The Beta-Carotene and Retinol Efficacy Trial: Incidence of Lung Cancer and Cardiovascular Disease Mortality During 6-Year Follow-up After Stopping β-Carotene and Retinol Supplements

Gary E. Goodman, Mark D. Thornquist, John Balmes, Mark R. Cullen, Frank L. Meyskens, Jr., Gilbert S. Omenn, Barbara Valanis, James H. Williams, Jr.

Background: **The Beta-Carotene and Retinol Efficacy Trial (CARET) tested the effect of daily β-carotene (30 mg) and retinyl palmitate (25 000 IU) on the incidence of lung cancer, other cancers, and death in 18 314 participants who were at high risk for lung cancer because of a history of smoking or asbestos exposure. CARET was stopped ahead of schedule in January 1996 because participants who were randomly assigned to receive the active intervention were found to have a 28% increase in incidence of lung cancer, a 17% increase in incidence of death and a higher rate of cardiovascular disease mortality compared with participants in the placebo group.** ***Methods:*** **After the intervention ended, CARET participants returned the study vitamins to their study center and provided a final blood sample. They continue to be followed annually by telephone and mail self-report. Self-reported cancer endpoints were confirmed by review of pathology reports, and death endpoints were confirmed by review of death certificates. All statistical tests were two-sided.** ***Results:*** **With follow-up through December 31, 2001, the post-intervention relative risks of lung cancer and all-cause mortality for the active intervention group compared with the placebo group were 1.12 (95% confidence interval [CI] = 0.97 to 1.31) and 1.08 (95% CI = 0.99 to 1.17), respectively. Smoothed relative risk curves for lung cancer incidence and all-cause mortality indicated that relative risks remained above 1.0 throughout the post-intervention follow-up. By contrast, the relative risk of cardiovascular disease mortality decreased rapidly to 1.0 after the intervention was stopped. During the post-intervention phase, females had larger relative risks of lung cancer mortality (1.33 versus 1.14; *P* = .36), cardiovascular disease mortality (1.44 versus 0.93; *P* = .03), and all-cause mortality (1.37 versus 0.98; *P* = .001) than males.** ***Conclusions:*** **The previously reported adverse effects of β-carotene and retinyl palmitate on lung cancer incidence and all-cause mortality in cigarette smokers and individuals with occupational exposure to asbestos persisted after drug administration was stopped although they are no longer statistically significant. Planned subgroup analyses suggest that the excess risks of lung cancer were restricted primarily to females, and cardiovascular disease mortality primarily to females and to former smokers. [J Natl Cancer Inst 2004;96:1743–50]**

Lung cancer is the leading cause of cancer death in the United States, accounting for 29% of deaths from cancers and 9% of all deaths *(1)*. In 2004, an estimated 90 000 men and 67 000 women will die from lung cancer. Although there has been a small but detectable improvement in lung cancer treatment over the past 20 years, the 5-year survival among individuals who received a lung cancer diagnosis between 1989 and 1996 was only 14% *(1)*. The National Cancer Institute (NCI) has encouraged an active research program in chemoprevention, i.e., the use of agents to prevent, arrest, or reverse lung cancer carcinogenesis.

In the early 1980s, the dietary constituent β-carotene was one of the agents with the strongest supportive evidence suggesting that it was a chemoprevention agent. Results of observational epidemiologic studies as well as those of less numerous animal studies *(2)* led to the initiation of the large intervention trials designed to test the effect of supplemental β-carotene. The Physicians' Health Study Trial tested the effect of β-carotene and aspirin on cancer and cardiovascular disease in a healthy male population. The Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Trial, which was conducted in Finland, and the Beta-Carotene and Retinol Efficacy Trial (CARET), which was conducted in the United States, were designed to test the effect of β-carotene on lung cancer incidence. ATBC used a 2 × 2 study design to test the combination of 20 mg of β-carotene and 50 mg of alpha-tocopherol in 29 133 male current cigarette smokers *(3)*. CARET compared the combination of 30 mg of β-carotene and 25 000 IU of retinyl palmitate (retinol) with placebo in 18 314 male and female current and recent ex-smokers and male asbestos-exposed workers *(4)*. In 1994, after a mean follow-up of 6.1 years, ATBC reported that subjects in the treatment arms that received β-carotene had a 16% increased incidence of lung cancer (relative risk [RR] = 1.16, 95% confidence interval [CI] = 1.02 to 1.33; *P* = .02) and an 8% increase in all-cause mortality (RR = 1.08, 95% CI = 1.01 to

Affiliations of authors: Fred Hutchinson Cancer Research Center, Seattle, WA (GEG, MDT); Swedish Cancer Institute, Seattle (GEG); Department of Medicine, University of California at San Francisco (JB); Yale School of Medicine, Yale University, New Haven, CT (MRC); UC Irvine Medical Center, Orange, CA (FLM, JHW); University of Michigan Medical School, Ann Arbor (GSO); Kaiser Permanente Center for Health Research, Portland, OR (BV).

Correspondence to: Gary E. Goodman, MD, MS, Fred Hutchinson Cancer Research Center, 1100 Fairview Ave. North, Bldg. M, M1-B514, Seattle, WA 98109 (e-mail: gary.goodman@swedish.org).

See "Notes" following "References."

DOI: 10.1093/jnci/djh320

1.16; $P = .02$) compared with subjects who had received placebo *(3,5)*. In January 1996, CARET reported similar results: after a mean follow-up of 4.0 years, the group that received the active study vitamins had a 28% greater incidence of lung cancer (RR = 1.28, 95% CI = 1.04 to 1.57; $P = .02$), 17% more deaths from all causes (RR = 1.17, 95% CI = 1.03 to 1.33; $P = .02$), and more deaths from cardiovascular disease (RR = 1.26, 95% CI = 0.99 to 1.61; $P = .06$) than the group that received placebo *(6)*. These two trials clearly established that supplements containing β-carotene were harmful to cigarette smokers, causing increases in the incidence of lung cancer and in overall mortality.

Ever since the study interventions were stopped (in 1994 for ATBC and 1996 for CARET), a major question has remained: How will discontinuation of the study supplements affect the elevated incidence of lung cancer, cardiovascular disease mortality, and all-cause mortality in those who received β-carotene? ATBC investigators have recently reported that, with 6 years of follow-up after ending the vitamin intervention, the adverse effects they found on lung cancer incidence had disappeared *(8)*. We now report the findings for CARET with 6 years of post-intervention follow-up.

