

# The Effect of Alpha-Tocopherol and Beta-Carotene Supplementation on COPD Symptoms

MATTI RAUTALAHTI, JARMO VIRTAMO, JARI HAUKKA, OLLI P. HEINONEN, JOUKO SUNDVALL, DEMETRIUS ALBANES, and JUSSI K. HUTTUNEN

National Public Health Institute, Helsinki, Finland; and National Cancer Institute, Bethesda, Maryland

The effects of alpha-tocopherol (50 mg/d) and beta-carotene (20 mg/d) supplementation on symptoms of chronic obstructive pulmonary disease were studied among the 29,133 participants of the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study undertaken to investigate the effects of these two substances in the prevention of lung and other cancers. During the follow-up the supplementations did not affect the recurrence or incidence of chronic cough, phlegm, or dyspnea. The prevalence of chronic bronchitis and dyspnea at baseline was lower among those with high dietary intake of beta-carotene (OR = 0.78 and 0.67, respectively) or vitamin E (OR = 0.87 and 0.77) and high serum beta-carotene (OR = 0.59 and 0.62) and alpha-tocopherol (OR = 0.76 and 0.82). High intake and serum levels of retinol were associated with low prevalence of dyspnea (OR = 0.84 and 0.80, respectively) but not with chronic bronchitis. The results indicate no benefit from supplementation with alpha-tocopherol or beta-carotene on the symptoms of chronic obstructive pulmonary disorders but support the beneficial effect of dietary intake of fruits and vegetables rich in these compounds. Rautalahti M, Virtamo J, Haukka J, Heinonen OP, Sundvall J, Albanes D, Huttunen JK. The effect of alpha-tocopherol and beta-carotene supplementation on COPD symptoms.

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The hypothesis that oxidants might play a decisive role in the pathogenesis of lung damage and pulmonary diseases has been intensely studied for the past few decades. In the natural, oxygen-rich environment of lungs, the toxic effects of oxidants are carefully balanced by several antioxidant defense systems (1). Maintenance of this balance is critical for the support of normal cellular function. Thus, an increase in exogenous or endogenous oxidative stress or decrease in antioxidant capacity can lead to a series of events in the lung that culminates in tissue damage and dysfunction.

Cigarette smoke is considered to be the most important cause of chronic obstructive bronchopulmonary diseases (COPD). Although the mechanisms by which smoking causes COPD are not fully understood, there are several possibilities. Cigarette smoke increases the oxidant burden on the lungs and can stimulate neutrophils and macrophages to increase production of proteases and also oxidants, which can inactivate the protease inhibitors (2). These changes could lead to a protease-antiprotease imbalance within the alveolar structures (3) and to the development of chronic inflammation and emphysema (4). The established role of oxidants in tissue injury and disease processes has promoted the idea of antioxidants acting as preventive agents. The purpose of this study was to investigate whether supplementation with two antioxi-

dants, alpha-tocopherol and beta-carotene, could affect the recurrence and incidence of COPD-related symptoms and whether dietary intake and serum levels of these substances are related to the prevalence of these symptoms in a cohort of smokers.

## METHODS

### Participants

The study population consisted of 29,133 male smokers, 50-69 yr old, randomized into the Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) Study during the years 1985-1988 to receive supplementation with dl-alpha-tocopherol (AT; 50 mg/d), beta-carotene (BC; 20 mg/d), both AT (50 mg/d) and BC (20 mg/d), or placebo (5). The primary objective of the ATBC Study was to investigate the efficacy of alpha-tocopherol and beta-carotene in cancer prevention. At baseline, all members of the study population were current smokers of at least five cigarettes daily and had smoked for a median of 36 yr. Smoking cessation was allowed and encouraged from the very beginning of the follow-up.

The baseline prevalences of COPD symptoms were studied in relation to the dietary intakes of beta-carotene, retinol, and vitamin E, and serum levels of beta-carotene, retinol, and alpha-tocopherol.

