Chapter 6

ANTIOXIDANT NUTRIENTS AND LUNG CANCER

Susan T. Mayne and Regina G. Ziegler

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I. INTRODUCTION TO LUNG CANCER

Lung cancer is the most common tumor worldwide.[1] In the U.S., it has been the leading cause of cancer death in men since the early 1950s and in women since 1987. The American Cancer Society estimates that in 1996, 177,000 Americans will be diagnosed with lung cancer and 158,700 will die of the disease.[2] The three cornerstones for reducing cancer-related morbidity and mortality are prevention, early detection, and treatment. Early detection of lung cancer has not been realized; only 15% of lung cancers are discovered when the disease is still localized.[2] The lack of success in treatment for lung cancer is evident given that the 5-year relative survival rate for lung cancer is only 13%.[2] These data emphasize the importance of prevention for reducing lung cancer morbidity and mortality.

A large body of literature has established cigarette smoking as the dominant risk factor for lung cancer; 90.3% of lung cancer deaths in U.S. men and 78.5% in U.S. women are attributable to cigarette smoking.[3] In the U.S., cigarette use among men has declined. A corresponding decline in lung cancer incidence has been observed in U.S. males, from a high of 87 new cases of lung cancer/100,000 in 1984 to 81 cases in 1992.[2] However, many individuals have difficulty quitting, and even those who quit face an increased risk of lung cancer relative to nonsmokers. In most studies, the risk of lung cancer among former smokers remained elevated above the risk in never smokers, even in the longest periods of abstinence evaluated.[4] Thus,

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an active area of cancer prevention research is the search for interventions to reduce the risk for lung cancer in current and former smokers.

The gas phase of cigarette smoke is known to be highly oxidative, possessing an array of free radical species.[5] Other exposures involved in lung carcinogenesis, such as asbestos (which contains iron, a catalyst of lipid oxidation), ozone, environmental tobacco smoke, and air pollution, may also involve oxidative mechanisms. This suggests a potential role for antioxidant nutrients in the prevention of lung cancer. As detailed below, many descriptive studies, case-control studies, cohort studies, and intervention trials of antioxidant nutrients and lung cancer have been published. The following summarizes and integrates this literature.

II. OBSERVATIONAL STUDIES OF ANTIOXIDANT NUTRIENTS AND LUNG CANCER

Observational studies of lung cancer have examined the association between antioxidant nutrient intake and disease incidence or between blood or tissue levels of antioxidant nutrients and disease incidence. One type of observational study is prospective, with the antioxidant intake and/or status measured before the clinical diagnosis of lung cancer. Another type of observational study is retrospective, wherein individuals with lung cancer and comparable controls are asked to recall usual dietary intake prior to the development of the disease (cases) or prior to interview (controls). Retrospective studies of nutrients and lung cancer typically rely on recalled dietary intake rather than blood nutrient levels to assess exposure, as altered blood nutrient status could easily be a consequence, rather than a cause, of the disease.

A comprehensive review of the literature on nutrition and lung cancer has been published recently,[6] with considerable attention given to the observational studies. Perhaps the most striking finding to emerge from the observational studies of lung cancer is the apparent protective effect of fruits and vegetables, i.e., persons who consume greater quantities of fruits and vegetables are far less likely to develop lung cancer than persons who consume lesser quantities of fruits and vegetables. The prospective studies of dietary factors and lung cancer risk are summarized in Table 6.1, and the retrospective studies of dietary factors and lung cancer risk are summarized in Table 6.2. Table 6.3 summarizes results of prospective studies of blood micronutrient levels and lung cancer risk. As shown in the tables, intake of fruits and vegetables and/or carotenoids (a marker of consumption of fruits and vegetables) was associated with reduced lung cancer risk in 8 of 8 prospective studies and 18 of 20 retrospective studies reviewed.[6] The reduced risk is observed in men and women, in various countries, in current smokers, ex-smokers, and never-smokers, and for all histologic types of lung cancer.