METHODS

CARET Study Design

The strategy, design, methods, eligibility criteria, pilot study findings, recruitment success, safety monitoring data, endpoints ascertainment and review process, and initial findings for CARET have been published *(4,6,9–12)*. Briefly, CARET was initiated in 1983 with two pilot studies that tested two doses of β-carotene with or without vitamin A (retinol) in two high-risk populations: 816 men with substantial occupational exposures to asbestos and 1029 men and women who were either current or former cigarette smokers (<6 years since quitting) with a smoking history of at least 20 pack-years. Synthetic β-carotene, retinol (tested in the pilot studies), and retinyl palmitate (tested in CARET) were all manufactured by Hoffmann-LaRoche (Nutley, NJ). In 1988, after successful completion of the pilot studies, all pilot study participants who had been randomly assigned to the active intervention groups were reassigned to receive 30 mg of β-carotene plus 25 000 IU of retinyl palmitate daily, the active regimen chosen for CARET. Recruitment to CARET was then expanded 10-fold at the six U.S. study centers located in Seattle (WA), Portland (OR), San Francisco (CA), Irvine (CA), Baltimore (MD), and New Haven (CT). Recruitment ended in 1994, when 18 314 participants had been randomly assigned to the active intervention or placebo groups. Written informed consent was obtained from all participants when they joined the trial. During the trial, CARET activities were reviewed annually by the institutional review boards at the six CARET study centers and by an external Safety and Endpoints Monitoring Committee (SEMC). The primary endpoints of CARET were lung cancer incidence, cardiovascular mortality, and all-cause mortality. After a planned interim analysis in 1995 and in consultation with the NCI and the SEMC, the CARET Steering Committee decided to end active intervention on January 11, 1996, because of adverse findings *(13)*. All participants were asked to stop taking the intervention agents and to return them to their study center, where a final blood sample was collected from each participant and written informed consent was obtained for post-intervention follow-up. Participants were followed annually by telephone (September 1996 through March 2000) and by mail thereafter to collect self-reported health information.

Endpoint Data Collection

CARET had a well-established endpoint assessment protocol *(11)*. We obtained the medical records and pathology reports from the diagnosing institutions for all participants who reported a cancer diagnosis during the intervention phase or post-intervention follow-up period. Central pathology review was conducted on all lung cancer cases (the CARET primary endpoint) diagnosed through December 31, 1997. For all other cancers (and lung cancer cases diagnosed on or after March 1, 1998), we reviewed pathology reports obtained from the diagnosing institutions. For cancer cases diagnosed prior to March 1, 1998, endpoint materials were reviewed independently by three physician adjudicators, who were required to reach a consensus on the site of the primary cancer, its histology, and the date of diagnosis to consider the cancer diagnosis as confirmed. For cancer cases diagnosed on or after October 1, 1998, the endpoint adjudication system was modified to allow review by endpoint specialists from the CARET Coordinating Center staff and a single physician adjudicator. The date and underlying cause of death were determined by reviewing medical records and death certificates.

Statistical Analysis

During the endpoint review process, the month and year of cancer diagnosis were adjudicated from the medical records; however, we found that the day of diagnosis could not be consistently determined. For analytic purposes, the date of cancer diagnosis was taken to be the 15th of the month unless there was evidence (such as a date of death prior to that date) indicating that the 15th was not possible, in which case the day of diagnosis was defined as the midpoint of the range of days consistent with the available information. The primary endpoints during the post-intervention phase were incidences of lung cancer, all-cause mortality, and mortality from cardiovascular disease.

Relative risk (RR) estimates for the intervention and post-intervention phases of CARET were obtained through Cox proportional hazards regression models. We determined that the assumptions were satisfied through examination of the log-log (survival) versus log time plot. For the intervention phase analysis, time was measured from the date of randomization into CARET to the date of the first diagnosis for the endpoint of interest; the date of death; or January 11, 1996, the date the intervention phase formally ended, whichever occurred first. For the post–intervention phase analysis, time was measured from January 12, 1996, to the date of first diagnosis, the date of death, or December 31, 2001, whichever occurred first. Participants who died or were diagnosed with cancer during the intervention phase were not included in the post–intervention phase analysis for that outcome, although they were included in analyses of other outcomes. Thus, for example, a participant who was diagnosed with two primary lung cancers, one during the intervention phase and the other during the post-intervention phase, would not be included in the post–intervention phase analysis of the lung cancer endpoint but would be included in the analysis

of the death endpoint. We assessed the statistical significance of differences in relative risks between subgroups by using a single model for the total population with main effects for the subgroup of interest that were evaluated by the change in partial likelihood. Smoking history was defined at the time of randomization and was not changed if smoking status changed during the trial.

To assess the time course of disease risk in calendar time, we estimated smoothed relative risks from a generalized additive model *(11)*. We computed incidence rates for each 1-month period of follow-up and smoothed the monthly relative risk estimates with a tricube kernel smoother. The span width for the smoother varied by disease outcome and subgroup examined; outcomes with relatively few endpoints required a larger span than outcomes with more endpoints. For all of the primary outcomes in this article, a span of 0.35 produced smooth curves that still retained fine detail: smaller spans produced lumpier curves whereas larger spans produced more linear curves that lacked fine detail. For each smoothed curve, we also computed the pointwise 95% confidence intervals (CIs); specifically, for each monthly smoothed relative risk, we computed the 95% confidence interval for that estimate from the generalized additive model. This analytic method was similar to that employed by the ATBC Study Group in its report on post-intervention follow-up *(8)*, in which they found that a span of 0.40 provided the best description of their data. These statistical analyses were performed using S-Plus software, version 6.2 (Insightful Corporation, Seattle, WA). All statistical tests were two-sided.