The recurrence of the pulmonary symptoms during follow-up was studied in relation to the supplementations with beta-carotene and alpha-tocopherol. These analyses were done in the total study population of 29,133 men.

The incidence of COPD symptoms was followed up among the 10,284 men who at baseline reported no current symptoms of chronic cough or phlegm or dyspnea. These men were evenly distributed in the four intervention groups (AT 2,596, BC 2,564, ATBC 2,581, placebo 2,543).

The follow-up continued until drop-out, death, or end of supplementation (April 1993) and lasted for 5 to 8 yr with a median of 6.1 yr. A total of 9,061 men (31.1%) dropped out of the study before the end

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Correspondence and requests for reprints should be addressed to Matti Rautalahti, National Public Health Institute, Mannerheimintie 166, SF-00300 Helsinki, Finland. E-mail: Matti.Rautalahti@ktl.fi

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of follow-up, evenly distributed in the intervention groups. No symptom-based endpoint ascertainment was available after the drop-out occurred.

## Methods

Various personal, dietary, and health-related information was collected at baseline and during the regular follow-up visits. The dietary data were collected with a food use questionnaire developed for the study (6). Together with available comprehensive nutrient data (7), this method facilitated quantitative estimation of various nutrient intakes, including vitamin E, beta-carotene, and vitamin A. Information on use of vitamin and trace element supplements was also recorded at baseline.

The symptoms of cough, phlegm, and dyspnea were queried with a modified Medical Research Council (MRC) questionnaire (8) at baseline and annually thereafter. Ascertainment of symptoms was based on interviews conducted by specially trained, registered nurses. Participants were considered to have cough if they reported coughing almost daily for at least 3 mo every year and unusual production of phlegm if they reported bringing up phlegm almost daily for at least 3 mo every year. The symptom-based definition of chronic bronchitis required both the cough and phlegm symptoms described above. Although there is no specific symptom for emphysema, it is the predominant lesion in persons with COPD-related airflow obstruction (9). Dyspnea was used as a proxy for emphysema and it was defined as having to walk slower than people of the same age on the level or up a slight hill because of breathlessness. Participants reporting to have asthma, angina pectoris, cardiac insufficiency, cardiomegaly, valvular disease, or arrhythmias were not considered for COPD-related dyspnea diagnosis at baseline or during follow-up. A participant was considered to have stopped smoking when he reported not having smoked during the past 8 mo.

Information on hospital admissions due to unspecified bronchitis (ICD-9 490), chronic bronchitis (ICD-9 491), emphysema (ICD-9 492), or other unspecified COPD (ICD-9 496) during the follow-up period was collected from the National Hospital Discharge Register. It contains the dates of hospital admission and discharge and up to four ICD-coded discharge diagnoses, the first of them being the principal cause of the hospital stay.

Blood samples were collected from all participants during the baseline visit and third annual visit and serum aliquots were stored at  $-70^{\circ}$  C. Concentrations of alpha-tocopherol, beta-carotene, and retinol were measured by high-performance liquid chromatography assay (10).

## Statistical Methods

In the statistical analyses of the effects of AT and BC supplements, the participants were compared by supplementation: (1) the men receiving alpha-tocopherol (AT) versus the men not receiving alpha-tocopherol (no AT); (2) the men receiving beta-carotene (BC) versus the men not receiving beta-carotene (no BC).

**Models for prevalent symptoms.** The prevalences of chronic cough, phlegm, bronchitis, and dyspnea and self-reported diagnosis of chronic bronchitis and emphysema were analyzed with logistic regression models. Baseline values of age (5-yr intervals), alcohol consumption (0, > 0–30 g/d, > 30 g/d), urbanization (rural, urban), body mass index (BMI; < 24, 24–27, > 27), cigarette consumption (5–10, 11–20, over 20/d), serum alpha-tocopherol (< 10.3, 10.3–12.8, > 12.8 mg/l), beta-carotene (< 129, 129–225, > 225  $\mu$ g/l), retinol (< 526, 526–630, > 630  $\mu$ g/l), and dietary intake of vitamin E (< 6.8, 6.8–12.4, > 12.4 mg/d), beta-carotene (< 1.2, 1.2–2.3, > 2.3 mg/d), and retinol (< 0.9, 0.9–2.0, > 2.0 mg/d) were used as explanatory variables in the models.

