Selenium Supplementation and Lung Cancer Incidence: An Update of the Nutritional Prevention of Cancer Trial[1]

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Abstract

Interest in the chemopreventive effects of the trace element selenium has spanned the past three decades. Of >100 studies that have investigated the effects of selenium in carcinogen-exposed animals, two-thirds have observed a reduction in tumor incidence and/or preneoplastic endpoints (G. F. Combs and S. B. Combs, The Role of Selenium in Nutrition Chapter 10, pp. 413–462. San Diego, CA: Academic Press, 1986, and B. H. Patterson and O. A. Levander, Cancer Epidemiol. Biomark. Prev., *6:* 63–69, 1997). The Nutritional Prevention of Cancer Trial, a randomized clinical trial reported by Clark *et al.* (L. C. Clark *et al.*, JAMA, *276:* 1957–1963, 1996), showed as a secondary end point, a statistically significant decrease in lung cancer incidence with selenium supplementation. The adjusted hazard ratio (HR) was 0.56 [95% confidence interval (CI), 0.31–1.01; $P = 0.05$]. These results were based on active follow-up of 1312 participants.

This reanalysis used an extended Nutritional Prevention of Cancer Trial participant follow-up through the end of the blinded clinical trial on February 1, 1996. The additional 3 years added 8 cases to the selenium-treated group and 4 cases to the placebo group, and increased follow-up to 7.9 years. The relative risk of 0.70 (95% CI, 0.40–1.21; $P = 0.18$) is not statistically significant. Whereas the overall adjusted HR is not significant (HR = 0.74; 95% CI, 0.44–1.24; $P = 0.26$), and the HR for current and former smokers was not significant, the trend is toward a reduction in risk of incident lung cancer with selenium supplementation. In a subgroup analysis there was a nominally significant HR among subjects with baseline plasma selenium in the lowest tertile (HR = 0.42; 95% CI, 0.18–0.96; $P = 0.04$). The analysis for the middle and highest tertiles of baseline showed HRs of 0.91 and 1.25. The current reanalysis indicates that selenium supplementation did not significantly decrease lung cancer incidence in the full population, but a significant decrease among individuals with low baseline selenium concentrations was observed.

Introduction

Interest in the chemopreventive effects of the trace element selenium has spanned the last 3 decades. Of >100 studies that have investigated the effect of selenium on tumor burden in carcinogen-exposed animals, two-thirds have observed a reduction in tumor incidence and/or preneoplastic endpoints (1, 2).

Results from ecologic studies (3–6), epidemiological studies, human clinical intervention trials, and *in vitro* and *in vivo* animal models clearly support a protective role of selenium against cancer development (7). Although selenium compounds have been shown to suppress carcinogenesis in many animal models and cell culture systems, the mechanisms by which selenium may exert its chemopreventive activity still remain unclear.

Many prospective studies of serum selenium concentrations and lung cancer risk have been published. Most of the studies used a nested case-control approach (8–22), whereas two studies evaluated prediagnostic concentrations of selenium in toenail clippings and their association with lung cancer (23, 24). Knekt *et al.* (9, 22) found a significant inverse association between serum selenium and subsequent lung cancer occurrence in men within the cohort studied in the Finnish Mobile Health Examination Survey. However, this study showed no association between estimated selenium intake and lung cancer risk (25). A strong inverse association between toenail selenium level and lung cancer incidence in men and women was observed in a longitudinal observational study from the Netherlands (23). Other published studies (10, 18, 20, 21) suggested inverse trends in lung cancer risk with increasing selenium status but were nonsignificant because of small numbers of cases. Conversely, nonsignificant positive correlations between serum selenium and lung cancer risk have been observed (11, 17). Garland *et al.* (24) reported significantly lower toenail selenium concentrations among lung cancer case patients compared with control subjects. However, after controlling for smoking history, this association was reversed. Methodological issues, including the use of toenail selenium, must be considered.

Overall, observational data tend to show a nonsignificant inverse association between selenium concentrations and lung cancer. The more consistent findings of nested case-control studies warrant additional evaluation of the role of dietary selenium in lung cancer prevention.

