (all cardiovascular disease: hazard ratio (HR) = 1.03, 95% confidence interval (CI): 0.78, 1.37; myocardial infarction: HR = 0.94, 95% CI: 0.61, 1.44; stroke: HR = 1.02, 95% CI: 0.63, 1.65; all cardiovascular disease mortality: HR = 1.22, 95% CI: 0.76, 1.95). The lack of significant association with cardiovascular disease endpoints was also confirmed when analyses were further stratified by tertiles of baseline plasma selenium concentrations. These findings indicate no overall effect of selenium supplementation on the primary prevention of cardiovascular disease in this population.

antioxidants; cardiovascular diseases; clinical trials; primary prevention; risk; selenium

Abbreviations: CI, confidence interval; HR, hazard ratio; NPC, Nutritional Prevention of Cancer.

The role of oxidative stress in atherogenesis has been established by a large body of experimental research with animal models and epidemiologic data in humans (1–4). Consequently, several antioxidants have been investigated as possible preventive agents of cardiovascular disease. However, observational studies on the effects of antioxidants have produced inconsistent results, and clinical trials have been mostly negative to date (5–7). Selenium, which is an essential component of a number of enzymes with antioxidant functions, has drawn particular interest for its potential role in the prevention of chronic disease based on its documented chemopreventive properties (8–11). In the past two decades, a number of observational studies have examined the association between selenium status and risk of cardiovascular disease, especially coronary heart disease (12–20). Although some of these studies suggest that low selenium status may represent a risk factor for cardiovascular disease, the evidence for a role of selenium in cardiovascular disease prevention remains controversial and inconclusive (9, 21, 22).

Despite the large number of observational studies, very few clinical trials have examined the efficacy of selenium

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supplementation in cardiovascular disease prevention; most have produced negative results (23–27). To our knowledge, there have been no randomized, placebo, controlled clinical trials to date that have specifically tested the relation between selenium supplementation alone and the risk of cardiovascular disease. The present report examined the effect of selenium supplementation on primary prevention of cardiovascular disease in the Nutritional Prevention of Cancer (NPC) Trial, a double-blind, randomized, clinical trial designed primarily to evaluate the efficacy of selenium supplementation in skin cancer prevention (28). Specifically, cardiovascular disease incidence and mortality were assessed as secondary endpoints through the entire blinded phase of the trial (1983–1996) among participants who were free of cardiovascular disease at baseline ($n = 1{,}004$).

MATERIALS AND METHODS

Study design and participants

The rationale, design, and methods of the NPC Trial have been described in detail in the original report by Clark et al. (28). Briefly, this study was a double-blind, randomized, placebo, controlled trial conducted among 1,312 participants recruited in 1983–1991 from seven dermatology clinics in low-selenium areas of the eastern United States. Randomization was blocked by time and stratified by clinic. Subjects were eligible if they had confirmed histories of nonmelanoma skin cancers within the year prior to randomization, had an estimated 5-year life expectancy, and had had no reported internal cancer within the previous 5 years. Exclusion criteria included a history of significant liver or kidney disorders. Although recruitment was gender neutral, approximately three fourths of the participants were male, and 1,312 of the 1,316 total subjects who were recruited were successfully randomized. At the end of the blinded period of treatment on February 1, 1996, no participants were lost to vital follow-up, generating a total of 9,301 person-years of follow-up. Participant-reported compliance indicated that 79.3 percent of participants (80.3 percent in the placebo group and 78.4 percent in the selenium group) had missed taking a pill less than twice a month (29).

Only participants with a valid baseline selenium value drawn from 4 days before to 4 days after the date of randomization ($n = 1{,}250$ of 1,312 total subjects) were included in the analysis. However, there were no significant differences in the baseline characteristics between the total NPC Trial cohort of 1,312 participants and the subsample of 1,250 participants with valid baseline selenium levels.

The main findings of this report focus on the 1,004 participants out of 1,250 total subjects who were free of cardiovascular disease at baseline (504 from the selenium treatment group and 500 from the placebo group).

Prevalent cardiovascular disease at baseline was ascertained by a self-reported diagnosis of myocardial infarction, angina, stroke, coronary artery bypass graft surgery, percutaneous transluminal coronary angioplasty, or carotid endarterectomy prior to randomization with subsequent evaluation of medical records ($n = 246$).

