



## Development of a Comprehensive Dietary Antioxidant Index and Application to Lung Cancer Risk in a Cohort of Male Smokers

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In many observational studies, a higher intake of individual antioxidants is inversely associated with lung cancer risk. Data from in vitro and animal experiments suggest that there are biochemical interactions among antioxidant nutrients; therefore, consideration of multiple antioxidants simultaneously may be important in terms of risk estimation. The authors constructed a dietary antioxidant index and evaluated its ability to predict lung cancer risk within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort. At baseline (1985–1988), 27,111 Finnish male smokers aged 50–69 years completed a dietary questionnaire that assessed usual frequency of consumption and portion sizes for the previous 12 months. A total of 1,787 incident cases of lung cancer were identified during a follow-up period of up to 14.4 years (1985–1999). Principal components analyses were individually applied to the carotenoid, flavonoid, and vitamin E nutrient groups, and summation of retained principal component scores, plus selenium and vitamin C, yielded the composite antioxidant index. In multivariate proportional hazards models, the relative risks for lung cancer according to increasing quintiles of the antioxidant index were 1.00 (referent), 1.00 (95% confidence interval (CI): 0.87, 1.14), 0.91 (95% CI: 0.79, 1.05), 0.79 (95% CI: 0.68, 0.92), and 0.84 (95% CI: 0.72, 0.98) ( $p$  for trend = 0.002). These findings support the hypothesis that a combination of dietary antioxidants reduces lung cancer risk in male smokers.

antioxidants; cohort studies; lung neoplasms; principal component analysis

Abbreviations: ATBC, Alpha-Tocopherol, Beta-Carotene; CI, confidence interval.

Antioxidants are characterized by their ability to scavenge free radicals and other oxidative species (1), and they may play an important role in lung cancer prevention. Increased consumption of nutrients with antioxidant activity may be particularly important for smokers, since cigarette smoke contains free radicals and causes oxidative DNA damage in lung epithelial cells (2). Cigarette smoke also depletes circulating concentrations of several antioxidant nutrients independently of dietary intake, rendering smokers more prone to oxidative stress (3). Furthermore, it is well established that smokers consume fewer antioxidant-rich foods than do nonsmokers (4).

Increased consumption of individual antioxidant nutrients has been inversely associated with lung cancer risk in the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study cohort (5–7) and in other populations (8). However, the independent effects of antioxidant nutrients are difficult to ascertain with accuracy because intakes of many of these nutrients are highly correlated with one another and with intakes of other anticarcinogenic phytochemicals found in the same food sources. Although single nutrients may play a role in lung cancer etiology, biologic interactions among dietary antioxidants are likely and should also be considered. The concept of an integrated

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antioxidant network is tenable, given that antioxidants of varying solubilities are located in adjacent regions of a cell and seem capable of regenerating one another (9). For example, *in vitro* experiments have demonstrated that  $\alpha$ -tocopheroxyl radicals—generated when the parent molecule reduces free radicals—are repaired efficiently by several carotenoids, which are in turn repaired by vitamin C (10). Flavonoids, including quercetin and catechins, may also participate in this process, since they have been shown to regenerate  $\alpha$ -tocopherol from the  $\alpha$ -tocopheroxyl radical (11). If nutrient interactions occur *in vivo*, they might enable a more comprehensive and sustained antioxidant response to chronic oxidative stress associated with cigarette smoking and other high-risk behaviors.

Several observational studies have examined the joint effects of dietary antioxidant nutrients on lung cancer risk and mortality. Some evaluated risk by cross-classifying individuals according to intakes of two or three antioxidants (12–15), while others created a single score that represented combined nutrient intake (16). Many studies utilized total carotenoid, total flavonoid, and/or total vitamin E summary measures in which intakes of structurally related nutrients were added directly together. Recently, antioxidant and prooxidant nutrient consumption was integrated into a dietary oxidative balance score, which was subsequently applied to total cancer mortality (17). Each of these studies was limited by the range of antioxidant nutrients examined. Furthermore, in those studies presenting results for a single summary variable, details on the rationale behind the construction of a particular index were often lacking.

We constructed a dietary antioxidant index that summarized the combined intake of individual carotenoids, flavonoids, tocopherols, tocotrienols, selenium, and vitamin C and evaluated its ability to predict lung cancer risk among male smokers participating in the ATBC Cancer Prevention Study. We specifically utilized principal components analysis to develop the index because it reduces a large number of highly correlated variables to a smaller set of components that capture as much of the variability in the data as possible. To our knowledge, this is the most comprehensive antioxidant nutrient index constructed to date, and this study is one of the first to evaluate whether a more comprehensive combination of dietary antioxidants is associated with a lower risk of incident lung cancer.