Results

The baseline characteristics of participants in CARET at the time of randomization were well balanced between the intervention groups (Table 1). When the intervention was stopped in January 1996, the intervention groups were still well balanced with respect to all characteristics (Table 1). The major changes in the population characteristics between the time of randomization and the start of the post-intervention period were increases in time-related measures (e.g., age, number of pack-years of cigarette smoking) that were related to the duration of the intervention phase, and a decrease in the percentage of participants who continued to actively smoke cigarettes. During the intervention phase of CARET, current cigarette smokers had a net smoking cessation rate (i.e., the rate calculated by subtracting the number of relapsers from the number of quitters) of 5% per year, based on self-report.

A total of 1174 participants who were enrolled in CARET did not contribute person-years of follow-up to this post-intervention analysis; of these, 1092 (93%) died during the intervention phase and 82 (7%) were lost to follow-up. In the ongoing post-intervention follow-up in CARET, 93% of the living participants are being followed actively through mailed questionnaires; the remainder (including those considered lost to follow-up during the intervention phase) are being followed passively through searches of local cancer registries and the National Death Index. In Table 2, we present our findings for the post-intervention phase for the primary endpoints and compare them with the findings previously reported at the end of the active intervention phase of CARET in January 1996. During the post-intervention phase, the relative risk of lung cancer for the active intervention group compared with the placebo group was 1.12 (95% CI = 0.97 to 1.31; $P = .13$), which was lower than it was during the intervention phase (1.28, 95% CI = 1.04 to 1.57; $P = .02$). Figure 1, A, illustrates how the ratio of the lung cancer risk in the

Table 1. Characteristics of the Beta-Carotene and Retinol Efficacy Trial (CARET) participants at randomization and at the start of the post-intervention period*

Characteristic	At randomization		At start of post-intervention period	
	Active group	Placebo group	Active group	Placebo group
Smoker cohort	n = 7376	n = 6878	n = 6902	n = 6545
Median age, y (IQR)†	58 (53–63)	57 (53–62)	62 (57–67)	62 (57–66)
% female	43.4	44.8	44.1	45.3
% non-white	5.1	5.7	5.1	5.7
% current smoker‡	66.5	66.1	51.4	52.7
Median No. of pack-years of smoking (IQR)§	45 (35–60)	44 (35–60)	47 (37–63)	47 (36–62)
Median No. of years since quitting smoking (IQR)‖	3 (1–5)	3 (1–5)	5 (2–8)	5 (2–8)
Asbestos-exposed cohort	n = 2044	n = 2016	n = 1842	n = 1851
Median age, y (IQR)†	57 (51–63)	57 (51–63)	62 (56–68)	62 (56–68)
% non-white	11.7	12.0	11.5	11.6
% current smoker‡	38.2	38.5	27.6	28.7
Median No. of pack-years of smoking (IQR)§	39 (26–54)	39 (26–54)	41 (27–56)	41 (26–53)
Median No. of years since quitting smoking (IQR)‖	8 (4–13)	8 (4–13)	11 (6–17)	11 (6–17)
Median No. of years since first asbestos exposure (IQR)¶	35 (29–42)	35 (29–42)	40 (34–47)	40 (34–47)
Median No. of years in high-risk trade (IQR)#	19 (6–29)	20 (7–30)	19 (6–29)	20 (7–29)
% with positive x-ray**	66.1	64.8	64.5	63.9

*IQR = interquartile range.

†Eligibility criterion for CARET was age 50–69 years for heavy smokers and 45–69 years for asbestos-exposed individuals (eligibility criterion for the pilot study was age 45–74 years for asbestos-exposed individuals).

‡All other participants were former smokers except for 133 never smokers from the pilot study of asbestos-exposed individuals (69 participants in the active group and 64 participants in the placebo group at randomization; 58 participants in the active group and 58 participants in the placebo group at the start of post-intervention).

§Ever smokers only. Eligibility criterion was ≥20 pack-years for heavy smokers (no eligibility criterion for asbestos-exposed men).

‖Former smokers only. Eligibility criterion was ≤6 years for heavy smokers and ≤15 years for asbestos-exposed individuals (no eligibility criterion for the pilot study in asbestos-exposed individuals).

¶Eligibility criterion was ≥15 years since first exposure.

#Eligibility criterion was ≥5 years in high-risk trade completed at least 10 years prior to randomization.

**An x-ray was considered positive if the profusion rating was ≥1/0, there were diaphragm abnormalities in at least one lung, there was calcification in at least one lung, there was circumscribed or diffuse pleural thickening of width B or greater in at least one lung, or there was circumscribed or diffuse pleural thickening of width A2 or greater in both lungs.

Table 2. Number of events, relative risk (RR), and 95% confidence intervals (CIs) for active group versus placebo group at the time the intervention was stopped and after a 6-year post-intervention period for all Beta-Carotene and Retinol Efficacy Trial (CARET) participants

	Post-intervention phase			Intervention phase
	Number of events			
Endpoint	Active group (n = 8744)	Placebo group (n = 8396)	RR (95% CI)	RR (95% CI)
Lung cancer incidence	376	311	1.12 (0.97 to 1.31)	1.28 (1.04 to 1.57)
Mortality from all causes*	1225	1047	1.08 (0.99 to 1.17)	1.17 (1.03 to 1.33)
Mortality from lung cancer	294	227	1.20 (1.01 to 1.43)	1.46 (1.07 to 2.00)
Mortality from cardiovascular disease	354	319	1.02 (0.88 to 1.19)	1.26 (0.99 to 1.61)
Mortality from other causes†	543	466	1.07 (0.95 to 1.21)	0.99 (0.79 to 1.25)

*Cause-specific mortality does not add up to all-cause mortality because of open endpoint cases in which the cause of death has not yet been adjudicated.
†Excludes death from lung cancer and cardiovascular disease.

active intervention arm to the risk in the placebo arm changed after the active intervention ended on January 11, 1996. The smoothed relative risk of lung cancer was greater than 1.1 throughout the first 4 years after the end of the intervention, and the pointwise 95% confidence intervals excluded 1.0 between November 1997 and August 1999.

During the post-intervention phase, the overall relative risk of death from any cause for the active intervention group compared with the placebo group was 1.08 (95% CI = 0.99 to 1.17; P = .07) (Table 2; Fig. 2, A), which was lower than it was during the intervention phase (1.17, 95% CI = 1.03 to 1.33; P = .02). Thus, these data are suggestive of, but not definitive for, a continued modestly elevated risk of death from all causes for participants in the active intervention group.