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The NPC[4] reported by Clark *et al.* (26) represented the first randomized clinical trial to suggest a decrease in lung cancer incidence with selenium supplementation. This finding was an unanticipated secondary end point in a trial designed to prevent nonmelanoma skin cancer recurrence in men and women living in a United States region with low soil selenium. The unadjusted RR observed by Clark *et al.* was 0.54 (95% CI, 0.30–0.96; $P = 0.04$), and the adjusted HR was 0.56 (95% CI, 0.31–1.01; $P = 0.05$). These results were based on the active follow-up of 1312 participants followed through December 31, 1993. This reanalysis is based on an extended follow-up of NPC participants through the end of the blinded clinical trial on February 1, 1996, when all of the subjects were informed of their treatment group and all of the participants were offered the opportunity to take selenium. This extension of follow-up extended the mean treatment years on the trial from 4.5 to 7.9 years. In addition to evaluating the main effect of selenium supplementation on lung cancer incidence, this investigation will determine the role of baseline selenium status in mediating the effects of supplementation.

Materials and Methods

The methods of subject recruitment and follow-up have been reported previously in Clark *et al.* (26). Briefly, subjects were recruited from seven dermatology practices located in cities in low-selenium areas in the Eastern United States. Subjects had confirmed histories of nonmelanoma skin cancers within the year before study enrollment. All of the subjects had an estimated 5-year life expectancy and had no reported internal cancer within the previous 5 years. Subjects were randomized in a double-blind fashion to either 200 μg/day of selenium in 0.5-gram high selenium baker's yeast (Nutrition 21) or a yeast placebo. Randomization occurred between the years 1983 and 1991.

All of the lung cancer cases were rereviewed recently by a thoracic oncologist. These reviews were based on an updated database obtained on all of the cancers that included these additional years of follow-up data; only cases confirmed as primary lung cancers presenting during the 13-year study period were included in this analysis. Cases with multiple primary lung tumors, with the first tumor occurring 5 years before randomization, were not included even if the second primary lung cancer occurred during the course of the trial. Lung cancers were staged according to the International System for Staging Lung Cancer (27). Histology is divided into non-small cell lung carcinoma, small cell lung carcinoma, undifferentiated carcinoma, carcinoid tumors, and lung neoplasms that had only clinical diagnoses because no biopsy was performed. Non-small cell lung carcinoma included adenocarcinoma, squamous cell, large cell, mixed, and not otherwise specified. Lung cancers were stratified by treatment group. The most prevalent histologies were adenocarcinoma ($n = 14$), squamous cell ($n = 22$), and small cell carcinoma ($n = 11$). Approximately 40% of the cases from each treatment group were confirmed by pathology report, whereas medical record notes confirmed the remaining 60%. There were no significant differences between the treatment groups for histological categories or in the method of case confirmation. The staging of each tumor type by treatment group showed no significant differences or trends between the treatment groups.

PY of follow-up were calculated only on subjects with valid baseline plasma selenium values collected on the day of randomization, plus or minus 4 days. For subjects without lung cancer, PY extended through to February 1, 1996 or the date of death, whichever came first. Follow-up for cases of lung cancer was based on the date of diagnosis of lung cancer from (in order of priority) a pathology report, or surgical or medical report.

Plasma samples were collected at baseline and at each 6-month clinical follow-up visit. Total selenium content was measured by automated electrothermal atomic absorption spectrophotometer (Perkin-Elmer 3030; Perkin-Elmer Corp., Norwalk, CT). Only subjects with a valid baseline selenium value drawn ± 4 days from the date of randomization ($n = 1250$ of 1312 total subjects) were included in the analysis. Baseline plasma selenium was evaluated as a continuous and dichotomous variable and by tertiles.

At the baseline interview, smoking status (current, former, and never), numbers of cigarettes smoked per day, and years of smoking were ascertained. In addition, a thorough medical and medication history was taken at baseline and updated at each follow-up visit.

The statistical methods included comparison of categories and means to determine differences between the baseline characteristics of all of the NPC participants. Incident rate ratios and Cox proportional hazards models were also used. In addition, Kaplan-Meier and Nelson-Aalen cumulative survival curves with the value for the log-rank tests for significance were generated. The program STATA (28) was used to perform all of the statistical tests.

Results

Table 1 summarizes baseline characteristics of the NPC participants by treatment group. The total NPC sample ($n = 1312$) and the subsample of subjects with a valid baseline selenium level at randomization ($n = 1250$) are also presented. There were no significant differences between the baseline characteristics of the total NPC sample and the subsample with a valid baseline level. There were also no significant differences in the critical baseline characteristics between the treatment groups. Approximately 75% of the sample was male, and the mean age of subjects at randomization was 63 years. Mean baseline plasma selenium was ~114 ng/ml across the treatment groups, and the mean number of years of follow-up was 7.9. Smoking status differed slightly between the treatment groups, with a small but nonsignificant excess of current smokers in the placebo group. The restriction of the study sample to subjects with valid baseline selenium values eliminated 2 lung cancer cases from the reanalysis, bringing the total to 60 cases.