## MATERIALS AND METHODS

### Study population

The ATBC Cancer Prevention Study was a randomized, double-blinded, placebo-controlled primary chemoprevention trial with a  $2 \times 2$  factorial design that tested whether supplementation with  $\beta$ -carotene (20 mg/day) and/or vitamin E (50 mg of DL- $\alpha$ -tocopherol/day) reduced the incidence of lung cancer. Details regarding the study design, methods, participant characteristics, and compliance have been reported previously (18). Briefly, 29,133 participants meeting all eligibility criteria (male residents of southwestern Finland aged 50–69 years who smoked five or more cigarettes per day and were willing to provide written

informed consent) were successfully randomized into the trial between 1985 and 1988. Reasons for exclusion included a history of cancer (other than nonmelanoma skin cancer or carcinoma *in situ*) or other diseases/conditions that might limit participation in a long-term intervention trial, use of vitamin E, vitamin A, or  $\beta$ -carotene supplements in excess of predefined amounts, and treatment with anticoagulants. The trial ended on April 30, 1993, with passive case ascertainment continuing thereafter. The present analysis is based on the 27,111 cohort subjects with complete baseline dietary information. Person-years of observation were calculated from the date of randomization to the date of lung cancer diagnosis, death, or April 30, 1999. The institutional review boards of both the National Public Health Institute of Finland and the US National Cancer Institute approved the study, and written informed consent was obtained from each participant prior to randomization.

### Data collection

Prior to randomization, all subjects were asked to provide detailed information on demographic, medical, smoking-related, and occupational factors and to complete a dietary questionnaire. The food use questionnaire inquired about the usual frequency of consumption and portion sizes of 276 common food items/mixed dishes and beverages during the past year. A color picture booklet was provided to each participant to assist with portion size estimation. Daily nutrient intakes were calculated using the food composition database of the National Public Health Institute of Finland. Most antioxidant nutrient values were based on analyses of Finnish foods, although values for flavonols (quercetin, kaempferol, and myricetin) were based primarily on Dutch composition analyses (19, 20), while values for catechin ((+)-catechin and (-)-epicatechin) were derived from US and German analyses (21–23). The dietary questionnaire was developed specifically for use in the ATBC Cancer Prevention Study and was validated against food consumption records in a pilot study conducted among middle-aged Finnish men (24). Energy-adjusted correlation coefficients (corrected for attenuation) were 0.55, 0.70, 0.76, 0.50, and 0.59 for vitamin A, vitamin C, vitamin E, selenium, and flavonols, respectively (6, 24).

### Case ascertainment

Incident primary cancers of the lung or bronchus were ascertained via the Finnish Cancer Registry, which provides almost 100 percent case ascertainment nationwide (25). Administration of a chest radiograph at baseline ensured that men with apparent lung cancer were excluded from the trial. Chest radiographs were also performed at two follow-up visits during the trial and at study exit to facilitate ascertainment of lung cancer cases. Upon identification of a case, all relevant medical records were obtained and reviewed independently by one or two study physicians. A total of 1,933 lung cancer cases were identified during a follow-up period of up to 14.4 years (median, 11.3 years). Of these cases, 146 had incomplete baseline dietary data, leaving 1,787 cases for

the present analysis. Histologic verification was available for 95 percent of all cases.

### Statistical analysis

The following antioxidant nutrients were frequently consumed in this cohort and were included in the analyses:  $\alpha$ -carotene,  $\beta$ -carotene,  $\beta$ -cryptoxanthin,  $\gamma$ -carotene, lutein + zeaxanthin, and lycopene (all broadly classified as “carotenoids”); catechin, epicatechin, kaempferol, myricetin, and quercetin (all broadly classified as “flavonoids”);  $\alpha$ -tocopherol,  $\gamma$ -tocopherol,  $\alpha$ -tocotrienol, and  $\beta$ -tocotrienol (all broadly classified as “vitamin E”); selenium; and vitamin C. Intake of each antioxidant nutrient was standardized by subtracting the mean and dividing by the standard deviation. Principal components analyses were individually applied to the carotenoid, flavonoid, and vitamin E groups because each group is composed of structurally related and correlated nutrients (26). Pearson correlation coefficients within nutrient groups ranged from 0.17 to 0.99 for the carotenoids, from 0.61 to 0.99 for the flavonoids, and from 0.007 to 0.85 for the vitamin E isomers. For a given set of nutrients, the resulting principal components represented linear combinations of the original variables. In other words, each principal component was computed by first multiplying the standardized intake of a specific nutrient by its corresponding weight, or factor loading, and subsequently summing across all contributing nutrients to obtain a score for each study participant. The first principal component within each nutrient group accounted for the largest proportion of the total variance in intake (carotenoids: 54 percent; flavonoids: 81 percent; vitamin E: 59 percent) and was retained for further analysis. Specific factor loadings and variance proportions corresponding to each retained principal component are given in table 1. Summation of the principal component scores, plus selenium and vitamin C, was carried out to derive the composite antioxidant index. The three principal components (table 1) and both nutrients (selenium, vitamin C) were equally weighted in the summed index. The index was subsequently adjusted for energy via the residual method (27).