We classified cause-specific mortality according to mortality from lung cancer, mortality from cardiovascular disease, and mortality from causes other than lung cancer or cardiovascular disease. Survival after a diagnosis of lung cancer did not differ by intervention group (RR = 1.00, 95% CI = 0.88 to 1.15, P = .94); thus, the numerical excess of lung cancers diagnosed dur-

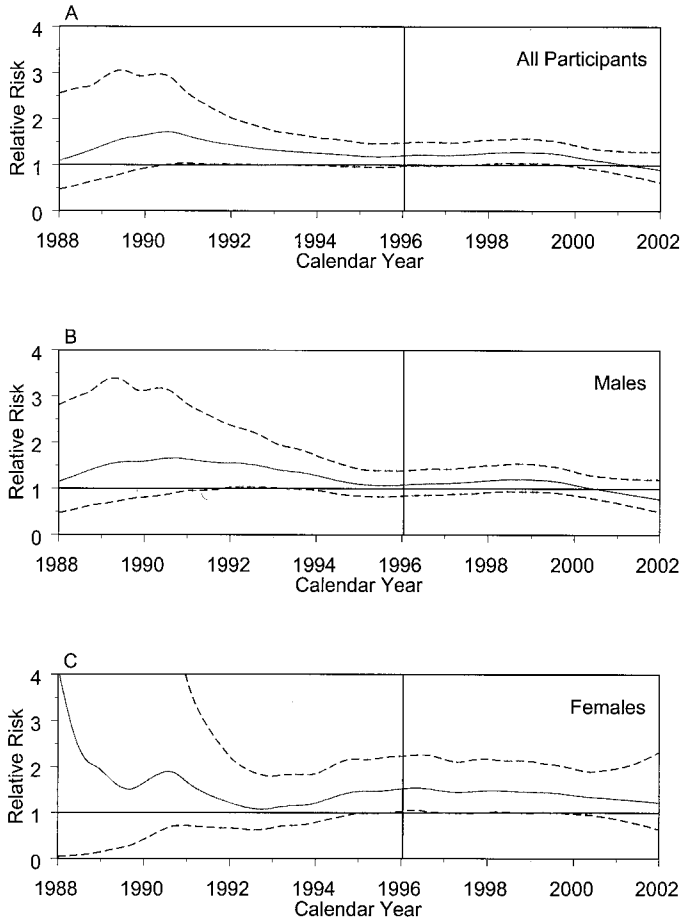

Fig. 1. Smoothed lung cancer incidence relative risks (**solid lines**) and pointwise 95% confidence intervals (**dashed lines**) by calendar time for all participants (**A**), males (**B**), and females (**C**). **Vertical line** indicates the end of the β-Carotene and Retinol Efficacy Trial (CARET) intervention period on January 11, 1996.

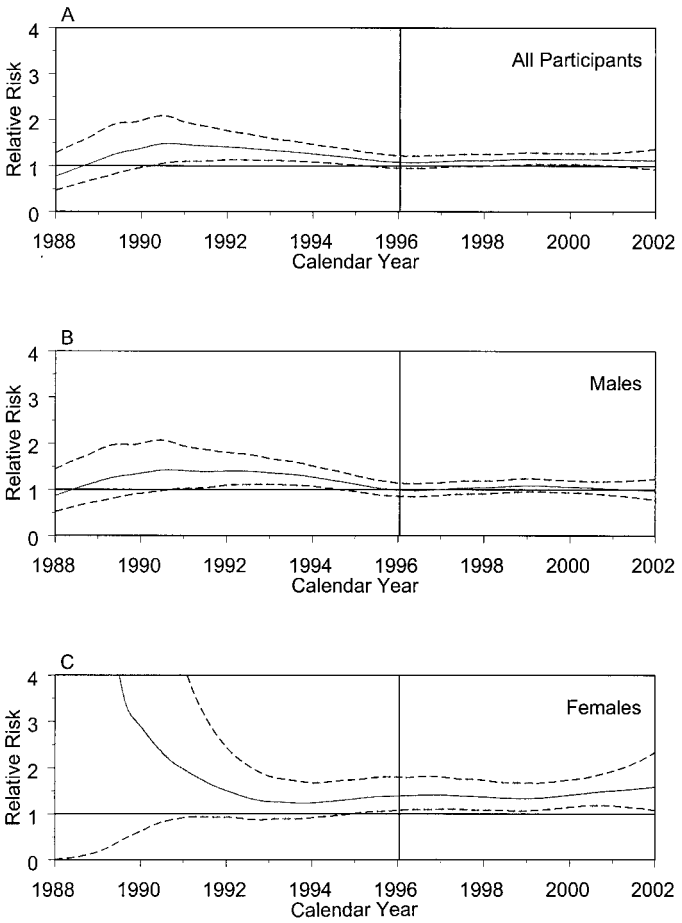

Fig. 2. Smoothed all-cause mortality relative risks (**solid line**) and pointwise 95% confidence intervals (**dashed lines**) by calendar time for all participants (**A**), males (**B**), and females (**C**). **Vertical line** indicates the end of the β-Carotene and Retinol Efficacy Trial (CARET) intervention period on January 11, 1996.

ing the post-intervention phase in the active intervention group translated into an excess of lung cancer deaths in that group (Table 2). By contrast, the excess mortality from cardiovascular disease in the active intervention group declined from a relative risk of more than 1.3 between 1990 and 1994 to a relative risk of less than 1.1 after the end of the intervention period (Table 2 and Fig. 3, A), at least for the study population as a whole. Compared with the placebo group, the active intervention group had more deaths from causes other than lung cancer or cardiovascular disease, although that difference was not statistically significant.