Fruits and vegetables are known to contain numerous substances with purported antioxidant activity including carotenoids, vitamin C, and other phytochemicals such as polyphenols.[7] What specific role these substances/groups of substances play in lung cancer prevention is unknown. As detailed in the tables, many studies have examined the association of lung cancer risk with total carotenoid intake and/or blood β-carotene levels, with vitamin C intake and/or blood levels, with vitamin E

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intake and/or blood levels, and with selenium status as measured biochemically. Individual studies suggest protective effects for each of these nutrients, although the totality of the evidence is not convincing at present for any one of these antioxidant micronutrients.[6]

There is clearly more information to be gleaned from observational studies of nutrition and lung cancer. For example, a recent analysis of intake of fruits and vegetables and lung cancer risk in a large population of nonsmoking (never and former smokers) men and women indicated that the lung cancer risk reduction was greater for fruits and vegetables typically consumed in a raw form, as compared to those typically cooked or processed in some manner.[8] Cooking appears to increase the bioavailability of some substances in fruits and vegetables, such as α- and β-carotene;[9] however, other substances in fruits and vegetables are heat labile, including vitamin C[10] and some of the xanthophyllic carotenoids.[11,12] Epidemiologic studies that specifically address the effects of cooking/processing of fruits and vegetables on lung cancer risk are needed, along with laboratory studies of effects of food preparation on levels of other phytochemicals in foods. Also, future epidemiological studies are needed to identify specific fruits and vegetables, if any, that are associated consistently with lung cancer risk reduction.

Observational epidemiologic studies of specific dietary components depend upon food composition data, which are generally adequate for most of the macronutrients and vitamins found in foods. However, data regarding the concentrations of various phytochemicals in foods are lacking. The U.S. Department of Agriculture recently released an individual carotenoid database, which includes levels of the five major carotenoids found in foods.[13] The release of this database has facilitated new research studies of the association between dietary intake of individual carotenoids and lung cancer risk. Le Marchand and colleagues found that dietary intake of α-carotene, β-carotene, and lutein was associated with reduced lung cancer risk in both men and women.[14] Ziegler and colleagues also found significant inverse trends for dietary α- and β-carotene, and a marginally significant effect for lutein.[15] It is notable that in both studies, high intake of a variety of vegetables was more strongly associated with reduced risk than high intake of the individual carotenoids. A food composition database for some of the major phytochemical classes is being developed; this will open new avenues of investigation for observational studies of dietary antioxidants and lung cancer.

III. INTERVENTION TRIALS OF ANTIOXIDANT NUTRIENTS AND LUNG CANCER

Intervention trials of antioxidant nutrients and lung cancer prevention consist predominantly of β-carotene trials, with some trials using β-carotene as a single agent, and others using it in combination with other antioxidant nutrients. Beginning with trials using premalignant endpoints (see Table 6.4), the Tyler (Texas) Chemoprevention Trial randomized 755 asbestos workers to receive β-carotene (50 mg/day) and retinol (25,000 IU every other day) vs. placebo to see if the combination of the two nutrients could reduce the prevalence of atypical cells in sputum. After a mean intervention period of 58 months, the prevalence of sputum atypia was nonsignificantly

TABLE 6.1
Prospective Studies of Vegetable, Fruit, Carotenoid, and Micronutrient Intake and Lung Cancer [a,b,c]

Study	Year	Location/ population	No. of cases[d]	Years of follow-up	Dietary assessment	Vegetables + fruits	Vegetables	Fruits
Bjelke et al.	1975	Norway	M + F: 153	9 to 12	FFQ		↓	0
Kvale et al.	1983						↓?	↓?
Shekelle et al.	1981	Chicago, IL	M: 33	19	DH			↓↓↓
Kromhout et al.	1987	Netherlands	M: 63	25	DH			↓
Paganini-Hill et al.	1987	Los Angeles, CA, U.S.	M: 125	8	FFQ	↑	↑	↓↓
Shibata et al.	1992	Retirement community	F: 70			↓	↓↓	
Shibata et al.	1992							↓↓↓
Fraser et al.	1991	California, U.S. Seventh-day Adventists	M: 61	6	FFQ			
Knekt et al.	1991	Finland	M: 117	14-20	DH		↓?	↓?
Chow et al.	1992	U.S. White Lutherans	M: 219	20	FFQ		↑	↓
Steinmetz et al.	1993	Iowa, U.S. 55+ years old	F: 138	4	FFQ	↓↓↓	↓↓↓	↓↓