**Models for recurrent symptoms.** Symptoms during follow-up over time have locally a Poisson distribution, conditional of its past, and thus the past was taken into account by Poisson regression modeling. Several events could occur in succession. We considered every follow-up period as a sampling, each zero indicating that no events were observed during the period, and ones indicating an event occurring and each forming a risk set. Both the time since last symptom and the number of previous symptoms were included in the Poisson regression models. Other variables in the models are supplementation, follow-up period, age (5-yr intervals), number of daily cigarettes at a given visit (0, 1–20, > 20), and alcohol consumption (0, > 0–30, > 30 g/d) at baseline. All variables were categorized to allow the aggregation of data.

The number of previous symptoms and the time since last symptom were both log-transformed in the models. The time since last symptom was not available before the first symptom had occurred, thus an interaction term between an indicator variable of one or more previous symptoms and time since last symptoms were included in the model. The results of Poisson regression analysis are shown as relative risks of getting a symptom compared to those with no symptoms. We used log-linear models, which produce multiplicative models, to model incidence of symptoms (11–13).

TABLE 1  
BASELINE CHARACTERISTICS OF THE STUDY PARTICIPANTS BY TREATMENT GROUPS\*

	AT (n = 7,286)		BC (n = 7,282)		ATBC (n = 7,278)		Placebo (n = 7,287)	
Age, yr	57.7	(5.1)	57.8	(5.1)	57.8	(5.0)	57.6	(5.1)
Smoking								
Age at starting	19.5	(4.7)	19.5	(4.9)	19.5	(4.6)	19.4	(4.8)
Regular smoking, yr	35.9	(8.5)	36.0	(8.5)	35.9	(8.5)	35.9	(8.4)
Amount, cigarettes/d	20.6	(8.8)	20.4	(8.9)	20.4	(8.9)	20.4	(8.7)
Serum								
Alpha-tocopherol, mg/L	11.9	(3.7)	12.0	(3.7)	12.0	(3.4)	11.9	(3.5)
Beta-carotene, $\mu$ g/L	214.3	(422.9)	212.6	(185.9)	218.0	(424.9)	211.4	(182.5)
Retinol, $\mu$ g/L	586.9	(131.7)	588.6	(134.0)	587.8	(128.0)	589.5	(129.1)
Total cholesterol, mmol/L	6.2	(1.2)	6.2	(1.2)	6.2	(1.2)	6.2	(1.2)
Intake								
Vitamin E, mg/d	12.0	(5.7)	12.1	(5.8)	12.1	(5.7)	12.0	(5.7)
Beta-carotene, mg/d	2.1	(1.6)	2.1	(1.6)	2.1	(1.5)	2.2	(1.5)
Vitamin A, mg/d	1.8	(1.5)	1.8	(1.5)	1.9	(1.5)	1.8	(1.5)
Alcohol, g/d	18.1	(21.6)	18.0	(22.0)	17.8	(21.2)	18.0	(21.5)
BMI, kg/m <sup>2</sup>	26.3	(3.8)	26.2	(3.8)	26.3	(3.7)	26.3	(3.9)
Type of residence area, %								
Rural	64.0		63.6		63.6		64.0	
Urban	36.0		36.4		36.4		36.0	

Definition of abbreviations: AT = alpha-tocopherol only; BC = beta-carotene only; ATBC = alpha-tocopherol and beta-carotene combined.

\* n = 29,133. Means (standard deviations), or proportions (%).