Table 2 shows the unadjusted RR and HR estimates from lung cancer from the 1983–1993 analysis published in 1996 (26), and the reanalysis for 1983–1996. The addition of 3 years of follow-up added 8 cases of lung cancer in the selenium-treated group and 4 cases in the placebo group. The RR for the reanalysis is 0.70 (95% CI, 0.40–1.21), $P = 0.18$, which is not statistically significant. The Cox proportional hazards model, which adjusted for age as a continuous variable and smoking status (never, former, and current) at randomization showed a nonsignificant HR of 0.74 (95% CI, 0.44–1.24), $P = 0.26$. Fig. 1 shows the overall cumulative incidence curve for lung cancer by treatment group; the P for the log-rank test is 0.17. Whereas the treatment group curves are not statistically different, they

[4] The abbreviations used are: NPC, Nutritional Prevention of Cancer Trial; CI, confidence interval; HR, hazard ratio; PY, person-years; Mtase, DNA cytosine-methyltransferase.

Table 1 Baseline characteristics of NPC subjects at randomization

Variable	Total NPC $n = 1312$	Valid baseline selenium $n = 1250$	Valid baseline selenium	
			Selenium supplemented group $n = 621$	Placebo supplemented group $n = 629$
Gender, males (%)	981 (74.8)	934 (75.0)	461 (74.0)	473 (75.0)
Age, mean (SD)	63.2 (10.1)	63.2 (10.0)	63.1 (10.2)	63.3 (9.9)
Years of follow-up (PY)	7.9 (2.6)	7.9 (2.6)	8.0 (2.5)	7.8 (2.6)
Baseline plasma selenium, ng/ml, mean (SD)	114.9 (23.0)	114.2 (22.0)	114.0 (22.6)	114.4 (21.5)
Baseline smoking status, *n* (%)				
Current	375 (28.6)	355 (28.4)	166 (26.7)	189 (30.1)
Former	514 (39.2)	497 (39.8)	246 (39.6)	251 (39.9)
Never	423 (32.2)	398 (31.8)	209 (33.7)	189 (30.1)
Age at smoking initiation, mean (SD)[a]	17.8 (6.2)	17.7 (6.1)	18.0 (6.4)	17.5 (5.8)
Years smoked, mean (SD)[a]	45.0 (11.1)	45.0 (11.1)	44.9 (11.2)	45.2 (11.0)
Cigarettes/day, mean (SD)[a]	24.7 (15.7)	24.9 (15.8)	25.0 (16.3)	24.9 (15.3)
Pack years, mean (SD)[a]	56.0 (39.4)	56.6 (39.6)	56.8 (40.4)	56.5 (38.9)
Alcoholic drinks/week, mean (SD)[b]	13.0 (20.6)	12.9 (20.5)	11.9 (19.4)	13.8 (21.5)

[a] Calculated excluding never-smokers.
[b] Calculated excluding nondrinkers.

Table 2 Summary of lung cancer risk estimates by treatment group

Analysis period	Number of cases selenium/placebo		RR (95% CI)	*P*	HR[a] (95% CI)	*P*
1983–1993[b]	17	31	0.54 (0.30, 0.98)	0.04	0.56 (0.31, 0.76)	0.05
1983–1996	25	35	0.70 (0.40, 1.21)	0.18	0.74 (0.44, 1.24)	0.26

[a] Cox proportional hazards model; adjusted for age, smoking status (current, former, never).
[b] Clark *et al.* (3).

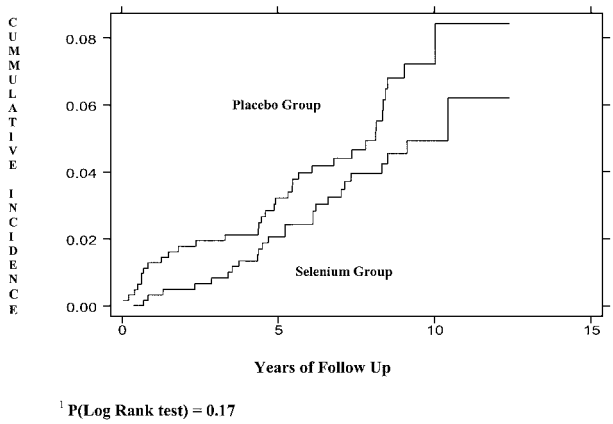


Fig. 1. Cumulative incidence curve for lung cancers by treatment group. *P* (log-rank test) = 0.17.

show that in the first 4 years after randomization, more lung cancers were diagnosed in the placebo group.