Table 3 presents the relative risks for the active intervention group compared with the placebo group during the post-intervention period for lung cancer incidence, all-cause mortality, and cause-specific mortality for preplanned analyses of key subpopulations of study participants (i.e., females, males, and current and former smokers). There were no statistically significant differences in relative risks between asbestos-exposed participants (all of whom were male) and males in the smoker cohort; only the relative risks of lung cancer incidence showed a numeric difference greater than 0.05. For this reason, all analyses comparing sexes combined males in the asbestos-exposed and smoker cohorts. Although females had a numerically higher relative risk of lung cancer if they used β-carotene

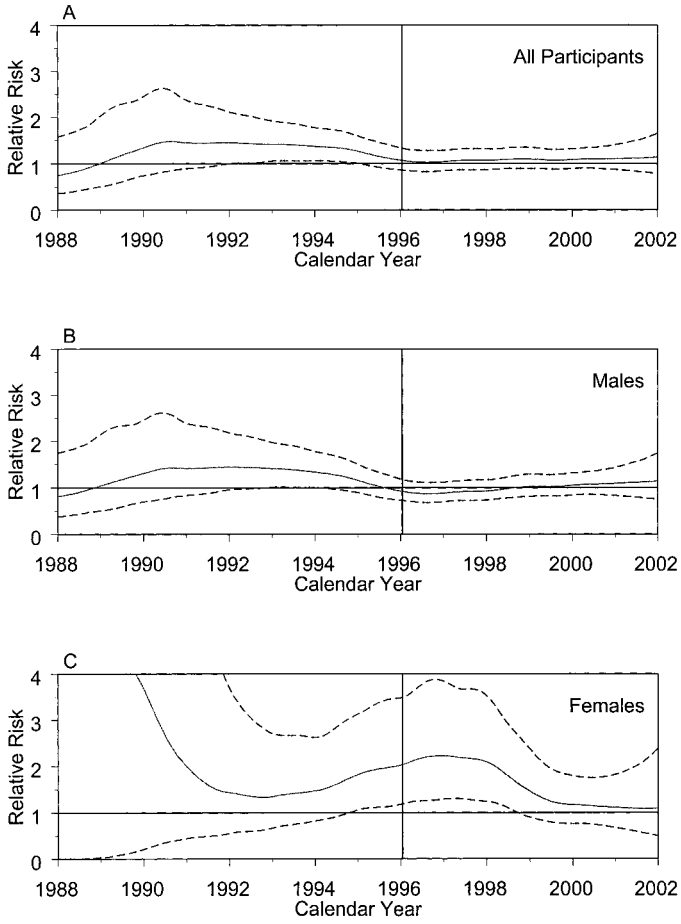

Fig. 3. Smoothed cardiovascular disease mortality relative risks (**solid lines**) and pointwise 95% confidence intervals (**dashed lines**) by calendar time for all participants (**A**), males (**B**), and females (**C**). **Vertical line** indicates the end of the β-Carotene and Retinol Efficacy Trial (CARET) intervention period on January 11, 1996.

than male heavy smokers (1.33, 95% CI = 1.01 to 1.75 versus 1.03, 95% CI = 0.86 to 1.24), the difference between relative risks was not statistically significant ($P = 0.09$). Figure 1, B and C, shows the smoothed lung cancer relative risks for males and females as a function of time. The point estimates of the lung cancer relative risk for males showed a modest increase for approximately the first 4 years after the end of the intervention. The point estimates of the lung cancer relative risk for females were much higher than those for males, they showed little evidence of decline, even at 5 years after the end of intervention. The very high estimated smoothed relative risks for females during the early years of the intervention period were due to instability in the smoothed estimates that arose from the very small number of outcomes in that subgroup prior to 1990 (e.g., number of lung cancers diagnosed in active group versus in placebo group, 2 versus 0; number of deaths from any cause in active group versus in placebo group, 3 versus 0; number of deaths from cardiovascular disease in active group versus in placebo group, 1 versus 0).

During the post-intervention phase, current and former smokers had similar relative risks of all-cause mortality of 1.09 and 1.18, respectively (Table 3). However, current smokers had a numerically higher relative risk of lung cancer mortality than former smokers (1.27 versus 1.12) that reflected their higher relative risk of lung cancer incidence compared with that of former smokers (1.22 versus 1.11), whereas former smokers had a much higher relative risk of cardiovascular disease mortality than current smokers (1.44 versus 0.87). A test for the statistical significance of the difference in the relative risk of cardiovascular disease mortality between current and former smokers gave a nominal P value of .01. The point estimate of the relative risk of cardiovascular disease mortality during the intervention phase was also greater in former smokers (RR = 1.45) than in current smokers (RR = 1.07; P for difference = .21).

Figure 2, B and C, shows that the smoothed relative risk for all-cause mortality was larger in females (smokers cohort) than males (asbestos-exposed and smoker cohort) since the end of intervention (nominal P value for difference = .003). Table 3 shows that, during the post-intervention phase, females had larger relative risks than males for lung cancer mortality (1.33 versus 1.14; $P = .31$), for cardiovascular disease mortality (1.44 versus 0.93; $P = .03$), and for all-cause mortality (1.37 versus 0.93; $P = .001$). Figure 3, B and C, shows the smoothed relative risks of cardiovascular disease mortality for males and females, respectively, which depict the immediate disappearance of the excess risk of cardiovascular disease mortality among males after January 11, 1996, and the excess risk of cardiovascular disease mortality in females continuing through much of the post-intervention period. The data thus suggest that whereas the excess lung cancer incidence and mortality risks seen during the intervention phase may have resolved in male smokers during the post-intervention phase, they persisted in female smokers.

Discussion

Before the findings of ATBC *(3,5)*, CARET *(6,12)*, and the Physicians Health Study (PHS) *(14)* were reported, β-carotene was widely considered by many researchers and the general public to be protective against cancer(s) and cardiovascular diseases. The hypothesis that β-carotene is chemopreventive was tested by ATBC, CARET, and the PHS. The first two trials

Table 3. Relative risks (RRs) and 95% confidence intervals (CIs) for active group versus placebo group for asbestos-exposed participants, smokers, and subpopulations of smokers during the post-intervention period