The index was divided into quintiles based on the distribution in the entire cohort, and Cox proportional hazards models were utilized to estimate relative risks and 95 percent confidence intervals for each quintile relative to the referent category (quintile 1). Tests for linear trend were carried out by taking the median values of all quintiles and modeling the index as a continuous variable. Confounders were identified as those covariates associated with both the index and lung cancer risk that, upon addition to the base model, altered risk estimates by more than 10 percent. The base model was specified a priori and included age, number of cigarettes smoked per day, number of years of smoking, and intervention assignment, in addition to quintiles of the antioxidant index. Putative confounders included body mass index (weight (kg)/height (m)<sup>2</sup>), educational level (primary school, high school, vocational school, or university), energy-adjusted intakes of dietary fat and cholesterol, place of residence (small town or large town), pack-years of smoking, smoking inhalation (never/seldom, often, or always), and

**TABLE 1. Factor loadings and variance proportions from principal components analyses of antioxidant nutrient groups in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1999**

	Factor loading*		
	Carotenoids	Flavonoids	Vitamin E
$\alpha$ -Carotene	0.45		
$\beta$ -Carotene	0.48		
$\beta$ -Cryptoxanthin	0.22		
$\gamma$ -Carotene	0.44		
Lutein + zeaxanthin	0.37		
Lycopene	0.44		
Catechin		0.48	
Epicatechin		0.48	
Kaempferol		0.48	
Myricetin		0.39	
Quercetin		0.40	
$\alpha$ -Tocopherol			0.58
$\gamma$ -Tocopherol			0.50
$\alpha$ -Tocotrienol			0.42
$\beta$ -Tocotrienol			0.49
% of variance explained	54.0	81.1	59.3

\* Corresponds to the first principal component within each nutrient group.

smoking cessation (defined as reporting having quit smoking at three consecutive visits (i.e., 1 year) during the trial). Body mass index and educational level were the only covariates that met the criterion for confounding described above, and they were therefore included in all multivariate models.

At baseline, a small proportion of participants reported taking vitamin or trace mineral supplements at doses low enough to prevent exclusion from the trial. To address this issue, we created multivariate models with and without adjustment for supplement use, performed stratified analyses, excluded supplement users altogether, and integrated baseline use of  $\beta$ -carotene, vitamin C, vitamin E, and selenium supplements with dietary intakes of these nutrients. None of these procedures altered the association between the dietary antioxidant index and lung cancer risk. We did not incorporate trial supplements into the antioxidant index because of the observed increase in lung cancer risk among men receiving  $\beta$ -carotene (28), but we did perform stratified analyses by treatment arm (see below). The validity of the proportional hazards assumption was tested with a cross-product term between follow-up time and the antioxidant index.

We stratified analyses by intervention assignment ( $\alpha$ -tocopherol, no  $\alpha$ -tocopherol,  $\beta$ -carotene, no  $\beta$ -carotene), smoking intensity (cigarettes/day), histologic type (squamous cell carcinoma, small cell carcinoma, adenocarcinoma, other carcinoma), stage of disease (tumor-node-metastasis stages I–IV), alcohol consumption (0, >0– $\leq$ 7.4, >7.4– $\leq$ 22.9, or >22.9 g/day), age ( $\leq$ 57 years vs. >57 years), and body mass index ( $\leq$ 25 vs. >25) to evaluate potential effect modifi-

cation. We carried out formal tests for interaction by adding the relevant cross-product term (with the exception of histologic type and stage of disease, which were assessed by chi-squared tests) to main-effects models. We also chose a priori to evaluate whether intake of the two most common putative prooxidant nutrients, polyunsaturated fatty acids derived from fish and heme iron, modified the association between the antioxidant index and lung cancer risk. We focused on heme iron rather than total iron because it is the most bioavailable form of dietary iron and, as such, is the greatest contributor to physiologic iron stores (29). We chose to analyze eicosapentaenoic acid and docosahexaenoic acid— $\omega$ -3 polyunsaturated fatty acids found predominantly in fatty fish—rather than total polyunsaturated fatty acids from all contributing food sources because they are the most highly unsaturated and therefore more susceptible to peroxidation (30). Because information on heme iron was not available in the Finnish nutrient database, we estimated its consumption using previously published information on the heme iron contents of cooked poultry, beef, veal, pork, and frankfurters (31, 32). We approximated the amount of heme iron present in standard portions of liver and fish—two foods that were frequently consumed in this cohort—since these values were not available in the literature. For liver, we multiplied the amount of total iron found in 1 g of this organ meat by a factor of 0.55, which represents the estimated percentage of total iron considered to be heme iron. The heme iron content of fish was assigned a value equivalent to that of poultry. All reported  $p$  values are two-sided.

## RESULTS

Baseline lifestyle and dietary characteristics are shown in table 2 by quintile of the antioxidant index. Participants in the highest quintile of the antioxidant index were younger, more likely to live in an urban area, and better educated, had a slightly higher body mass index, smoked fewer cigarettes per day and for fewer years, and were more likely to have stopped smoking during the trial than those in lower quintiles. Intakes of all individual antioxidant nutrients, fruits, vegetables, and fish increased with successive quintiles of the index, while fat consumption decreased with successive quintiles. Tests for linear trend across quintiles of the antioxidant index were statistically significant for all baseline covariates ( $p < 0.05$ ).

Associations between the dietary antioxidant index and lung cancer risk, stratified by trial intervention group, are presented in table 3. Within each arm of the trial, risks of lung cancer were 13–18 percent lower among participants in the highest antioxidant index quintile compared with the lowest. There was no evidence of heterogeneity in these risk estimates across the intervention groups ( $p$  for interaction = 0.99). In combined analysis of the entire cohort, multivariate relative risks for lung cancer according to increasing quintiles of the antioxidant index were 1.00 (referent), 1.00 (95 percent confidence interval (CI): 0.87, 1.14), 0.91 (95 percent CI: 0.79, 1.05), 0.79 (95 percent CI: 0.68, 0.92), and 0.84 (95 percent CI: 0.72, 0.98) ( $p$  for trend = 0.002).