Study	Dark green vegetables	Yellow-orange vegetables	Carotenoids	Retinol	Vitamin A	Vitamin C	Total vitamin C	Vitamin E	Total vitamin E
Bjelke et al.		↓↓			↓↓↓	0			
Kvale et al.			↓↓↓	0		↓?			
Shekelle et al.			0	0		↓↓↓			0
Kromhout et al.			0			0	0		↓↓
[illegible]	↑	↓				[illegible]	↓↓		

Study	Dark green vegetables	Yellow-orange vegetables	Carotenoids	Retinol	Vitamin A	Vitamin C	Total vitamin C	Vitamin E	Total vitamin E
Bjelke et al.		↓↓			↓↓↓	0			
Kvale et al.									
Shekelle et al.			↓↓↓	0		↓?			
Kromhout et al.			0	0		↓↓↓			
Paganini-Hill et al.	↑	↓	0			0	0		0
Shibata et al.	↓↓	↓↓	↓↓			↓↓	↓↓		↓↓
Shibata et al.									
Fraser et al.									
Knekt et al.	0	↓?	↓?	↑?	↑?	0		0	
Chow et al.		↓	0	0	0	↓			
Steinmetz et al.	↓↓↓	↓	↓			0	0		

From Ziegler, R. G., Mayne, S. T., and Swanson, C. A., *Pharmacol. Toxicol.*, 72(Suppl), S116, 1993.

[a] To be included, studies had to assess diet in sufficient detail for the intake of several food groups and/or micronutrients to be evaluated. When sequential analyses of one cohort have been published, results based on the largest number of lung cancer cases or deaths are used.

[b] ↓↓↓ (and ↑↑↑) indicate inverse (and positive) associations with statistically significant trends. ↓↓ (and ↑↑) indicate inverse and positive) associations with marginally significant trends or apparent trends that do not reach statistical significance. ↓ (and ↑) indicate inverse (and positive) associations with no evidence of a trend. ↓? (and ↑?) indicate inverse (and positive) associations with insufficient data presented to evaluate trend. 0 indicates no association.

[c] None of these studies assessed folate or fiber intake. Carotenoids, retinol, vitamin A, vitamin C, and vitamin E refer to intake from foods. Total vitamin C and total vitamin E refer to combined intake from foods and supplements.

[d] M, males; F, females.

[e] FFQ, food frequency questionnaire; DH, diet history.

TABLE 6.2
Retrospective Studies of Vegetable, Fruit, Carotenoid, and Micronutrient Intake and Lung Cancer [a,b,c]

Study	Year	Location/ population	No. of cases[d]	Study design[e]	Dietary assessment[f]	Vegetables + fruits	Vegetables
Mettlin et al.	1979	Buffalo. NY, U.S.	M: 427	Hos	FFQ		
Byers et al.	1984						
Hinds et al.	1984	Hawaii, U.S.	M: 261	Pop	DH		
Kolonel et al.	1985	Multi-ethnic	F: 103				
Ziegler et al.	1984	New Jersey, U.S.	WM: 736	Pop	FFQ	↓↓↓	↓↓↓
Ziegler et al.	1986	Whites and Blacks	WF: 860				↓↓↓
Dorgan et al.	1993		BM: 269				0
			BF: 86				↓
Samet et al.	1985	New Mexico, U.S.	WM + F: 322	Pop	FFQ		
		Whites and Hispanics	HM + F: 125				
Wu et al.	1985	Los Angeles, CA, U.S.	W: 210	Com	FFQ		
		Whites					
Byers et al.	1987	Western New York, U.S.	M: 296	Pop	FFQ		
			F: 154				
Gao et al.	1987	Shanghai, China	F: 672	Pop	FFQ		
Pastorino et al.	1987	Milan, Italy	F: 47	Hos	FFQ		
Fontham et al.	1988	Louisiana, U.S.	M + F: 1253	Hos	FFQ	↓↓↓	↓↓
		Whites and Blacks					
Koo et al.	1988	Hong Kong Chinese	F: 88	Com	FFQ		
		Never-smokers					
Le Marchand et al.	1989	Hawaii, U.S.	M: 230	Pop	DH		↓↓↓
		Multi-ethnic	F: 102				↓↓↓
Dartiques et al.	1990	France	M + F: 143	Hos	FFQ		
Jain et al.	1990	Toronto, Canada	M + F: 839	Pop	DH		↓↓↓
Kalandidi et al.	1990	Athens, Greece	F: 91	Hos	FFQ		0
		Never-smokers					
Wu-Williams et al.	1990	Northeast China	F: 963	Pop	FFQ		0
		Air pollution					
Harris et al.	1991	Oxford, U.K.	M: 96	Hos	FFQ	↓↓	
Candelora et al.	1992	Florida, U.S.	F: 124	Pop	FFQ		↓↓↓
		Never-smokers					
Forman et al.	1992	Yunnan, China	M: 428		FFQ		
Swanson et al.	1992	Tin miners					
Alavanja et al.	1993	Missouri, U.S.	F: 429	Pop	FFQ		0
		Nonsmokers					
Mayne et al.	1994	New York State, U.S.	M: 201	Pop	FFQ	↓↓↓[g]	0
		Nonsmokers	F: 212			↓↓↓[g]	↓↓↓