TABLE 2  
PREVALENCE (%) OF SYMPTOMS RELATED TO COPD  
AMONG THE STUDY PARTICIPANTS AT BASELINE

	AT n = 7,286	BC n = 7,282	ATBC n = 7,278	Placebo n = 7,287
Cough almost every day or night for at least 3 mo every year	40.0	40.3	40.5	40.7
Phlegm almost every day or night for at least 3 mo every year	43.1	42.2	43.1	42.8
Chronic bronchitis*	31.1	31.4	31.6	31.0
Dyspnea when hurrying on level or walking up a slight hill <sup>†</sup>	24.7	27.2	25.7	25.6

Definition of abbreviations: AT = alpha-tocopherol only; BC = beta-carotene only; ATBC = alpha-tocopherol and beta-carotene combined.

\* Cough and sputum almost every day or night for at least 3 mo every year.

<sup>†</sup> Men with cardiac problems and asthma at baseline have been excluded.

**Models for incident symptoms.** The incidence of chronic cough, phlegm, bronchitis, and dyspnea was analyzed with Cox regression models. Follow-up started in the beginning of the supplementation and only persons without any of the symptoms described above at baseline were followed. All events were recorded with a one-year interval. Baseline values of age (5-yr intervals), alcohol consumption (0, < 30 g/d, > 30 g/d), urbanization (rural/urban), body mass index (less than 24, 24–27, > 27), cigarette consumption (5–10, 11–20, over 20/d), total serum cholesterol, alpha-tocopherol, beta-carotene, retinol (each tertile), and dietary intake of vitamin E, beta-carotene and retinol (each tertile) were used as explanatory variables in the models. All persons were smokers at baseline and thus smoking cessation during the study was treated as a time-dependent explanatory variable.

Assumption of proportional hazards was tested and no reason to reject it was detected. Possible interaction between supplementation groups and effect modification between various background variables and supplementations were tested with Cox regression models.

## RESULTS

Baseline characteristics of the study population are presented in Table 1. There were no differences between the four inter-

vention groups. All participants were long-term smokers (median 36 yr) with little variation in their smoking histories and smoked mostly manufactured cigarettes (median 20 cigarettes/d). Prevalence of pulmonary symptoms (Table 2) were also similarly distributed in the intervention groups at baseline. The intervention groups also had no differences in the baseline prevalences of self-reported, physician-made diagnosis of asthma (3.1%), emphysema (6.5%), chronic bronchitis (7.7%), farmer's lung (0.3%), angina pectoris (7.6%), cardiac insufficiency (4.2%), cardiomegaly (4.9%), valvular disease (1.0%), and arrhythmias (13.7%). During the follow-up, 21% of the participants stopped smoking, without differences in the intervention groups.

High dietary intake of beta-carotene or vitamin E and high serum beta-carotene and alpha-tocopherol were associated with low prevalence of chronic bronchitis symptoms and dyspnea at baseline (Table 3). High intake and serum levels of retinol were associated with low prevalence of dyspnea but not with chronic bronchitis symptoms. The odds ratios for chronic cough and phlegm were similar to those of their composite, chronic bronchitis. Adjustment for age, alcohol intake, type of residential area, BMI, and number of daily cigarettes did not affect the odds ratios. The effects of baseline dietary intakes and serum levels on the incidence of COPD symptoms during follow-up were analyzed in the placebo group. The relative risks were suggestive of similar associations at baseline, but with few exceptions, and were not statistically significant (data not shown).

The results of the Poisson regression models indicate that during the follow-up supplementation with AT or BC did not affect the probability of symptom recurrence (Table 4). The models included follow-up time, time since last symptom, number of previous symptoms, age, number of daily cigarettes, and baseline alcohol consumption. Baseline serum alpha-tocopherol and beta-carotene concentrations had no effect on the symptom recurrence and were left out of the final model. During the follow-up, 8,467 men had at least two recurrences of chronic bronchitis symptoms, and 11,117 men had at least two recurrences of dyspnea.