Table 3 summarizes the lung cancer risk estimates for current and former smokers separately. Among current smokers, there were 16 cases of lung cancer in the selenium supplemented group and 25 cases in the placebo group, yielding an HR of 0.67 (95% CI, 0.36–1.26; $P = 0.36$). Among former smokers there were 7 cases of lung cancer in the selenium supplemented group and 10 cases in the placebo group. The corresponding HR was 0.70 (95% CI, 0.26–1.81; $P = 0.45$). Fig. 2 shows the Kaplan-Meier curve for lung cancer incidence by baseline smoking status and treatment group. There were

Table 3 Summary of lung cancer risk estimates by smoking status and treatment group

Smoking status[a]	Number of cases selenium/placebo[b]	HR[c] (95% CI)
Current	16/25	0.67 (0.36, 1.26)
Former	7/10	0.70 (0.26, 1.81)

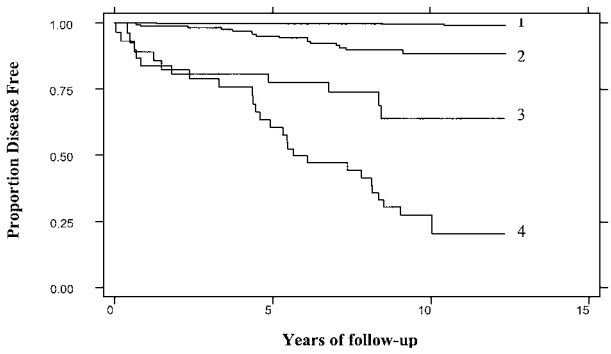
[a] Smoking status defined at baseline. Never-smokers represented only 2 cases of lung cancer.
[b] Two lung cancer cases in never-smokers from the selenium group were excluded from this analysis.
[c] Adjusted for age and baseline plasma selenium as continuous variables.

more lung cancer in the former and current smokers in the placebo group than the selenium supplemented group, although these differences were not statistically significant. Two cases of lung cancer in never-smokers, both in the selenium group, were excluded from this analysis.

The effect of selenium supplementation on lung cancer incidence in each tertile of baseline selenium is summarized in Table 4. After adjusting for age and smoking status, there is a statistically significant inverse association between supplementation and lung cancer incidence in the lowest tertile of baseline selenium (HR = 0.42; 95% CI, 0.18–0.96; $P = 0.04$). The models for the middle and highest tertiles of baseline showed HRs of 0.91 and 1.25, respectively. Fig. 3 shows the cumulative survival curve by treatment group for the incidence of lung cancers in the lowest tertile of baseline selenium with a *P* from the log-rank test of 0.04.

Discussion

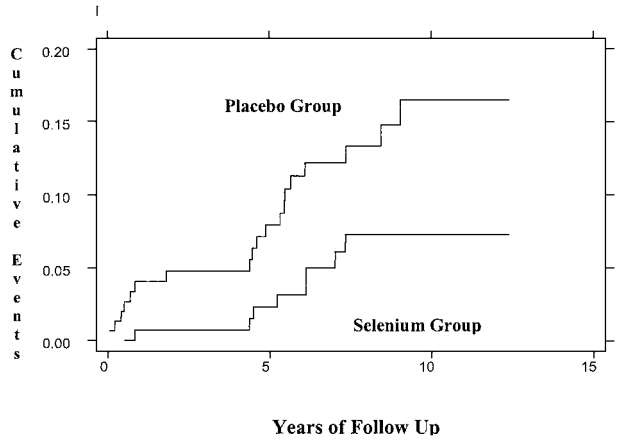
In the year 2001, an estimated 169,500 new cases of lung and bronchus cancer will be diagnosed in the United States (29).



1 = Former Smokers, Se Group; 2 = Current smokers, Se Group;
3 = Former Smokers, Placebo Group; 4 = Current Smokers, Placebo Group

[1]P(log rank test) = 0.26 for Current Smokers
[2]P(log rank test) = 0.45 for Former Smokers

Fig. 2. Kaplan-Meier curve for lung cancer incidence in current and former smokers by treatment group. *1*, former smokers, Se group; *2*, current smokers, Se group; *3*, former smokers, placebo group; *4*, current smokers, placebo group. *P* (log-rank test) = 0.26 for current smokers; *P* (log-rank test) = 0.45 for former smokers.