increased, not reduced, in the subjects receiving supplements.[16] In a 14-week randomized intervention trial in the Netherlands, however, heavy smokers who received supplemental β-carotene (20 mg/day) had a significant reduction in sputum micronuclei.[17] Micronuclei are indicative of DNA damage/chromosomal breakage; however, the relevance of a short-term effect on micronuclei to lung carcinogenesis, which is thought to take decades to develop, is unknown. Notably, in the same trial, β-carotene was not found to reduce *in vivo* oxidation as measured by urinary excretion

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have now been comp
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TABLE 6.2 (Continued)

Vegetables + fruits	Vegetables	Fruits	Dark green vegetables	Yellow-orange vegetables	Carotenoids	Retinol
					↓?	↓?
					↓?	
					↑?	
↓↓↓	↓↓↓	↓	↓↓↓	↓↓↓	↓↓	↑
	↓↓↓	↓↓↓			↓↓↓	
	0	↑↑↑			↑	
	↓	↑			↑↑	
					↓↓	0
					↑	0
					↓?	0
					↓↓↓	
					0	
			↓?		↑	
					↓?	↓?
↓↓↓	↓↓	↓↓↓			0	0
		↓↓↓	↓	↓		
	↓↓↓	0	↓↓↓	↓↓↓	↓↓↓	0
	↓↓↓	0	↓↓↓	↓↓↓	↓↓↓	0
					↓	↓
	↓↓↓	0			↓	0
	0	↓↓			0	↑?
	0	↑				
↓↓			0	↓↓	↓↓↓	↑
	↓↓↓	↓↓↓		↓?	↓↓↓	0
		0	↓↓↓	0		
	0	0			0	0
↓↓↓[g]	0	↓			↓?	0
↓↓↓[g]	↓↓↓	↓↓			↓?	0

of 8-oxo-7,8-dihydro-2′deoxyguanosine, suggesting that antioxidant activity was not responsible for the reduction in micronuclei.[18]

Two trials of antioxidant nutrients that had lung cancer as a primary endpoint have now been completed (see Table 6.4). The Alpha-Tocopherol Beta-Carotene (ATBC) trial[19] involved 29,133 males age 50 to 69 years old from Finland, who were heavy cigarette smokers at entry (average one pack/day for 36 years). The study design was a two-by-two factorial with participants randomized to receive

TABLE 6.2 (Continued)