TABLE 3  
ODDS RATIOS FOR COPD SYMPTOMS AT BASELINE IN RELATION TO INTAKE AND SERUM  
LEVELS OF BETA-CAROTENE, ALPHA-TOCOPHEROL/VITAMIN E, AND RETINOL

	(tertile limits)	Cough*	Phlegm <sup>†</sup>	Chronic Bronchitis <sup>‡</sup>	Dyspnea <sup>§</sup>
		OR <sup>¶</sup> (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
<b>Intake</b>					
Beta-carotene, mg/d	(1.2, 2.3)	0.75 (0.71, 0.80)	0.82 (0.77, 0.87)	0.78 (0.73, 0.83)	0.67 (0.62, 0.71)
Vitamin E, mg/d	(8.6, 12.4)	0.89 (0.84, 0.94)	0.91 (0.86, 0.97)	0.87 (0.82, 0.93)	0.78 (0.73, 0.84)
Retinol, mg/d	(0.9, 2.0)	1.09 (1.03, 1.16)	1.02 (0.97, 1.09)	1.08 (1.02, 1.15)	0.86 (0.80, 0.92)
<b>Serum</b>					
Beta-carotene, µg/L	(129, 225)	0.57 (0.54, 0.60)	0.67 (0.62, 0.70)	0.59 (0.55, 0.62)	0.62 (0.58, 0.66)
Alpha-tocopherol, mg/L	(10.3, 12.8)	0.73 (0.69, 0.77)	0.80 (0.76, 0.85)	0.76 (0.71, 0.80)	0.80 (0.75, 0.85)
Retinol, µg/L	(526, 630)	1.03 (0.97, 1.09)	0.97 (0.92, 1.03)	1.01 (0.95, 1.08)	0.81 (0.76, 0.87)

\* Cough almost every day or night for at least 3 mo every year.

<sup>†</sup> Phlegm almost every day or night for at least 3 mo every year.

<sup>‡</sup> Cough and phlegm almost every day or night for at least 3 mo every year.

<sup>§</sup> Dyspnea when hurrying on level or walking up a slight hill.

<sup>¶</sup> Odds ratios (OR) and their 95% confidence intervals (95% CI) are expressed as the highest versus lowest tertile.

TABLE 4

## RELATIVE RISKS FOR THE RECURRENCE OF COPD SYMPTOMS DURING FOLLOW-UP BY SUPPLEMENTATION

Symptom	Alpha-tocopherol versus no Alpha-tocopherol*		Beta-carotene versus no Beta-carotene†	
	RR‡	95% CI	RR‡	95% CI
Cough§	1.00	0.98, 1.03	1.00	0.97, 1.02
Phlegm§	1.01	0.98, 1.03	1.00	0.98, 1.03
Chronic bronchitis¶	1.01	0.98, 1.04	0.99	0.96, 1.02
Dyspnea	1.02	0.99, 1.05	1.00	0.97, 1.03

\* Supplementation with alpha-tocopherol (AT and ATBC groups combined) compared with no alpha-tocopherol supplementation (BC and placebo combined).

† Supplementation with beta-carotene (BC and ATBC groups combined) compared with no beta-carotene supplementation (AT and placebo groups combined).

‡ Poisson regression model including follow-up time, time since last symptom, number of previous symptoms, age, number of daily cigarettes at a given visit (0, 1–20, > 20), and baseline alcohol consumption (0, > 0–30, > 30 g/d).

§ Almost every day or night for at least 3 mo every year.

¶ Cough and phlegm almost every day or night for at least 3 mo every year.

|| Dyspnea when hurrying on level or walking up a slight hill.

The longer the participants were symptomless, the smaller was their risk of getting a relapse. Stopping smoking reduced the risk of recurrence of cough and phlegm symptoms even more. This was not the case with dyspnea where smoking cessation did not predict recurrence (data not shown). The number of symptom relapses was positively associated with yet another recurrence, i.e., the more often a participant reported symptoms, the greater the risk of having them on the next follow-up visit. With regard to cough and phlegm but not dyspnea, this was more pronounced among current smokers. Those who stopped smoking exhibited a similar pattern but on a somewhat lower level (data not shown). Symptom recurrence was also increased by age and with cough and phlegm by the level of alcohol intake at baseline.