Clinical examination and laboratory methods

The intervention agent was 200 μg of selenium per day, supplied in a 0.5-g high-selenium baker's yeast tablet provided by Nutrition 21 (La Jolla, California) through 1995 and by Cypress Systems (Fresno, California) thereafter, or a yeast placebo. The selenium content of each batch of pills was determined in the laboratories of Dr. G. F. Combs, Jr., and of Dr. I. S. Palmer (South Dakota State University, Brookings, South Dakota) by use of the diaminonaphthalene-fluorimetric procedure after nitric-perchloric acid digestion (30). The plasma selenium concentration was determined in the laboratory of Dr. Combs by an automated electrothermal atomic absorption spectrophotometer (Perkin-Elmer model 3030; Perkin-Elmer Corporation, Norwalk, Connecticut) equipped with an electrodeless discharge lamp and automatic Zeeman-effect background correction. Quality control included multiple aliquots of human plasma as external control samples. A coefficient of variation of less than 7 percent (for duplicate analyses) was the criterion for acceptance (31).

Participants visited their respective clinics biannually to provide blood samples and to report new illnesses and medications. Patients' medical records from each clinic were periodically reviewed to ascertain information from both study and nonstudy visits to ensure the completeness and accuracy of the information. For participants who became inactive, annual contact was attempted by use of the National Death Index and ChoicePoint (Alpharetta, Georgia; formerly Equifax, Inc.) to determine vital status and to identify new illness diagnoses. At the baseline interview, information on sociodemographic, anthropometric, and behavioral variables, including education (number of years of schooling: 0–18), body mass index (calculated as weight (kg)/height $(m)^2$), use of vitamin supplements, numbers of alcoholic drinks per day, smoking status (never, former, current), and pack-years of smoking, was collected from the participants.

Cardiovascular disease ascertainment and follow-up

Cardiovascular disease events were drawn from participants who had a new diagnosis of fatal and nonfatal myocardial infarction, coronary artery bypass graft surgery, or percutaneous transluminal coronary angioplasty, collectively called coronary heart disease; had a diagnosis of stroke or carotid endarterectomy, collectively called cerebrovascular accidents; or died from cardiovascular disease through the entire blinded phase of the trial (September 15, 1983, to February 1, 1996). For combined endpoints (e.g., all cardiovascular disease, all coronary heart disease, and all cerebrovascular accidents), only the first cardiovascular disease event after randomization was registered as an endpoint. To verify a reported cardiovascular disease event or procedure, whether it was pre- or postrandomization, we applied standard medical record requirements. Multiple sources of information were required to fully document a report. Research nurses requested medical and surgical records from physicians and hospitals for documentation. Review and coding of all records were blinded to treatment

group status. Death certificates were requested and recorded on all deceased patients. A nosologist coded the death certificates, recording the underlying cause of death. Review and coding of all death certificates were also completed in a blinded manner by use of the *International Classification of Diseases* (32, 33).

Person-years of follow-up were accrued from the date of randomization, as the start date, to the date of first event, the date of death, or the end of the blinded period of the trial (February 1, 1996) if free of a cardiovascular disease event.

Statistical analysis

For continuous and categorical variables, respectively, *t* tests and χ^2 tests were conducted to determine the statistical significance of any differences in the distribution of baseline variables between treatment groups. Tests for interaction with baseline covariates (e.g., age, gender, education, body mass index, smoking, alcohol, and use of vitamin supplements) were not statistically significant. Cardiovascular disease incidence and mortality data were statistically analyzed by calculating hazard ratios and 95 percent confidence intervals, using the Cox proportional hazard model, which allowed adjustment for age (continuous variable), gender, and smoking status (never, former, current) at baseline as covariates. We decided a priori to adjust for these cardiovascular disease risk factors regardless of whether they differed between treatment groups.

The statistical association between cardiovascular disease incidence and mortality and concentrations of baseline plasma selenium was also assessed. Based on the distribution among the 1,004 participants with valid selenium values and free of cardiovascular disease at baseline, baseline plasma selenium concentrations were divided by tertiles ($\leq$105.2 ng/ml, 105.3–122.4 ng/ml, and $>$122.4 ng/ml).

All analyses were implemented by use of the Statistical Package for Social Sciences (SPSS-12.0; SPSS, Inc., Chicago, Illinois).

RESULTS

Selected baseline characteristics of participants at randomization, by treatment group, are displayed in table 1 for individuals who were free of cardiovascular disease at baseline (n = 1,004). The treatment groups were well balanced for all the evaluated baseline characteristics, with no significant differences in the distribution of either continuous or categorical variables between treatment groups.

Table 2 shows the adjusted hazard ratio estimates for cardiovascular disease incidence and mortality, as well as for non-cardiovascular disease and all-cause mortality among participants without prevalent cardiovascular disease at baseline. Throughout the entire blinded phase of the trial (1983–1996), a total of 199 cardiovascular disease events (103 selenium- and 96 placebo-related events) and 71 cardiovascular disease deaths (40 selenium- and 31 placebo-related deaths) were accrued during a mean follow-up of 7.6 (standard deviation: 2.8) years. Myocardial infarction was the most frequent cardiovascular disease event (n = 84), followed by stroke (n = 67). No statistically significant association was found between treatment assignment and overall or specific cardiovascular disease endpoints (all cardiovascular disease: hazard ratio (HR) = 1.03 (95 percent confidence interval (CI): 0.78, 1.37); total myocardial infarction: HR = 0.94 (95 percent CI: 0.61, 1.44); stroke: HR = 1.02 (95 percent CI: 0.63, 1.65); all cardiovascular disease mortality: HR = 1.22 (95 percent CI: 0.76, 1.95)).