Study	Year	Location/ population	No. of cases[d]	Study design[e]	Dietary assessment[f]	Vitamin A	Vitamin C	To
Mettlin et al.	1979	Buffalo, NY, U.S.	M: 427	Hos	FFQ	↓?	0	
Byers et al.	1984							
Hinds et al.	1984	Hawaii, U.S.	M: 261	Pop	DH	↓?		
Kolonel et al.	1985	Multi-ethnic	F: 103			0		
Ziegler et al.	1984	New Jersey, U.S.	WM: 736	Pop	FFQ	0		
Ziegler et al.	1986	Whites and Blacks	WF: 860					
Dorgan et al.	1993		BM: 269					
			BF: 86					
Samet et al.	1985	New Mexico, U.S.	WM + F: 322	Pop	FFQ	↓↓↓		
		Whites and Hispanics	HM + F: 125			0		
Wu et al.	1985	Los Angeles, CA, U.S.	W: 210	Com	FFQ	0		
		Whites						
Byers et al.	1987	Western New York, U.S.	M: 296	Pop	FFQ	↓	↓	
			F: 154			0	0	
Gao et al.	1987	Shanghai, China	F: 672	Pop	FFQ	↓?		
Pastorino et al.	1987	Milan, Italy	F: 47	Hos	FFQ			
Fontham et al.	1988	Louisiana, U.S.	M + F: 1253	Hos	FFQ		↓↓↓	
		Whites and Blacks						
Koo et al.	1988	Hong Kong Chinese	F: 88	Com	FFQ			
		Never-smokers						
Le Marchand et al.	1989	Hawaii, U.S.	M: 230	Pop	DH	↓↓↓	↓↓↓	
		Multi-ethnic	F: 102			↓↓	0	
Dartiques et al.	1990	France	M + F: 143	Hos	FFQ			
Jain et al.	1990	Toronto, Canada	M + F: 839	Pop	DH	0	0	
Kalandidi et al.	1990	Athens, Greece	F: 91	Hos	FFQ	0	↓	
		Never-smokers						
Wu-Williams et al.	1990	Northeast China	F: 963	Pop	FFQ			
		Air pollution						
Harris et al.	1991	Oxford, U.K.	M: 96	Hos	FFQ			
Candelora et al.	1992	Florida, U.S.	F: 124	Pop	FFQ	↓↓	↓↓↓	
		Never-smokers						
Forman et al.	1992	Yunnan, China	M: 428		FFQ			
Swanson et al.	1992	Tin miners						
Alavanja et al.	1993	Missouri, U.S.	F: 429	Pop	FFQ		0	
		Nonsmokers						
Mayne et al.	1994	New York State, U.S.	M: 201	Pop	FFQ			
		Nonsmokers	F: 212					

either supplemental vitamin E (50 mg/day), β-carotene (20 mg/day), the combination, or placebo for 5 to 8 years. Unexpectedly, participants receiving β-carotene (alone or in combination with vitamin E) had a significantly higher incidence of lung cancer (RR = 1.18; 95% CI = 1.03 to 1.36) and total mortality (RR = 1.08, 95% CI = 1.01 to 1.16) than participants receiving placebo. Supplemental β-carotene had no significant effect on the incidence of other major cancers occurring in this population (prostate, bladder, colon/rectum, stomach). Tumor site-specific data were not presented regarding the effects of supplemental β-carotene on less common cancers. Within the placebo group, higher β-carotene intake and higher serum

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TABLE 6.2 (Continued)

Vitamin A	Vitamin C	Total vitamin C	Vitamin E	Total vitamin E	Folate	Fiber
↓?	0					0
↓?		↓?				
0		↑?				
0						
↓↓↓						
0						
0						
↓	↓	↓		↓		↓↓↓
0	0	0		0		↓
↓?						
	↓↓↓					
↓↓↓	↓↓↓	↓↓↓			↓↓	↓↓
↓↓	0	↑			↓↓	↓↓
0	0					0
0	↓	↓				
↓↓	↓↓↓					
	0	0	↑↑	↑		↓
				↓		
				↓		

β-carotene concentrations at baseline were each associated with a lower subsequent lung cancer incidence, consistent with the observational literature.

The finding of an increased incidence of lung cancer in β-carotene supplemented smokers has now been replicated in another major trial.[20] The Carotene and Retinol Efficacy Trial (CARET) was a multicenter lung cancer prevention trial of supplemental β-carotene (30 mg/day) plus retinol (25,000 IU/day) vs. placebo in asbestos workers and smokers. The CARET vitamin combination was terminated nearly 2 years early because interim analyses of the data indicated that should the trial have continued for its planned duration, it was highly unlikely that the intervention would

TABLE 6.2 (Continued)

From Ziegler, R. G., Mayne, S. T., and Swanson, C. A., *Cancer Causes and Control*, 7, 157, 1996.

[a] To be included, studies had to assess diet in sufficient detail for the intake of several food groups and/or micronutrients to be evaluated. When several analyses of one study population have been published, results based on the largest number of lung cancer cases or deaths are used.