Supplementation with beta-carotene or alpha-tocopherol did not have a major effect on the incidence of chronic cough, phlegm, bronchitis, or dyspnea during follow-up among those men who were free of these symptoms at baseline (Table 5). The results are, however, suggestive of increased risks. The point estimates of risk were above unity but did not reach statistical significance. These results did not change when adjusted for baseline age, serum AT and BC levels, alcohol intake, number of daily cigarettes, type of residence area, BMI, follow-up time, and smoking cessation during follow-up. No interaction between the supplementations was found. No consistent picture of effect modification emerged with smoking, age, baseline serum levels of AT and BC, changes of serum levels of AT and BC during follow-up, and intakes of vitamin E, beta-carotene, and alcohol.

Smoking cessation had a striking effect on the risk of developing COPD symptoms. Among the men who stopped smoking, relative risk for cough was 0.17 (95% confidence interval [CI] 0.13, 0.21; n = 65), for phlegm, 0.22 (95% CI 0.18, 0.28; n = 94), for chronic bronchitis, 0.16 (95% CI 0.12, 0.22; n = 45), and for dyspnea, 0.61 (95% CI 0.52, 0.70; n = 198) compared with the men who continued smoking. All these relative risks were adjusted for supplementation, dietary intakes of BC, vitamin E, and retinol, serum levels of AT, BC, and retinol, age, alcohol intake, type of residence area, BMI, and number of daily cigarettes. Smoking cessation did not modify the effects of beta-carotene or alpha-tocopherol supplementations.

The number of participants admitted to a hospital due to a COPD diagnosis during the follow-up was smaller compared

TABLE 5

## NUMBER OF NEW CASES AND RELATIVE RISK OF THE INCIDENCE OF COPD SYMPTOMS DURING FOLLOW-UP BY SUPPLEMENTATION

Symptom	Supplementation			
	AT	No AT	BC	No BC
Cough*				
Number of new cases	1,540	1,477	1,547	1,470
Relative risk	1.04	1.00	1.07	1.00
95% confidence interval	0.96, 1.11	—	0.99, 1.15	—
Phlegm*				
Number of new cases	1,710	1,655	1,713	1,652
Relative risk	1.02	1.00	1.05	1.00
95% confidence interval	0.96, 1.10	—	0.98, 1.13	—
Chronic bronchitis†				
Number of new cases	1,145	1,067	1,138	1,074
Relative risk	1.07	1.00	1.07	1.00
95% confidence interval	0.98, 1.16	—	0.99, 1.17	—
Dyspnea‡				
Number of new cases	1,467	1,369	1,411	1,425
Relative risk	1.07	1.00	0.98	1.00
95% confidence interval	0.99, 1.15	—	0.91, 1.06	—

Definition of abbreviations: AT = supplementation with alpha-tocopherol (AT and ATBC groups combined); No AT = no supplementation with alpha-tocopherol (BC and placebo groups combined); BC = supplementation with beta-carotene (BC and ATBC groups combined); No BC = no supplementation with beta-carotene (AT and placebo groups combined).

\* Almost every day or night for at least 3 mo every year.

† Cough and phlegm almost every day or night for at least 3 mo every year.

‡ Dyspnea when hurrying on level or walking up a slight hill.

with the symptom-based definition of cases: 102 cases of unspecified bronchitis, 1,588 cases of chronic bronchitis, 91 cases of emphysema, and only nine cases of unspecified COPD. Thus, the diagnostic groups were combined for analyses of supplementation effects. Neither of the antioxidant supplements had a statistically significant effect on the risk of being admitted to a hospital due to a COPD diagnosis. The relative risk for alpha-tocopherol was 1.03 (95% CI 0.91, 1.15) and for beta-carotene, 0.99 (95% CI 0.88, 1.11).