Exposure (n)	Post-Intervention phase			Intervention phase
	No. of cases in active group	No. cases in placebo group	RR (95% CI)	RR (95% CI)
Lung cancer incidence				
Asbestos-exposed (3693)	61	66	0.92 (0.65 to 1.30)	1.40 (0.95 to 2.07)
Smokers† (13 447)	315	245	1.18 (0.99 to 1.39)	1.23 (0.96 to 1.56)
Current smokers (7000)	198	153	1.22 (0.98 to 1.51)	1.42 (1.07 to 1.87)
Former smokers (6447)	117	92	1.11 (0.85 to 1.47)	0.80 (0.48 to 1.31)
Males (7440)	188	157	1.08 (0.87 to 1.34)	1.25 (0.91 to 1.73)
Females (6007)	127	88	1.33 (1.01 to 1.75)	1.19 (0.82 to 1.72)
All-cause mortality				
Asbestos-exposed (3693)	283	293	0.96 (0.81 to 1.13)	1.25 (1.01 to 1.56)
Smokers† (13 447)	942	754	1.13 (1.02 to 1.24)	1.13 (0.96 to 1.32)
Current smokers (7000)	512	435	1.09 (0.96 to 1.24)	1.15 (0.96 to 1.38)
Former smokers (6447)	430	319	1.18 (1.02 to 1.37)	1.06 (0.76 to 1.48)
Males (7440)	590	515	1.00 (0.89 to 1.13)	1.10 (0.90 to 1.34)
Females (6007)	352	239	1.37 (1.16 to 1.62)	1.16 (0.88 to 1.52)
Lung cancer mortality				
Asbestos-exposed (3693)	50	43	1.16 (0.77 to 1.75)	1.29 (0.75 to 2.22)
Smokers† (13 447)	244	184	1.21 (1.00 to 1.47)	1.55 (1.06 to 2.28)
Current smokers (7000)	145	107	1.27 (0.99 to 1.64)	1.66 (1.07 to 2.56)
Former smokers (6447)	99	77	1.12 (0.83 to 1.52)	1.27 (0.56 to 2.87)
Males (7440)	153	121	1.14 (0.89 to 1.45)	1.62 (0.98 to 2.68)
Females (6007)	91	63	1.33 (0.96 to 1.84)	1.46 (0.81 to 2.62)
Cardiovascular disease mortality				
Asbestos-exposed (3693)	95	103	0.91 (0.69 to 1.21)	1.43 (0.97 to 2.12)
Smokers (13 447)	259	216	1.07 (0.89 to 1.29)	1.16 (0.85 to 1.58)
Current smokers (7000)	129	137	0.87 (0.68 to 1.10)	1.07 (0.75 to 1.53)
Former smokers (6447)	130	79	1.44 (1.08 to 1.91)	1.45 (0.77 to 2.74)
Males (7440)	176	162	0.93 (0.75 to 1.16)	1.05 (0.73 to 1.52)
Females (6007)	83	54	1.44 (1.02 to 2.04)	1.42 (0.80 to 2.54)
Mortality from other causes*				
Asbestos-exposed (3693)	126	132	0.95 (0.74 to 1.21)	1.13 (0.78 to 1.63)
Smokers (13 447)	417	334	1.12 (0.97 to 1.30)	0.92 (0.69 to 1.22)
Current smokers (7000)	220	175	1.15 (0.94 to 1.41)	0.97 (0.69 to 1.35)
Former smokers (6447)	197	159	1.10 (0.89 to 1.35)	0.78 (0.44 to 1.39)
Males (7440)	249	220	0.98 (0.81 to 1.18)	0.95 (0.66 to 1.35)
Females (6007)	168	114	1.37 (1.08 to 1.75)	0.85 (0.52 to 1.39)

*Excludes death from lung cancer and cardiovascular disease.

clearly showed that β-carotene supplements of 20 or 30 mg/day increased the incidence of lung cancer and cardiovascular disease in cigarette smokers and the PHS showed that doses of 50 mg of β-carotene every other day provided no protective benefits against these diseases in former and never smokers. These findings have raised many questions about the assumed benefits and safety of high-dose vitamin supplements.

When the initial findings of CARET were reported, there was no clear explanation for the adverse effects seen in CARET and ATBC. Most investigators concluded that the adverse effects observed with the combination of β-carotene and retinyl palmitate in CARET was primarily due to β-carotene because similar adverse effects were seen in the β-carotene–containing arms of ATBC and because a skin cancer trial that administered even higher doses of retinol than that administered in CARET reported no adverse effects (although that trial was not powered to study lung cancer incidence) *(15)*. The fact that CARET administered a higher dose of β-carotene and reported a greater relative risk of lung cancer (30 mg of β-carotene and RR = 1.28, respectively) than ATBC (20 mg of β-carotene and RR = 1.18, respectively) also suggested a possible dose–response relationship for the adverse effect on lung cancer incidence during the intervention phase. Thus, although it remains possible that it was the combination of retinyl palmitate and β-carotene, and not the higher dose of β-carotene, that was responsible for the higher relative risk of lung cancer observed in CARET, the similar spectrum of adverse effects seen in the β-carotene-containing arms of ATBC and CARET suggests that β-carotene was the agent responsible for the adverse effects.

The rapid increase in lung cancer incidence observed in both ATBC and CARET suggested an effect on the growth of preclinical tumors rather than induction of *de novo* tumors because it is unlikely that tumors of the latter type would become clinically evident within a year or two of the start of drug administration. If only preclinical tumors were affected, however, then stopping the intervention should have resulted in a rapid return to pretreatment cancer risk. Indeed, in ATBC participants, the relative risk of lung cancer returned to 1.0 at 4 years after the end of the intervention. However, in CARET participants, the relative risk of lung cancer remained elevated (although not statistically significantly) 4 years after the end of the intervention. One possible explanation for the more prolonged adverse effect in CARET is the higher dose of β-carotene used; participants may have had a greater deposition of

β-carotene in fat tissue, one of the major sites of β-carotene storage *(15),* than ATBC participants, given that the median body mass index in ATBC participants was 26.0 kg/m^2 *(3),* whereas in CARET participants, it was 27.1 kg/m^2 [27.7 kg/m^2 in males and 25.7 kg/m^2 in females *(11)*]. Alternatively, the concentration of β-carotene may remain elevated in tissue compartments that are crucial to lung carcinogenesis but decline rapidly in compartments that are critical to cardiovascular disease, the relative risk for which rapidly fell to 1.0 in both trials.

The results of our current analyses of data from 6 years of follow-up after the intervention was stopped suggest that the lung cancer relative rate among CARET participants has decreased at a slower rate than that among ATBC participants. This finding is compatible with the hypothesis that β-carotene may cause cellular changes that persist after serum concentrations return to baseline levels. On the other hand, we found that the relative risks of cardiovascular disease mortality among CARET participants rapidly returned to near 1.0, as was also reported for ATBC participants *(8),* which is consistent with the hypothesis that this disease mechanism requires the continued presence of the active agent.