[b] ↓↓↓ (and ↑↑↑) indicate inverse (and positive) associations with statistically significant trends. ↓↓ (and ↑↑) indicate inverse (and positive) associations with marginally significant trends or apparent trends that do not reach statistical significance. ↓ (and ↑) indicate inverse (and positive) associations with no evidence of a trend. ↓? (and ↑?) indicate inverse (and positive) associations with insufficient data presented to evaluate trend. 0 indicates no association.

[c] Carotenoids, retinol, vitamin A, vitamin C, vitamin E, folate, and fiber refer to intake from foods. Total vitamin C and total vitamin E refer to combined intake from foods and supplements.

[d] M, males; F, females; W, Whites; B, Blacks.

[e] Hos, hospital-based; Pop, population-based; Com, community-based.

[f] FFQ, food frequency questionnaire; DH, diet history.

[g] Trends were seen for raw vegetables and fruits.

have been found to be beneficial, given the results as of late 1995. Furthermore, the interim results indicated that the supplemented group was developing more lung cancer, not less, consistent with the results of the Finnish trial. Overall, lung cancer incidence was increased by 28% in the supplemented subjects (RR = 1.28; 95% CI = 1.04 to 1.57) and total mortality was also increased (RR = 1.17; 95% CI = 1.03 to 1.33). Nonlung cancers were distributed nearly evenly between the two groups. While the p-value for the lung cancer increase ($p = 0.032$) is less than the conventional p-value of 0.05, it cannot be concluded that this trial provided statistically significant evidence of harm. Interim analyses, particularly if done repeatedly, could occasionally indicate a treatment difference ($p < 0.05$) even when one does not truly exist, just due to random fluctuations in the data.[21] Thus, in order to preserve an overall significance level of 0.05, interim analyses typically have very stringent significance levels ($p = 0.007$ for the 1995 CARET analysis).

The CARET investigators reported that baseline serum β-carotene levels were inversely correlated with the subsequent incidence of lung cancer in both the placebo and β-carotene groups.[20] CARET results also suggested effect modification by smoking status. Supplemental β-carotene increased lung cancer risk significantly in the participants who were smoking at the time of randomization (RR = 1.42; 95% CI = 1.07 to 1.87). In contrast, supplemental β-carotene was nonsignificantly protective in those smokers who had given up smoking prior to randomization (RR = 0.80; 95% CI = 0.48 to 1.31).[20] Risk estimates for lung cancer in former smokers are not available from the ATBC study, which restricted eligibility to current smokers.

Major findings of one additional trial, the Physicians' Health Study of supplemental β-carotene vs. placebo in 22,071 male U.S. physicians, were also published[22] along with the CARET results. There was no significant effect — positive or negative — of 12 years of supplementation of β-carotene (50 mg every other day) on cancer or total mortality. With regard to lung cancer, there was no indication of excess lung cancer in the β-carotene supplemented individuals, even among smokers. The relative risk for lung cancer in current smokers randomized to β-carotene was 0.90 (95% CI = 0.58 to 1.40). Among nonsmokers, the relative risk was 0.78 (0.34 to

1.79). The apparen
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(95% CI = 0.26
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1.79). The apparent lack of an effect of long-term supplementation of β-carotene on lung cancer incidence, even in baseline smokers who took the supplements for up to 12 years, is noteworthy. While only 11% of the participants were current smokers at entry, an additional 39% were former smokers. As discussed further below, the lack of effect of supplemental β-carotene on lung cancer in this trial may be an important clue in trying to understand the overall relationship between β-carotene and lung cancer.

The findings of the Physicians' Health Study illustrate the potential problems involved in overinterpreting results based upon interim analyses. After 9 years of intervention, an interim analysis was done to look at any cancers that developed during years 5 to 9 of the intervention. Discounting the first few years of intervention is typical and reasonable, as cancers that develop shortly after randomization were probably pre-existing yet undiagnosed. When this analysis was completed, the β-carotene supplemented group had 500 total cancers vs. 567 in the placebo group ($p = 0.03$).[22] Rather than closing the trial at this point, the finding of benefit was used as a grounds for continuation. At the conclusion of the trial, the reduction in total cancer was no longer statistically significant or clinically relevant in the total study population. This highlights why caution must be used in interpreting results obtained from trials before their planned conclusion.