To explore whether a specific constituent was responsible for the association between the antioxidant index and lung

cancer risk, we removed each nutrient group's principal component, as well as selenium and vitamin C, from the index one at a time (data not shown). We also systematically deleted combinations of retained components, selenium, and vitamin C from the full antioxidant index. With the following exceptions, the resulting subindices exhibited similar associations with lung cancer risk when compared with the original index. When the carotenoid, flavonoid, and vitamin E principal components were removed simultaneously, leaving only selenium and vitamin C, the relative risk of lung cancer for the highest antioxidant index quintile versus the lowest was essentially null (relative risk = 0.94, 95 percent CI: 0.80, 1.10;  $p$  for trend = 0.52). Similarly attenuated relative risks were observed with concurrent exclusion of carotenoids, flavonoids, and vitamin C or flavonoids, vitamin E, and vitamin C from the index.

Consumption of heme iron, a putative prooxidant nutrient, modified the association between the antioxidant index and lung cancer risk (table 4;  $p$  for interaction = 0.05). The inverse relation between the index and lung cancer was strongest among subjects in the upper quartiles of heme iron intake but was not apparent among men in the lower quartiles of heme iron intake. The index also appeared to be most protective among men consuming higher quantities of fish polyunsaturated fatty acids (25–30 percent reductions in the risk of lung cancer for the highest antioxidant index quintile versus the lowest), though no significant effect modification was observed (data not shown;  $p$  for interaction = 0.40).

Age, body mass index, number of cigarettes smoked per day, alcohol consumption, and disease stage did not significantly modify the association between the antioxidant index and lung cancer risk, although risk estimates and associated trends were slightly stronger among younger and leaner persons (data not shown). When the relation between the antioxidant index and lung cancer risk was examined according to histologic type (distribution of cases: 43 percent squamous cell carcinoma, 23 percent small cell carcinoma, 16 percent adenocarcinoma, and 17 percent other carcinomas combined), a significant trend in risk was observed among men with squamous cell tumors but not among those with small cell carcinomas, adenocarcinomas, or other carcinomas combined (chi-squared  $p$  value for heterogeneity = 0.009).

The composite antioxidant index predicted lung cancer risk better than several alternative antioxidant nutrient measures (including individual principal components and direct summation of intakes of related nutrients) but similarly to total fruit and vegetable intake (table 5). The dose-response trend for the antioxidant index was more pronounced than trends for any of the alternative methods. Risk estimates for the antioxidant index were proportional over time.

## DISCUSSION

In this prospective cohort study of male smokers, we developed a comprehensive antioxidant index that summarized the combined intake of carotenoids, flavonoids, tocopherols, tocotrienols, selenium, and vitamin C. We found that men with the highest index scores had a significant 16

**TABLE 2. Age-adjusted mean values and proportions for baseline characteristics, by quintile of the dietary antioxidant index, in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1999**

	Quintile of antioxidant index				
	1	2	3	4	5
No. of participants	5,422	5,422	5,423	5,422	5,422
Age (years)	57.5	57.5	57.2	57.0	56.7
Body mass index*	25.6	26.1	26.3	26.4	26.5
Smoking history					
No. of years of regular smoking	37.0	36.3	35.8	35.5	35.0
No. of cigarettes smoked per day	22.6	20.8	20.1	19.8	18.8
Pack-years of smoking	42.1	37.9	36.1	35.4	33.5
Smoking inhalation (%)					
Never/seldom	6.9	8.1	8.8	9.0	11.1
Often	31.4	36.3	39.2	41.0	42.6
Always	61.7	55.4	51.8	49.8	46.1
Smoking cessation† (%)	11.7	14.1	16.3	17.4	20.7
Urban residence (%)	34.1	38.2	42.8	45.9	51.3
Educational level (%)					
Primary school	78.2	72.0	65.9	59.0	48.0
High school	5.3	6.5	8.4	8.4	9.7
Vocational school	15.2	19.5	22.4	26.8	31.8
University	1.3	1.9	3.2	5.7	10.2
Daily dietary intake‡					
Energy (kcal)	2,967	2,712	2,730	2,769	2,896
Total fat (g)	128	125	123	121	118
Fruits/vegetables§ (g)	151	228	284	343	453
Red meat¶ (g)	144	149	148	147	142
Fish and shellfish (g)	30.3	36.5	39.3	43.6	47.5
Total carotenoids (µg)	2,832	3,890	4,801	5,948	8,320
α-Carotene	229	401	556	761	1,180
β-Carotene	1,126	1,543	1,937	2,469	3,594
β-Cryptoxanthin	13.6	23.1	31.4	39.4	55.0
γ-Carotene	16.2	27.5	38.4	50.9	78.1
Lutein + zeaxanthin	1,096	1,315	1,435	1,565	1,790
Lycopene	309	522	722	957	1,460
Total flavonoids (µg)	6,737	10,249	13,642	19,178	36,005
Catechin	505	1,005	1,611	2,684	6,288
Epicatechin	822	1,685	2,750	4,617	10,922
Kaempferol	277	535	877	1,495	3,637
Myricetin	469	629	759	959	1,598
Quercetin	4,296	5,745	6,792	8,326	12,101
Tocopherols/tocotrienols (mg)					
α-Tocopherol	7.2	9.1	10.5	11.8	13.4
γ-Tocopherol	3.9	6.5	8.6	10.2	11.9
α-Tocotrienol	1.86	1.98	2.02	2.07	2.05
β-Tocotrienol	2.31	2.52	2.61	2.67	2.71
Selenium (µg)	83.2	89.3	90.9	92.2	93.3
Vitamin C (mg)	61.8	81.0	94.8	110.9	142.1
Heme iron (mg)	1.82	1.97	2.03	2.10	2.15