## DISCUSSION

The results of this study indicate that a relatively short-term, albeit intensive supplementation with antioxidants cannot control or prevent the development of chronic cough, phlegm, or dyspnea among men with decades of accumulated smoking years. Diet rich in antioxidants, measured both as intake and serum levels of alpha-tocopherol and beta-carotene and reflecting a more long term intake, is inversely associated with the prevalence of smoking-related, chronic pulmonary symptoms.

The methods used in this study for end-point assessment pose certain limitations. The validity and reliability of the MRC and similar questionnaires have been extensively documented (14). While the survey methods can give reliable information on chronic productive cough, i.e., chronic bronchitis, the other clinical feature of COPD, emphysema, constitutes a more problematic entity. Dyspnea is a relatively unreliable indicator of emphysema, since it is a common symptom in many cardiovascular diseases. Although the prevalences of various cardiac disorders at baseline were balanced in the four intervention groups and men with cardiac problems at baseline were excluded from the dyspnea cases, they inevitably included also those with a nonpulmonary etiology. Thus our results regarding dyspnea are not specific to emphysema.

The participants demonstrated no imbalance at baseline in relation to the various known risk factors for chronic cough,

phlegm, and dyspnea between the intervention groups. This supports the presumption that randomization ensured the balance of essential factors. It is important to bear in mind that all participants of this study had smoked for decades with relatively little variation in their lifetime smoking history and current smoking habits. Smoking starts to cause functional and structural changes in the lungs already after a few years (9), and thus, it is obvious that practically all participants had some changes in their lungs regardless of the reported symptoms at baseline. The prevalence of COPD symptoms at the end of follow-up in this study is of the same magnitude found previously in elderly, smoking Finns (15).

There are several possible explanations why lower doses of dietary antioxidants are beneficial, but higher doses of supplementary antioxidants do not offer protection against free radicals. It is widely accepted that in a healthy organism there exists a balance between oxidants and various antioxidants. It is often forgotten that reactive oxygen species are not solely harmful, but they are involved in many defense reactions of the cells. High levels of antioxidants may disturb these reactions with unpredictable and unexpected consequences.

Length of usage of the antioxidant supplementation is another issue: how long must one use it to bring about the maximum or any effects. Also, there can be periods in the disease process that are more susceptible to the effects of antioxidants. Very little is known what comes to antioxidant supplementation and prevention of COPD symptoms. It is plausible that the antioxidant supplementation was given too late or for a too short period to time, considering the long smoking histories of the participants. The data available in this study does not permit further evaluation of these possibilities. While current dietary intakes of vitamin E and beta-carotene do not necessarily measure long-term intake accurately, they are in most cases at least reflections of a more chronic exposure to these and related substances. Dietary intakes could thus be valid measures of the preventive potential of antioxidants in relation to COPD.

A third possibility is that we chose wrong substances for supplementation or that we used wrong doses. Experimental studies have shown that tracheal epithelial cells can be stimulated to generate reactive oxygen species into the airway lumen, but these substances are detoxified (16) and the epithelial cells are protected against oxidant-induced injury (17). Alpha-tocopherol has been shown to be important in these defense mechanisms (18). In humans, respiratory tract lining fluid contains antioxidants secreted by epithelial cells (19). Smoking has been shown to decrease the vitamin E concentration of alveolar fluid and the difference to nonsmokers could not be corrected with massive, 3 wk supplementation (20). It has been pointed out that while vitamin E decreases the toxicity of oxidizing factors, it appears to be unable to completely counteract excessive levels (21).