Our follow-up analysis generated several provocative new findings for subgroups of CARET participants. First, we found that the elevated post-intervention relative risk of lung cancer for the total population was due in large part to the statistically significant excess risk of lung cancer among females who took β-carotene and retinyl palmitate. The relative risks of lung cancer and all-cause mortality in females remained elevated compared with those in males throughout the post-intervention follow-up, consistent either with more persistent storage of excess study vitamins in women than in men or with a different mechanism of the adverse effect that resulted in a persisting elevated risk in women. Second, throughout the post-intervention follow-up, the relative risk of cardiovascular disease mortality remained higher in females than in males. The temporal pattern of relative risk in females suggests that β-carotene was cleared from the body stores over a 2- to 4-year period, after which the excess risk in the active group disappeared. These findings contrast with the 6-year post-intervention follow-up findings of the all-male active smoker ATBC cohort *(8).* The relative risk of cardiovascular disease mortality in the β-carotene-containing arms of ATBC rapidly returned to placebo level, whereas the relative risk of lung cancer returned to placebo level more slowly. The mechanisms of the increased risk of cardiovascular disease during active intervention in both males and females and of the rapid return to placebo rates in males and a slower fall in females are unknown.

It has long been recognized that the epidemiology, demographics, and clinical behavior of lung cancer in females differ from those in males *(16,17).* Results of several studies suggest that females are more sensitive than men to the adverse effects of cigarette smoking *(18–20).* In addition, lung cancer in never smokers is more frequent in females than males *(21–23).* Taioli and Wynder *(22)* and Siegfried *(24)* have suggested that estrogens may play a role in the development of lung cancer in female smokers and never smokers. Results of other studies have suggested that hydroxylated estrogen metabolites, such as 4-hydroxyestradiol, can cause oxidative DNA damage and form DNA adducts *(25–27).* The mechanism for differential adverse effects in CARET females could potentially reflect an interaction of either β-carotene or retinyl palmitate with the endogenous or exogenous hormones. High doses of β-carotene in combination with estrogen metabolites may cause additive DNA damage. Alternatively, high-dose β-carotene may augment the metabolism of estradiol to active metabolites.

The Women's Health Study randomly assigned 39 876 women to receive aspirin, α-tocopherol, and β-carotene in a 2 × 2 × 2 factored study design, but the β-carotene component was ended when ATBC and CARET reported their initial findings *(28).* After a median treatment duration of 2.1 years, the investigators reported no effect of β-carotene on the incidence of all cancers (RR = 1.11, 95% CI = 0.78 to 1.58) or of cardiovascular disease (RR = 1.01, 95% CI = 0.62 to 1.63). However, that trial has limitations in confirming or refuting our findings in females because of the short duration of treatment, the low incidence of smoking, the small number of lung cancers, and the lack of post-intervention reports.

Although our findings of post-intervention relative-risk differences between former and current smokers and between females and males are provocative, they should be interpreted with caution because of the possibility of spurious findings due to the multiple statistical tests performed. However, several factors suggest that these differences are real. First, the higher relative risk of cardiovascular disease mortality in former smokers compared with current smokers is consistent with the findings during the intervention phase. Second, the differences in relative risks between females and males were consistent across multiple causes of death; if the difference in the relative risks of all-cause mortality between males and females were a spurious finding, we would anticipate more variability in the cause-specific findings. We emphasize that the subgroup and cause-specific analyses were planned in advance and were not motivated by the findings in the all-cause mortality analysis. Third, the relative risk point estimates in the male heavy smokers were similar to those in the asbestos-exposed participants (all of whom were male).

The findings of ATBC and CARET have stimulated investigators to explore the *in vivo* and *in vitro* effect of high doses of β-carotene and interactions with tobacco smoke. In 1999, Salgo et al. *(29)* suggested that the oxidative stress induced by cigarette smoke in the lung can result in a change in the metabolism of β-carotene, which may actually increase the mutagenicity of tobacco carcinogens. Wang et al. *(30)* studied the effect of β-carotene in cigarette-smoking ferrets and described detrimental effects with a high-dose β-carotene supplement, whereas the low-dose supplement had no effect or provided only weak protection. Paolini et al. *(31)* and Perocco *(33)* have both suggested that high doses of β-carotene can increase conversion of benzo-[*a*]-pyrene to active metabolites. These studies, stimulated by the results of CARET and ATBC, provide some potential mechanisms to explain the detrimental effects of β-carotene and the adverse interaction with tobacco smoke. Because of the rapid onset of adverse effects in both ATBC and CARET, these mechanisms may be important in the progression of preclinical tumors and in the persisting effect of increased lung cancer incidence, as we have seen in CARET. The persisting adverse effect in females versus males provides additional evidence of the potential of hormonal effects on lung cancer etiology.

In conclusion, the excess risks of lung cancer and all-cause mortality that existed at the end of the active intervention phase of CARET persisted after 6 years of post-intervention follow-up, albeit at lower and not statistically significant levels. Results of

planned subgroup analyses suggest that the excess risks of lung cancer were restricted primarily to females and of cardiovascular disease mortality primarily to females and to former smokers. However, results of our analyses of the primary endpoints in the full study population were not statistically significant, and results of secondary endpoint and subgroup analyses, although nominally statistically significant, may be spurious, because they resulted from multiple testing. Additional follow-up of CARET participants will be necessary to confirm or refute these trends.

When chemoprevention agents are administered to large, healthy populations, it is necessary to document long-term safety, efficacy and, importantly, the duration of the beneficial (or adverse) effect. This is especially true when the basic underlying molecular and genetic mechanism of the agent is unclear. The results of CARET and ATBC emphasize that chemoprevention trials require careful monitoring of all disease endpoints, both during active intervention and during long-term follow-up, even after the study intervention is discontinued.

References

(1) Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ. Cancer statistics, 2003. CA Cancer J Clin 2003;53:5–26.

(2) Peto R, Doll R, Buckley JD, Sporn MB. Can dietary beta-carotene materially reduce human cancer rates? Nature 1981;290:201–9.

(3) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N Engl J Med 1994;330:1029–35.