\* Weight (kg)/height (m)<sup>2</sup>.

† Defined as having stopped smoking for at least 1 year during the trial.

‡ Adjusted for energy intake.

§ Includes fruits, vegetables, and juices.

¶ Includes beef, pork, sausages and other cold cuts, and inner organs and blood.

**TABLE 3. Multivariate relative risk\* of lung cancer according to quintile of the dietary antioxidant index, stratified by trial intervention arm, in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1999**

Quintile of antioxidant index	Intervention arm											
	$\alpha$ -Tocopherol			No $\alpha$ -tocopherol			$\beta$ -Carotene			No $\beta$ -carotene		
	No. of cases	RR†	95% CI†	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI
1	233	1.0		222	1.0		242	1.0		213	1.0	
2	199	0.98	0.81, 1.19	210	1.01	0.84, 1.22	209	0.94	0.78, 1.13	200	1.06	0.87, 1.29
3	172	0.86	0.70, 1.05	174	0.97	0.80, 1.19	184	0.89	0.73, 1.08	162	0.94	0.77, 1.16
4	151	0.77	0.63, 0.95	142	0.82	0.66, 1.02	143	0.71	0.58, 0.88	150	0.89	0.72, 1.10
5	145	0.82	0.66, 1.01	139	0.87	0.70, 1.08	154	0.83	0.68, 1.03	130	0.84	0.67, 1.06
<i>p</i> for trend		0.01			0.07			0.02			0.05	

\* Adjusted for energy intake, age, number of cigarettes smoked per day, number of years of smoking, body mass index, and educational level.

† RR, relative risk; CI, confidence interval.

percent lower risk of lung cancer than men with the lowest scores, after adjustment for multiple confounders. Furthermore, among men ingesting higher quantities of heme iron, a putative prooxidant nutrient, risks of lung cancer were 25–30 percent lower for those in the highest quintile of the antioxidant index versus the lowest.

The finding that our dietary antioxidant index was associated with a reduced risk of lung cancer, whereas high-dose supplements of  $\beta$ -carotene and  $\alpha$ -tocopherol (both antioxidant nutrients) did not lower lung cancer risk in the same study, may at first seem contradictory. However, both  $\beta$ -carotene and  $\alpha$ -tocopherol are known to have numerous biologic functions that are unrelated to their antioxidant activity. For example,  $\beta$ -carotene seems to be an effective antioxidant at lower doses, such as those achieved through diet, but it loses this ability at higher concentrations (33). The adverse effect of high-dose  $\beta$ -carotene supplements on lung cancer risk observed in this study and in other trials is likely mediated by antioxidant-independent mechanisms, including induction of cytochrome P-450 enzymes and alter-

ation of normal retinoid signaling (34–36).  $\alpha$ -Tocopherol also possesses numerous properties that are independent of its chain-breaking antioxidant ability, including inhibition of protein kinase C activity, which affects cell proliferation, cell adhesion, immune responses, and gene expression (37). In addition, discrepancies might have arisen because dietary antioxidants are likely to exert their protective effects through interactions with other vitamins and phytochemicals found in the same food sources; supplements only contain large quantities of a single antioxidant nutrient and therefore are ingested without the added benefit of potentially important cofactors.

Although there is a substantial amount of in vitro and animal data suggesting biologic interactions between proximate antioxidant micronutrients, few studies have explored whether combinations of dietary antioxidants affect lung cancer morbidity and mortality in human populations. Our results are consistent with, though generally more modest than, findings from these studies. Using prospective data from the First National Health and Nutrition Examination

**TABLE 4. Multivariate relative risk\* of lung cancer according to quintile of the dietary antioxidant index, stratified by intake of heme iron, in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1999**

Quintile of antioxidant index	Intake of heme iron† (mg/day)											
	<1.47			1.47–1.91			1.92–2.42			>2.42		
	No. of cases	RR‡	95% CI‡	No. of cases	RR	95% CI	No. of cases	RR	95% CI	No. of cases	RR	95% CI
1	165	1.0		116	1.0		99	1.0		75	1.0	
2	98	0.94	0.73, 1.21	116	1.07	0.83, 1.39	107	0.91	0.69, 1.20	88	1.03	0.76, 1.41
3	75	0.85	0.65, 1.12	93	1.00	0.76, 1.32	89	0.82	0.61, 1.09	89	0.96	0.70, 1.30
4	48	0.63	0.45, 0.87	70	0.79	0.58, 1.07	84	0.79	0.59, 1.07	91	0.89	0.65, 1.22
5	75	1.12	0.84, 1.49	70	0.89	0.65, 1.20	71	0.73	0.53, 1.00	68	0.69	0.49, 0.97
<i>p</i> for trend		0.76			0.15			0.04			0.01	

\* Adjusted for energy intake, age, number of cigarettes smoked per day, number of years of smoking, intervention assignment, body mass index, and educational level.