[1] P(Log Rank test) = 0.04

Fig. 3. Cumulative incidence curve for lung cancers in the lowest tertile of baseline plasma selenium. *P* (log-rank test) = 0.04.

Table 4 Risk of lung cancer incidence by treatment group and tertile of baseline plasma selenium

Selenium tertiles	Selenium cases	Placebo cases	HR[a] (95% CI)	*P*
Lowest tertile 42–106 ng/ml	8	19	0.42 (0.18, 0.96)	0.04
Middle tertile 106–122 g/ml	7	8	0.91 (0.33, 2.52)	0.86
Highest tertile 122–220 g/ml	10	8	1.25 (0.49, 3.21)	0.64

[a] Cox proportional hazards model; adjusted for age, smoking status (current, former, never).

Among men, cancer of the lung and bronchus is estimated to be the second leading site for cancer incidence, accounting for 90,700 (14%) of new cancer cases. In 2001, for both men and women combined, lung cancer will be the most common cause of cancer deaths in the United States, accounting for 28% of all cancer deaths, and more deaths than from prostate, breast, and colorectal cancer combined (29). Lung cancer is a public health problem of great magnitude. Most lung cancers present at diagnosis in an advanced stage, and the vast majority of patients with lung cancer will succumb to the disease.

Numerous recent studies have indicated that lung cancer is not the result of a sudden transformation in the bronchial epithelium, but of a multistep accumulation of genetic and epigenetic alterations that are in most cases caused by chronic exposure to carcinogens in tobacco smoke (30–32). Histological mucosal changes in the large airways that may precede or accompany invasive carcinoma, include hyperplasia, metaplasia, dysplasia, and carcinoma *in situ* (33–36). Therefore, a strategy to arrest or reverse preneoplastic changes in the bronchial epithelium before invasive cancer develops is a critical element in reducing the burden of lung cancer. At present there are few promising chemopreventive agents that offer this potential protection.

When first published, the NPC trial showed as a secondary end point, a marginally significant reduction in lung cancer incidence associated with selenium supplementation. Whereas the exact anticarcinogenic mechanism was not known, these results promoted interest in the study of selenium as a well-tolerated chemopreventive agent for a variety of solid tumors, including lung.

The extended follow-up of NPC subjects through 1996 reports important results that may impact the design of future chemopreventive trials using selenium. Whereas the addition of 3 years of follow-up diminishes the apparent benefit of selenium supplementation in decreasing lung cancer incidence to nonsignificant levels, the trend continues to suggest a decrease in lung cancer risk with supplementation. In the lowest tertile of baseline plasma selenium, there was a statistically significant inverse relationship between supplementation with selenium and lung cancer incidence.

The average plasma selenium concentrations for United States residents included in an analysis of antioxidant nutrients and pulmonary function obtained from the National Health and Nutrition Examination Survey III was 123 ng/ml (37). Whereas the subjects in the NPC trial averaged selenium concentrations of 114 ng/ml (a range of 42–220), this represents a nutritionally adequate population. Ninety-eight percent of the NPC participants exceeded plasma selenium concentrations greater than the 70–90 ng/ml required to maximize plasma selenoproteins such as glutathione peroxidase and selenoprotein-P (38, 39). Therefore, benefits derived from supplementation in subjects in the lowest tertile of baseline selenium may not be because of alterations in glutathione peroxidase or selenoprotein-P but may be related to an as yet unidentified chemopreventive activity. This suggests that selenium supplementation for chemoprevention of lung cancer should be targeted at populations with low plasma selenium concentrations.

Whereas the exact mechanism by which selenium exerts a chemopreventive effect is not known, there are several possibilities suggested in the literature. El-Bayoumy (40) recently reviewed potential mechanisms for the protective role of selenium against cancer. In multiple organ systems in rodents, including the lungs, several forms of selenium have inhibited carcinogen-induced covalent DNA adduct formation, retarded oxidative damage to DNA, lipids and proteins, inhibited tumor cell growth, altered DNA, RNA, and protein synthesis, increased apoptosis, changed cell cycle, and p53 and COX-2 expression, modified transcriptional factors activator protein P

and nuclear factor κB, decreased aberrant crypt foci, and decreased Mtase activity (40).

DNA hypermethylation, increased COX-2 expression, and decreased apoptosis are three possible mechanisms that have been implicated in lung carcinogenesis. At present, there is evidence, primarily from animal and *in vitro* systems, that selenium modulates these biomarkers, which may explain the effect that selenium supplementation has on lung cancer incidence.