(4) Omenn GS, Goodman G, Thornquist M, Grizzle J, Rosenstock L, Barnhart S, et al. The beta-carotene and retinol efficacy trial (CARET) for chemoprevention of lung cancer in high risk populations: smokers and asbestos-exposed workers. Cancer Res 1994;54(7b Suppl):2038s–2043s.

(5) Albanes D, Heinonen OP, Taylor PR, Virtamo J, Edwards BK, Rautalahti M, et al. Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the alpha-tocopherol, beta-carotene cancer prevention study: effects of base-line characteristics and study compliance. J Natl Cancer Inst 1996;88:1560–70.

(6) Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, et al. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 1996;334:1150–5.

(7) Omenn GS. Chemoprevention of lung cancer: the rise and demise of beta-carotene. Annu Rev Public Health 1998;19:73–99.

(8) Virtamo J, Pietinen P, Huttunen JK, Korhonen P, Malila N, Virtanen MJ, et al. Incidence of cancer and mortality following alpha-tocopherol and beta-carotene supplementation: a postintervention follow-up. JAMA 2003; 290:476–85.

(9) Omenn GS, Goodman GE, Thornquist MD, Rosenstock L, Barnhart S, Gylys-Colwell I, et al. The Carotene and Retinol Efficacy Trial (CARET) to prevent lung cancer in high-risk populations: pilot study with asbestos-exposed workers. Cancer Epidemiol Biomarkers Prev 1993;2:381–7.

(10) Goodman GE, Omenn GS, Thornquist MD, Lund B, Metch B, Gylys-Colwell I. The Carotene and Retinol Efficacy Trial (CARET) to prevent lung cancer in high-risk populations: pilot study with cigarette smokers. Cancer Epidemiol Biomarkers Prev 1993;2:389–96.

(11) Thornquist MD, Omenn GS, Goodman GE, Grizzle JE, Rosenstock L, Barnhart S, et al. Statistical design and monitoring of the Carotene and Retinol Efficacy Trial (CARET). Control Clin Trials 1993;14:308–24.

(12) Omenn GS, Goodman GE, Thornquist M, Balmes J, Cullen MR, Glass A, et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst 1996;88: 1550–9.

(13) Bowen DJ, Thornquist M, Anderson K, Barnett M, Powell C, Goodman G, et al. Stopping the active intervention: CARET. Control Clin Trials 2003; 24:39–50.

(14) Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, et al. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N Engl J Med 1996;334:1145–9.

(15) Moon TE, Levine N, Cartmel B, Bangert JL, Rodney S, Dong Q, et al. Effect of retinol in preventing squamous cell skin cancer in moderate-risk subjects: a randomized, double-blind, controlled trial. Southwest Skin Cancer Prevention Study Group. Cancer Epidemiol Biomarkers Prev 1997; 6:949–56.

(16) Zang EA, Wynder EL. Differences in lung cancer risk between men and women: examination of the evidence. J Natl Cancer Inst 1996;88:183–92.

(17) Patel JD, Bach PB, Kris MG. Lung cancer in US women: a contemporary epidemic. JAMA 2004;291:1763–8.

(18) Baldini EH, Strauss GM. Women and lung cancer: waiting to exhale. Chest 1997;112(4 Suppl):229S–34S.

(19) Brownson RC, Chang JC, Davis JR. Gender and histologic type variations in smoking-related risk of lung cancer. Epidemiology 1992;3:61–4.

(20) McDuffie HH, Klaassen DJ, Dosman JA. Female-male differences in patients with primary lung cancer. Cancer 1987;59:1825–30.

(21) Kirsh MM, Tashian J, Sloan H. Carcinoma of the lung in women. Ann Thorac Surg 1982;34:34–9.

(22) Taioli E, Wynder EL. Re: Endocrine factors and adenocarcinoma of the lung in women. J Natl Cancer Inst 1994;86:869–70.

(23) Sellers TA, Potter JD, Folsom AR. Association of incident lung cancer with family history of female reproductive cancers: the Iowa Women's Health Study. Genet Epidemiol 1991;8:199–208.

(24) Siegfried JM. Women and lung cancer: does oestrogen play a role? Lancet Oncol 2001;2:506–13.

(25) Cavalieri E, Frenkel K, Liehr JG, Rogan E, Roy D. Estrogens as endogenous genotoxic agents—DNA adducts and mutations. J Natl Cancer Inst Monogr 2000(27):75–93.

(26) Li KM, Todorovic R, Devanesan P, Higginbotham S, Kofeler H, Ramanathan R, et al. Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone in vitro and in female ACI rat mammary gland in vivo. Carcinogenesis 2004;25:289–97.

(27) Yager JD. Endogenous estrogens as carcinogens through metabolic activation. J Natl Cancer Inst Monogr 2000;(27):67–73.

(28) Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. J Natl Cancer Inst 1999;91:2102–6.

(29) Salgo M, Cueto R, Winston G, Pryor W. Beta-carotene and its oxidation products have different effects on microsome mediated binding of benzo-[A]pyrene to DNA. Free Radical Biol Med 1999;26:162–73.

(30) Wang XD, Liu C, Bronson RT, Smith DE, Krinsky NI, Russell M. Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. J Natl Cancer Institute 1999; 91:60–6.

(31) Liu C, Wang XD, Bronson RT, Smith DE, Krinsky NI, Russell RM. Effects of physiological versus pharmacological beta-carotene supplementation on cell proliferation and histopathological changes in the lungs of cigarette smoke-exposed ferrets. Carcinogenesis 2000;21:2245–53.

(32) Paolini M, Cantelli-Forti G, Perocco P, Pedulli GF, Abdel-Rahman SZ, Legator MS. Co-carcinogenic effect of beta-carotene. Nature 1999;398: 760–1.

(33) Perocco P, Paolini M, Mazzullo M, Biagi G, Cantelli-Forti G. beta-carotene as enhancer of cell transforming activity of powerful carcinogens and cigarette-smoke condensate on BALB/c 3T3 cells in vitro. Mutat Res 1999;440:83–90.

Notes

Supported by Public Health Service grant U01 CA63673 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services to G. E. Goodman.

Manuscript received July 2, 2004; revised September 23, 2004; accepted October 1, 2004.