† Adjusted for energy intake.

‡ RR, relative risk; CI, confidence interval.

**TABLE 5. Multivariate relative risk\* of lung cancer according to various antioxidant nutrient measures in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1999**

	Interquartile range	Relative risk	95% confidence interval	<i>p</i> for trend
Antioxidant index	4.81	0.90	0.85, 0.95	0.0008
First principal component†				
Carotenoids	1.99	0.93	0.88, 0.99	0.02
Flavonoids	1.42	0.94	0.91, 0.98	0.003
Vitamin E	1.37	0.95	0.90, 1.01	0.10
Total nutrient intake				
Carotenoids (μg/day)	3,090	0.96	0.91, 1.02	0.16
Flavonoids (μg/day)	12,762	0.94	0.91, 0.98	0.005
Tocopherols/tocotrienols (mg/day)	5.34	0.96	0.91, 1.02	0.15
Fruit/vegetable intake (g/day)	235.87	0.91	0.86, 0.96	0.001

\* Relative risks were based on a difference corresponding to the interquartile range of exposure and were adjusted for energy intake, age, number of cigarettes smoked per day, number of years of smoking, intervention assignment, body mass index, and educational level.

† Corresponds to the first principal component within each nutrient group.

Survey, Yong et al. (15) demonstrated that subjects in the highest quartiles of vitamin E, vitamin C, and carotenoid consumption had a 68 percent lower risk of lung cancer than those in the lowest quartiles of all three nutrients. Michaud et al. (16) created a total carotenoid score based on consumption of  $\alpha$ -carotene,  $\beta$ -carotene, lutein, lycopene, and  $\beta$ -cryptoxanthin, and in a pooled analysis of data from the Nurses' Health Study and Health Professionals Follow-up Study cohorts, they found that subjects in the highest category had a significantly lower risk of lung cancer (highest quintile vs. lowest: relative risk = 0.68, 95 percent CI: 0.49, 0.94; *p* for trend = 0.004). Results from three case-control studies indicated that joint intakes of 1)  $\beta$ -carotene and glutathione, 2) lutein,  $\alpha$ -carotene, and  $\beta$ -carotene, and 3)  $\alpha$ -carotene and  $\beta$ -carotene were each associated with a marked reduction in lung cancer risk (12–14).

A unique feature of our study is the manner in which the antioxidant index was constructed. To our knowledge, this is the first study in which principal components analysis was specified a priori as a means of developing a composite antioxidant index. This technique is appropriate when a large number of variables are highly correlated with one another and therefore redundant in the information they contain. Our antioxidant index is also unique because it incorporates a large number of antioxidant nutrients that are derived from a wide variety of food sources. Although carotenoids, flavonoids, and vitamin C are found primarily in fruits and vegetables, vitamin E and selenium are not. The best sources of vitamin E are vegetable oils and fats, grains and grain products, and nuts, whereas selenium is found in organ meats and seafood, as well as in cereals and grains grown in selenium-rich soil (38). Ideally, our index should discriminate between antioxidant-rich and antioxidant-poor dietary patterns rather than serve exclusively as a marker of fruit and vegetable intake. The coefficients for correlation between the antioxidant index and intakes of vegetables, fruits, and juices were 0.72, 0.52, and 0.13, respectively (all *p* values <

0.0001). The finding that the antioxidant index predicted lung cancer risk only marginally better than fruit and vegetable intake likely reflects our inherent assumption that all component nutrients had similar antioxidant efficiencies (see below).

The risk estimate presented in this paper, though statistically significant, was not as strong in magnitude as previously published estimates corresponding to individual antioxidant nutrients. Previous studies using ATBC data showed that lycopene (5), quercetin, myricetin, and kaempferol (6), and vitamin E (7) were each more strongly inversely associated with lung cancer risk than our antioxidant index was. These discrepancies may have arisen because we were unable to account for the relative antioxidant efficiencies of the different nutrients under investigation. For example, lycopene has been shown to quench singlet oxygen more effectively than any other carotenoid in vitro (39), whereas  $\alpha$ -tocopherol appears to be the most effective inhibitor of lipid peroxidation induced by peroxy radicals (40). However, in vitro results may not reflect in vivo activity, given differences in metabolism, absorption, and utilization. Ranking of micronutrients is further complicated by the fact that multiple oxidative species are implicated in carcinogenesis, and different isomers within the same nutrient group may have particular affinities for specific free radicals. For example,  $\alpha$ -tocopherol is the major chain-breaking antioxidant for inhibition of lipid peroxidation, while  $\gamma$ -tocopherol is better able to trap reactive nitrogen oxide species, such as those found in cigarette smoke (41). Therefore, generation of a complete antioxidant hierarchy is not possible at the present time, especially because the free-radical-scavenging abilities of other important dietary antioxidants have not been fully characterized. This represents an important area of continuing research.