Two other trials involving antioxidant nutrients were not designed as lung cancer prevention trials; nonetheless, their results are of relevance to the topic of lung cancer prevention. The first was a trial of selected nutrient combinations in the prevention of esophageal and gastric cancers in China.[23] The trial was conducted in residents from the general population of Linxian County, a population with several micronutrient deficiencies. Nearly 30,000 men and women aged 40 to 69 took part in the study, which tested the efficacy of four different nutrient combinations at inhibiting the development of esophageal and gastric cancers. The nutrient combinations included retinol plus zinc, riboflavin plus niacin, ascorbic acid plus molybdenum, and the combination of β-carotene, selenium, and vitamin E. After a 5-year intervention period, those who were given the combination of β-carotene, vitamin E, and selenium had a 13% reduction in cancer deaths (RR = 0.87; 95% CI = 0.75 to 1.00), a 9% reduction in total deaths (RR = 0.91; 95% CI = 0.84 to 0.99), a 4% reduction in esophageal cancer deaths (RR = 0.96; 95% CI = 0.78 to 1.18), and a 21% reduction in gastric cancer deaths (RR = 0.79; 95% CI = 0.64 to 0.99).[23] None of the other nutrient combinations reduced gastric or esophageal cancer deaths significantly in this trial. As for lung cancer, this trial had limited statistical power with only 31 total lung cancer deaths.[24] However, the relative risk of death from lung cancer was 0.55 (95% CI = 0.26 to 1.14) among those receiving the combination of β-carotene, α-tocopherol, and selenium. The smoking prevalence, including individuals who had ever smoked cigarettes for 6 months or more, was 30% in this study population.

Another study was a decade-long, double-blind trial of a daily oral supplement of selenium-enriched yeast (200 μg selenium/person/day) vs. placebo in 1300 older Americans. The study, designed as a skin cancer prevention trial, observed a significant reduction in the incidence of total cancer (RR = 0.59; $p = 0.0004$) and that of several cancer sites, including lung cancer (RR = 0.54; $p = 0.04$).[25] The reduction in lung cancer risk, while intriguing, was not a prior hypothesis and is presumably

TABLE 6.3
Prospective Studies of Blood Micronutrient Levels and Lung Cancer [a,b]

Study	Year	Location/ population	No. of cases[c]	Years of follow-up	β-Carotene	Vitamin A	Vitamin E	Vitamin C	Selenium
Stahelin et al.	1984	Basel, Switzerland	M: 68	12-14	↓↓↓	↓[d]	↓	0	
Stahelin et al.	1991								
Stahelin et al.	1991								
Nomura et al.	1985	Hawaii, U.S.	M: 74	~10	↓↓↓	0	0		0
Nomura et al.	1987	Japanese							
Menkes et al.	1986	Washington County, MD, U.S.	M + F: 99	9	↓↓↓	0	↓↓↓		↑↑
Wald et al.	1986	London, U.K.	M: 50	3-10	↓↓↓	0	0		
Wald et al.	1987								
Wald et al.	1988								
Friedman et al.	1986	San Francisco-Oakland, CA, U.S.	M + F: 151	7-14		0			
Virtamo et al.	1987	Finland							
		55+ years old	M: 38	9					↓?
Connett et al.	1989	22 centers in U.S.	M: 66	10	↓↓	0	↓		
Knekt et al.	1988	Finland	M: 144	5-9	0	↓?	↑?		↓↓↓
Knekt et al.	1990								
Knekt et al.	1990								0
Criqui et al.	1991	10 centers in U.S.	M + F: 27	8.5		0			↓↓↓[e]
Van den Brandt et al.	1993	Netherlands	M: 285	3					↓↓↓[e]
		55+ years old	F: 32						0
Kabuto et al.	1994	Japan	M + F: 77	11-13					↑↑[e]
Garland et al.	1995	U.S.	F: 47	3.5					

[illegible] 7, 157, 1996

Comstock et al.	1989	22 centers in U.S.	M: 66	10	↓↓	0	↓	
Knekt et al.	1988	Finland	M: 144	5-9	0	↓?	↑?	↓↓↓
Knekt et al.	1990							
Knekt et al.	1990							
Criqui et al.	1991	10 centers in U.S.	M + F: 27	8.5		0		0
Van den Brandt et al.	1993	Netherlands	M: 285	3				↓↓↓[c]
		55+ years old	F: 32					↓↓↓[c]
Kabuto et al.	1994	Japan	M + F: 77	11-13				0
Garland et al.	1995	U.S.	F: 47	3.5				↑↑[e]

From Ziegler, R. G., Mayne, S. T., and Swanson, C. A., *Cancer Causes and Control*, 7, 157, 1996.