Methylation is the main epigenetic modification in humans (41), and changes in methylation patterns play an important role in tumorigenesis. Hypermethylation of the tumor suppressor gene *p16*, the DNA repair gene *MGMT*, and genes related to metastasis and invasion, *DAP-K* and *TIMP3*, has been described in lung cancer cell lines and a few primary lung tumors (42–49). Methylation is a well-known fate of selenium metabolism (50). Fiala *et al.* (51) studied the direct effect of selenium on the activity of Mtase, the group of enzymes critical to the methylation of DNA (52). HCT116 cell lines that were treated with several forms of organic selenium compounds showed more inhibition of Mtase (50%) than the control. The effect of organic selenium compounds on Mtase activity may explain part of the inhibitory effect of selenium supplementation on lung cancer development.

COX-2 expression has been well documented in lung pathogenesis (53–58). COX-2 is up-regulated in carcinomas of the lung. The precise mechanisms by which COX-2 regulates lung cancer carcinogenesis are not clear, but are likely through increased proliferation, resistance to apoptosis (58–60), promotion of angiogenesis (58, 61, 62), and impairment of immune surveillance (58, 63). The published literature on selenium and inhibition of COX-2 expression has included several forms of selenium tested predominantly in the colon cancer model. However, because both lung and colon cancers are known to heavily express COX-2, this may explain some of the chemopreventive activity of selenium. The elevation of the relative number of apoptotic cells and bodies, an apoptotic index, has been shown to be an early event in lung carcinogenesis. The apoptotic index increases as normal bronchial epithelium develops a premalignant lesion; an increase in apoptotic activity is associated with the severity of the bronchial premalignant lesions (35). Selenium-induced apoptosis has been studied in a number of cancer cells in *in vitro* conditions. Both organic and inorganic forms of selenium have been shown to induce apoptosis in various cancer cells, including human lung cancer cells (64). The hypothesis that supranutritional doses of selenium can enhance apoptosis is consistent with the results of several studies (65–68).

Several forms of selenium have been used to determine the mechanisms that can explain the activity of selenium in preventing cancer. In the NPC trial, selenized baker's yeast was selected as a vehicle for selenium because it contains high concentrations of organic, bioavailable forms of selenium. Selenized baker's yeast contains a mixture of ~60% selenomethionine; the remaining selenium compounds (40%) are a mixture of other organic selenoproteins. Whereas many of these organoselenium compounds have not been completely identified, high-performance liquid chromatography analysis has revealed the presence of selenocysteine, se-methylselenocysteine and selenomethionine, which together account for 20% of the overall selenium content (46). Other as-yet-unidentified selenium-based agents are likely to be complex organic selenium compounds of which the chemopreventive properties are not known, continuing the active debate on the most efficacious form of selenium to use in chemoprevention trials. Whereas much time and resources are now being dedicated to clinical chemoprevention trials using selenomethionine, the evidence that this form alone explains the results of the NPC trial is limited.

The NPC sample had high percentages of former and current smokers. It is of particular interest that selenium supplementation appears to have chemopreventive effects in persons with relatively heavy tobacco use histories (median of 49 pack-years) and low baseline selenium concentrations. Thus, both current and former smokers may benefit from selenium supplementation if they have low plasma selenium concentrations.

A major limitation of this study is that it is based on an analysis involving only 60 cases of incident lung cancer and that lung cancer was a secondary end point in the NPC trial. Conversely, it is a strength of this study that the analysis is based on incident cases ascertained in a thorough and consistent manner, blinded to treatment group. A second limitation involves the generalizability of these results because the NPC sample consisted of subjects from the Eastern United States with a strong history of nonmelanoma skin cancer. A study sample that includes individuals with a substantial smoking exposure but without a history of any type of cancer would allow these results to be generalized to broader populations.

Overall, Khuri and Lippman (69), and Goodman (70) have suggested that selenium may have an important role in the chemoprevention of lung cancer. On the basis of the current reanalysis, selenium supplementation appears efficacious in decreasing lung cancer incidence in subjects whose baseline plasma selenium is ~106 ng/ml or below. Future research is needed to help confirm the role of selenium in lung cancer prevention, using multiple forms of selenium alone and in combination with other chemopreventive agents. In addition, clinical studies that investigate the molecular mechanisms driving the chemopreventive effects of selenium and the role of plasma selenium status in mediating these effects are clearly indicated.

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