We cannot be certain that the observed association between the antioxidant index and lung cancer risk is due to its antioxidant activity per se. Many other anticarcinogenic

functions have been ascribed to antioxidant nutrients. For example, selenium is a potent inducer of apoptosis and may also initiate DNA repair (42), while carotenoids seem to play an important role in cellular growth regulation (43).  $\alpha$ -Tocopherol and  $\gamma$ -tocopherol possess antiproliferative and antiinflammatory properties, respectively, which are independent of their antioxidant activities (41). The index might also be highly correlated with other known and unknown protective factors found in plant foods. However, our finding that a combination of dietary antioxidants was more strongly protective against lung cancer among persons consuming higher quantities of heme iron (which can contribute to increased oxidation) suggests that the index is, in fact, capturing antioxidant activity.

We were unable to assess whether secular changes in diet occurred during the long follow-up period because dietary intakes were estimated with a single baseline measurement. Any resulting exposure misclassification likely attenuated the association between the antioxidant index and lung cancer risk. Exposure misclassification due to imprecise measurement of selenium was also possible, given that the Finnish government began adding selenium to fertilizers in the fall of 1984. Another limitation involves use of the same data set to develop and test the antioxidant index. Although we could have split the original data set into two distinct parts for this purpose, we kept it intact to ensure robust risk estimates. Finally, investigating relations between diet and lung cancer in cohorts of smokers is sometimes problematic, given that modest effects may be overwhelmed by the strong association between cigarette smoking and lung cancer. Residual confounding by smoking cannot be discounted, even though multiple measures were utilized to control for this exposure.

The strengths of this study include its prospective design; a priori specification of lung cancer as the primary trial endpoint; the large number of lung cancer cases available for analysis, which ensured adequate statistical power to detect modest associations; use of a validated food use questionnaire to capture usual nutrient intakes; and adjustment for multiple confounders, which may have accounted for our more modest risk estimates as compared with prior reports of specific antioxidants. Although residual confounding by smoking was noted as a limitation above, smokers constitute an important group within which to explore the effects of antioxidant nutrients on primary prevention of lung cancer, given that 90 percent of the disease is attributable to smoking (44). Since oxidative stress also appears to play a role in the etiology of other cancers and chronic conditions, application of the antioxidant index to these diseases should be forthcoming and may generate new primary prevention strategies.

Lung cancer is the leading cause of cancer-related mortality and the most common incident cancer worldwide (8). Current screening approaches have not been proven to improve survival, and because most lung cancers have metastasized by the time they are detected, 5-year survival rates remain low at 15 percent (45). Our results indicate that a balanced diet rich in a variety of antioxidant nutrients may benefit smokers with regard to lung cancer prevention. Although these benefits appear to be modest, the potential public health impact is likely to be sizeable, given the world-

wide extent of this disease. Therefore, smokers should adhere to current guidelines recommending increased vegetable, fruit, and whole grain consumption. Use of antioxidant nutrient supplements does not seem warranted at the present time, given the results of recent cancer prevention trials (28, 46, 47). Of course, the fundamental strategy in the prevention of lung cancer is abstinence from smoking or smoking cessation.

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## REFERENCES

- Halliwel B, Gutteridge JM. Free radicals in biology and medicine. New York, NY: Oxford University Press, 1999.
- Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst* 1999;91:1194–210.
- Alberg A. The influence of cigarette smoking on circulating concentrations of antioxidant micronutrients. *Toxicology* 2002; 180:121–37.
- Dallongeville J, Marecaux N, Fruchart JC, et al. Cigarette smoking is associated with unhealthy patterns of nutrient intake: a meta-analysis. *J Nutr* 1998;128:1450–7.
- Holick CN, Michaud DS, Stolzenberg-Solomon R, et al. Dietary carotenoids, serum beta-carotene, and retinol and risk of lung cancer in the Alpha-Tocopherol, Beta-Carotene cohort study. *Am J Epidemiol* 2002;156:536–47.
- Hirvonen T, Virtamo J, Korhonen P, et al. Flavonol and flavone intake and the risk of cancer in male smokers (Finland). *Cancer Causes Control* 2001;12:789–96.
- Woodson K, Tangrea JA, Barrett MJ, et al. Serum alpha-tocopherol and subsequent risk of lung cancer among male smokers. *J Natl Cancer Inst* 1999;91:1738–43.
- American Institute for Cancer Research, World Cancer Research Fund. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research, 1997.
- Eastwood MA. Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease? *QJM* 1999;92:527–30.
- Bohm F, Edge R, Land E, et al. Carotenoids enhance vitamin E antioxidant efficiency. *J Am Chem Soc* 1997;119:621–2.
- Pedrielli P, Skibsted LH. Antioxidant synergy and regeneration effect of quercetin, (-)-epicatechin, and (+)-catechin on alpha-tocopherol in homogeneous solutions of peroxidating methyl linoleate. *J Agric Food Chem* 2002;50:7138–44.
- Stefani ED, Boffetta P, Deneo-Pellegrini H, et al. Dietary antioxidants and lung cancer risk: a case-control study in Uruguay. *Nutr Cancer* 1999;34:100–10.
- Ziegler RG, Colavito EA, Hartge P, et al. Importance of alpha-carotene, beta-carotene, and other phytochemicals in the etiology of lung cancer. *J Natl Cancer Inst* 1996;88:612–15.
- Le Marchand L, Hankin JH, Kolonel LN, et al. Intake of specific carotenoids and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 1993;2:183–7.
- Yong LC, Brown CC, Schatzkin A, et al. Intake of vitamins E, C, and A and risk of lung cancer: The NHANES I Epidemiol-