The functions and actions of beta-carotene and retinol have been extensively studied in general and particularly in relation to the mechanisms of carcinogenesis (22). However, their specific role in the defense mechanisms of the lungs is less well characterized. The lung tissue of rats fed a diet high in antioxidants including beta-carotene has been shown to exhibit protection against oxidative damage (18). Retinol functions as a key factor for the maintenance of cell differentiation of bronchopulmonary epithelium (23). Both beta-carotene and retinol are measurable from human lung tissue, but it does not appear to be the particular target organ with high concentrations (24). In experimental studies, beta-carotene has been shown to exhibit prooxidant properties in high oxygen pressure (25). It has been speculated that similar conditions could

exist in the lungs (25), though these results have been disputed (26). The beta-carotene dose used in the ATBC Study was about 10-fold compared with the mean dietary intake at baseline among the participants and increased the serum levels 17-fold compared with baseline values (5). Unpublished results from the ATBC Study show a 7-fold increase in the beta-carotene concentration of buccal mucosal cells due to beta-carotene supplementation. No information is available as to how the supplementation affected lung tissue concentrations, but based on other studies of the diet-tissue relationship (27), it can be assumed that they were also raised.

Antioxidants like alpha-tocopherol, beta-carotene, and superoxide dismutases can produce secondary radicals when reacting with oxidants (28), especially in high concentrations (29). The alpha-tocopherol dose in the ATBC Study was not extremely high, but it nevertheless increased the serum levels by 50% (5). The relatively modest intake of vitamin C among the study participants (5) might not have been enough to reduce alpha-tocopheroxyl radical formed in the AT-radical reaction back to functional AT. A similar interactive relationship might exist between beta-carotene and vitamin C (28). The imbalance in the serum concentrations of AT, BC, and vitamin C might have invalidated protective effects of high concentrations of AT and BC. Based on experimental work, it has been proposed that the benefits brought by an antioxidant might be obtained only within a certain range of tissue concentration (30).

It is also possible that the deleterious effects of cigarette smoke on the integrity of lungs are mediated by mechanisms other than damage induced by free radicals and other oxygen-derived species. For example, exogenous antioxidants cannot inhibit the protein modifications caused by aldehydes in smoke (31) and supplemental vitamin E could not decrease the enhanced cytotoxicity of alveolar macrophages of smokers (32).

The possibility that supplemental AT and BC could actually increase the incidence of COPD symptoms is intriguing. No experimental or epidemiological data are even suggestive of this kind of an effect. Smoking has major effects on respiratory tissues and mucosal function. It causes epithelial cell dysfunction, secretory cell metaplasia, increased mucus production, and recruitment of leukocytes, which in turn increase the production of oxygen radicals (33). These changes, together with the resulting ciliostasis, lead to disturbance in the mucociliary clearance (34) and contribute to the retention of mucus and inhaled impurities in the lungs. In theory, supplemental antioxidants with the resulting increase in the tissue levels could counteract the effects of the oxygen radicals on ciliary function. The improved ciliary clearance could result in mobilization of mucus. Clinically, this would be observed as productive cough. However, the definition of an incident case with new COPD symptoms is problematic in this study. As pointed out earlier, all participants of this study had a long history of smoking. It is possible and even probable that they had experienced symptoms previously but happened to be free of symptoms at baseline of this study due to the natural variation in the course of the disease. This warrants for caution in our conclusions of the incidence results.

Dietary factors can affect the long term incidence of chronic pulmonary disease as demonstrated in the Dutch Zutphen Study (35). Interestingly, however, while fruit intake was inversely and statistically significantly related to the incidence of the lung diseases, intakes of specific antioxidants including beta-carotene and vitamin C were not. No other results from antioxidant intervention trials on the pulmonary symptoms have been reported. It appears that while the consumption of fruits and vegetables containing antioxidant is associated with

lower prevalence and incidence of pulmonary conditions, the identification of individual effective substances has not been successful. Single dietary constituents that appear to be associated with disease prevalence and incidence, can actually be surrogates of more complex entities. This can also be the case with such measures of internal exposure as serum levels of antioxidants. Although in formal statistical analyses serum levels of both AT and BC showed a protective effect also in our study, it does not necessarily imply that they would be the actual effective substances.

In conclusion, supplementation with alpha-tocopherol or beta-carotene for 5–8 yr did not prevent the development of symptoms related to chronic obstructive pulmonary disease, but a diet rich in these substances offered some protection even among elderly, long-term smokers. The most effective way to prevent COPD remains smoking cessation.

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