TABLE 1. Baseline characteristics of participants at randomization by treatment group, Nutritional Prevention of Cancer Trial, 1983–1991*,†

Variable	Selenium	Placebo
Participants randomized (no.)	504	500
Age, years	62.5 (10.6)	62.1 (10.3)
Education, years	13.0 (3.3)	13.0 (3.4)
Body mass index, kg/m^2	25.5 (3.9)	25.4 (4.2)
Follow-up, years	7.6 (2.7)	7.5 (2.8)
Baseline plasma selenium, ng/ml	113.3 (21.6)	113.8 (22.1)
Alcohol, drinks/day	0.5 (1.3)	0.4 (0.9)
Gender, males (%)	71.0	71.2
Smoking status (%)		
Never	35.3	32.4
Former	38.1	37.6
Current	26.6	30.0
Pack-years of smoking	55.1 (39.0)	52.8 (37.3)
Use of vitamin supplements, yes (%)	38.4	41.3

* Values are given as mean (standard deviation) unless stated; no difference between treatment groups was statistically significant ($p \leq 0.05$).

† There were 1,004 participants without prevalent cardiovascular disease at baseline.

Because previous literature indicates that selenium deficiency may be particularly associated with disease (9, 21), the effect of selenium supplementation on cardiovascular disease incidence and mortality was also assessed within subgroups defined by tertiles of baseline plasma selenium, among participants without prevalent cardiovascular disease at baseline (table 3). No statistically significant association between treatment assignment and overall or specific cardiovascular disease endpoints was found across tertiles of baseline plasma selenium.

The lack of significant association with cardiovascular disease endpoints was confirmed even in participants (n = 246) who reported prevalent cardiovascular disease at baseline (data not shown).

DISCUSSION

In this study, we examined the effect of a dietary supplementation with 200 μg of selenium daily on major cardiovascular disease events among individuals without history of cardiovascular disease at baseline (mean follow-up of

TABLE 2. Cardiovascular disease incidence by treatment group, Nutritional Prevention of Cancer Trial, 1983–1996*

Cardiovascular disease	Cases (no.)		Adjusted†		
	Selenium group	Placebo group	Hazard ratio	95% confidence interval	*p* value
All cardiovascular disease	103	96	1.03	0.78, 1.37	0.81
All coronary heart disease	63	59	1.04	0.73, 1.49	0.81
Total myocardial infarction	41	43	0.94	0.61, 1.44	0.77
Fatal myocardial infarction	9	8	1.08	0.42, 2.80	0.88
Nonfatal myocardial infarction	32	35	0.91	0.56, 1.47	0.69
Coronary artery bypass graft	15	11	1.30	0.59, 2.84	0.51
Percutaneous transluminal coronary angioplasty	7	5	1.36	0.43, 4.31	0.60
All cerebrovascular accidents	40	37	1.02	0.65, 1.59	0.94
Stroke	35	32	1.02	0.63, 1.65	0.92
Carotid endarterectomy	5	5	0.98	0.28, 3.39	0.97
Cardiovascular disease mortality	40	31	1.22	0.76, 1.95	0.41
Non-cardiovascular disease mortality	70	80	0.84	0.61, 1.17	0.31
All-cause mortality	110	111	0.95	0.73, 1.24	0.71

* There were 1,004 participants without prevalent cardiovascular disease at baseline.
† The 95% confidence intervals and *p* values were derived from the Cox proportional hazards model adjusted for age (continuous), gender, and smoking status (never, former, current) at randomization.

TABLE 3. Cardiovascular disease incidence by tertiles of baseline plasma selenium, Nutritional Prevention of Cancer Trial, 1983–1996*