- logic Follow-up Study. *Am J Epidemiol* 1997;146:231–43.
16. Michaud DS, Feskanich D, Rimm EB, et al. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am J Clin Nutr* 2000;72:990–7.
  17. Van Hoydonck PG, Temme EH, Schouten EG. A dietary oxidative balance score of vitamin C, beta-carotene and iron intakes and mortality risk in male smoking Belgians. *J Nutr* 2002;132:756–61.
  18. The ATBC Cancer Prevention Study Group. The Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study: design, methods, participant characteristics, and compliance. *Ann Epidemiol* 1994;4:1–10.
  19. Hertog MG, Hollman PC, Katan MB. Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* 1992;40:2379–83.
  20. Hertog MG, Hollman PC, van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J Agric Food Chem* 1993;41:1242–6.
  21. Herrmann K. Occurrence and content of hydroxycinnamic and hydroxybenzoic acid compounds in foods. *Crit Rev Food Sci Nutr* 1989;28:315–47.
  22. Frankel EN, Waterhouse AL, Teissedre PL. Principal phenolic phytochemicals in selected California wines and their antioxidant activity in inhibiting oxidation of human low-density lipoproteins. *J Agric Food Chem* 1995;43:890–4.
  23. Arts IC, van De Putte B, Hollman PC. Catechin contents of foods commonly consumed in the Netherlands. 2. Tea, wine, fruit juices, and chocolate milk. *J Agric Food Chem* 2000;48:1752–7.
  24. Pietinen P, Hartman AM, Haapa E, et al. Reproducibility and validity of dietary assessment instruments. I. A self-administered food use questionnaire with a portion size picture booklet. *Am J Epidemiol* 1988;128:655–66.
  25. Korhonen P, Malila N, Pukkala E, et al. The Finnish Cancer Registry as follow-up source of a large trial cohort—accuracy and delay. *Acta Oncol* 2002;41:381–8.
  26. Hatcher L. A step-by-step approach to using the SAS system for factor analysis and structural equation modeling. Cary, NC: SAS Institute, Inc, 1994.
  27. Willett W. *Nutritional epidemiology*. New York, NY: Oxford University Press, 1998.
  28. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994;330:1029–35.
  29. Carpenter CE, Mahoney AW. Contributions of heme and non-heme iron to human nutrition. *Crit Rev Food Sci Nutr* 1992;31:333–67.
  30. Halliwell B, Chirico S. Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr* 1993;57(suppl):715S–24S.
  31. Lombardi-Boccia G, Martinez-Dominquez B, Aguzzi A. Total heme and non-heme iron in raw and cooked meats. *J Food Sci* 2002;67:1738–41.
  32. Mrudula Kalpalathika PV, Clark EM, Mahoney AW. Heme iron content in selected ready-to-serve beef products. *J Agric Food Chem* 1991;39:1091–3.
  33. Young AJ, Lowe GM. Antioxidant and prooxidant properties of carotenoids. *Arch Biochem Biophys* 2001;385:20–7.
  34. Wang XD, Liu C, Bronson RT, et al. Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. *J Natl Cancer Inst* 1999;91:60–6.
  35. Liu C, Wang XD, Bronson RT, et al. 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.
  36. Liu C, Russell RM, Wang XD. Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. *J Nutr* 2003;133:173–9.
  37. Ricciarelli R, Zingg JM, Azzi A. Vitamin E: protective role of a Janus molecule. *FASEB J* 2001;15:2314–25.
  38. Bowman BA, Russell RM. *Present knowledge in nutrition*. 8th ed. Washington, DC: International Life Sciences Institute, 2001.
  39. Di Mascio P, Kaiser S, Sies H. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys* 1989;274:532–8.
  40. Woodall AA, Britton G, Jackson MJ. Carotenoids and protection of phospholipids in solution or in liposomes against oxidation by peroxy radicals: relationship between carotenoid structure and protective ability. *Biochim Biophys Acta* 1997;1336:575–86.
  41. Jiang Q, Christen S, Shigenaga MK, et al. Gamma-tocopherol, the major form of vitamin E in the US diet, deserves more attention. *Am J Clin Nutr* 2001;74:714–22.
  42. Longtin R. Selenium for prevention: eating your way to better DNA repair? *J Natl Cancer Inst* 2003;95:98–100.
  43. Rock CL. Carotenoids: biology and treatment. *Pharmacol Ther* 1997;75:185–97.
  44. Alberg AJ, Samet JM. Epidemiology of lung cancer. *Chest* 2003;123(suppl):21S–49S.
  45. American Cancer Society. *Cancer facts and figures 2003*. Atlanta, GA: American Cancer Society, 2003.
  46. Hennekens CH, Buring JE, Manson JE, 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.
  47. Omenn GS, Goodman GE, Thornquist MD, 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.