Cardiovascular disease by selenium tertiles (ng/ml)	Cases (no.)		Adjusted†		
	Selenium group	Placebo group	Hazard ratio	95% confidence interval	*p* value
All cardiovascular disease	103	96	1.03	0.78, 1.37	0.81
≤105.2	40	40	1.15	0.74, 1.79	0.53
105.3–122.4	40	28	1.19	0.73, 1.94	0.47
>122.4	23	28	0.83	0.47, 1.45	0.52
All coronary heart disease	63	59	1.04	0.73, 1.49	0.81
≤105.2	25	23	1.24	0.70, 2.19	0.45
105.3–122.4	25	15	1.42	0.75, 2.70	0.28
>122.4	13	21	0.62	0.31, 1.25	0.18
Total myocardial infarction	41	43	0.94	0.61, 1.44	0.77
≤105.2	13	15	0.96	0.46, 2.03	0.92
105.3–122.4	18	14	1.12	0.55, 2.25	0.76
>122.4	10	14	0.72	0.32, 1.63	0.43
All cerebrovascular accidents	40	37	1.02	0.65, 1.59	0.94
≤105.2	15	17	1.02	0.51, 2.04	0.96
105.3–122.4	15	13	0.93	0.44, 1.97	0.85
>122.4	10	7	1.45	0.55, 3.85	0.45
Cardiovascular disease mortality	40	31	1.22	0.76, 1.95	0.41
≤105.2	15	8	2.07	0.88, 4.90	0.10
105.3–122.4	17	9	1.66	0.73, 3.77	0.22
>122.4	8	14	0.54	0.22, 1.29	0.16

* There were 1,004 participants without prevalent cardiovascular disease at baseline.
† The 95% confidence intervals and *p* values were derived from the Cox proportional hazards model adjusted for age (continuous), gender, and smoking status (never, former, current) at randomization.

7.6 years). Selenium supplementation was not significantly associated with any of the cardiovascular disease endpoints. Results were not altered by the examination of baseline plasma selenium concentrations. These findings indicate no overall effect of selenium supplementation on the primary prevention of cardiovascular disease.

Despite the large number of observational epidemiologic studies (12–20), very few intervention trials have examined the efficacy of selenium supplementation on cardiovascular endpoints; most of these have produced negative results (23–27). A significant increase in the high-density lipoprotein cholesterol:cholesterol ratio was observed in a small group of healthy individuals with an initial low-selenium status who received 96 μg of selenium daily for 2 weeks (23). In a double-blind, placebo, controlled study involving 81 patients with acute myocardial infarction who were taking selenium supplementation (100 μg daily) over a period of 6 months, four cardiac deaths were observed in the placebo group, whereas no patient died in the selenium-supplemented group. However, the difference between study groups was not statistically significant (24). Furthermore, no effect of selenium supplementation (200 μg daily) on platelet aggregation was found in a 6-week intervention trial involving a very small group of young healthy males (25). In a large nutrition intervention trial in a region of China with low-selenium status, a supplementation of selenium (50 μg daily) along with beta-carotene and vitamin E for 5 years was not significantly associated with mortality from cerebrovascular disease (26). More recently, findings from a double-blind primary prevention trial in a large cohort of French adults showed no effect of antioxidant supplementation including selenium (100 μg daily) on ischemic cardiovascular disease incidence after 7.5 years of follow-up (27).

The current study represents the only large randomized clinical trial so far that has examined the efficacy of selenium supplementation alone in the prevention of cardiovascular disease. Our results extend previous research based on smaller intervention trials focusing on cardiovascular risk factors. Our findings are consistent with those from previous studies that have shown no beneficial effect of selenium supplementation in combination with other antioxidants on different cardiovascular endpoints.

There are several limitations to this study. First, cardiovascular disease incidence and mortality were not primary endpoints of the NPC Trial (28). Therefore, our results must be cautiously interpreted, as they result from exploratory analyses, though from the largest randomized clinical trial available that has selenium only as the intervention. However, the ascertainment of the cardiovascular disease endpoints did not change throughout the entire blinded phase of the trial. In addition, the selected cardiovascular disease endpoints are all hard clinical outcomes and less subject to diagnostic misclassification. Second, although the incidence estimates were adjusted for potential confounders, such as age, gender, and smoking status, the lack of detailed information on unmeasured risk factors at baseline, such as blood pressure, serum cholesterol, and physical activity, is a potential limitation; however, randomization should minimize the likelihood of confounding by these factors as shown by the lack of significant differences in the evaluated baseline characteristics between treatment groups. A further limitation concerns the generalizability of these results considering the selected nature of the participants. In fact, the NPC Trial sample consisted of elderly individuals (mean age: 63.2 years) from low-selenium areas in the eastern United States who had a history of nonmelanoma skin cancer. It is possible that the selection of the usual late clinical endpoints in this cohort of elderly individuals could obscure the effect of selenium on the early stages of lesion development, limiting its efficacy in the primary prevention of cardiovascular disease (7). Conversely, the strengths of this study include the high compliance with the intervention, as indicated by the fact that the plasma selenium concentration of the placebo group remained constant throughout the trial, whereas the blood selenium levels in the selenium group were substantially higher than those in the placebo group (28).

In conclusion, this report adds important information to our knowledge of the role of selenium in cardiovascular disease prevention. Consistent with the findings from previously conducted clinical trials, our results indicate no overall benefit of supplementation by selenium alone in prevention of cardiovascular disease.

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Conflict of interest: none declared.

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