[a] Only analyses with at least 25 lung cancer cases or deaths are included. When sequential analyses of one cohort have been published, results based on the largest number of lung cancer cases or deaths are used.

[b] ↓↓↓ (and ↑↑↑) indicate inverse (and positive) associations with statistically significant trends. ↓↓ (and ↑↑) indicate inverse (and positive) associations with marginally significant trends or apparent trends that do not reach statistical significance. ↓ (and ↑) indicate inverse (and positive) associations with no evidence of a trend. ↓? (and ↑?) indicate inverse (and positive) associations with insufficient data presented to evaluate trend. 0 indicates no association.

[c] M, males; F, females.

[d] Only observed in men 60+ years of age.

[e] Selenium was measured in toenails.

TABLE 6.4
Chemoprevention Trials Relevant to Antioxidant Nutrients and Lung Cancer

Study, year	Population	Daily intervention	Total subjects	Endpoint	Result
		Premalignant Endpoints			
McLarty et al., 1995	Male asbestos workers	50 mg β-carotene + 25,000 IU Vitamin A[a]	755	Sputum atypia	OR = 1.24 (CI = 0.78 to 1.96)
Van Poppel et al., 1992	Male smokers	20 mg β-carotene	114	Sputum micronuclei	27% decrease (CI = 9 to 41%)
		Primary Prevention — Completed			
ATBC, 1994	Finnish male smokers	20 mg β-carotene ± 50 mg Vitamin E	29,133	Lung cancer	BC: RR = 1.18 (CI = 1.03 to 1.36) Vit. E: RR = 0.98 (CI = 0.86 to 1.12)
CARET, 1996	Smokers/asbestos workers	30 mg β-carotene + 25,000 IU Vitamin A	18,314	Lung cancer	RR = 1.28 (CI = 1.04 to 1.57)
Physicians' Health Study, 1996	Male physicians	50 mg β-carotene[a] ± 325 mg Aspirin[a]	22,071	Cancer	RR = 0.98 (CI = 0.91 to 1.06)
				Lung cancer[b]	RR = 0.90 (CI = 0.58 to 1.40)
Blot et al., 1994	General population Linxian, China	15 mg β-carotene + 30 mg Vitamin E + 50 µg Selenium	29,584	Lung cancer[c]	RR = 0.55[c] (CI = 0.26 to 1.14)
Clark et al., in press	Older Americans	200 µg Selenium	4,300	Lung cancer[c]	RR = 0.54 [illegible]
		Primary Prevention — Ongoing			
Women's Antioxidant Cardiovascular Study	High-risk women	500 mg Vitamin C + 600 IU Vitamin E + 50 mg β-carotene[c]	8000	Cancer, CHD	
Heart Protection Study	High-risk CHD U.K.	20 mg β-carotene+ 600 mg Vitamin E+ 250 mg Vitamin C	20,000+	Cancer, CHD	

		50 µg Selenium			(CI = 0.26 to 1.14)
Clark et al., in press	Older Americans	200 µg Selenium	>1300	Lung cancer[c]	RR = 0.54[c] (p = 0.04)
		Primary Prevention — Ongoing			
Women's Antioxidant Cardiovascular Study	High-risk women	500 mg Vitamin C ± 600 IU Vitamin E[a] + 50 mg β-carotene[a]	8000	Cancer, CHD	
Heart Protection Study	High-risk CHD U.K.	20 mg β-carotene+ 600 mg Vitamin E+ 250 mg Vitamin C	20,000+	Cancer, CHD	
		Secondary Prevention — Completed			
Pastorino et al., 1993	Stage I non-small cell lung cancer patients	300,000 IU Vitamin A	307	Second cancer	Longer time to second cancer (p = 0.045)
		Secondary Prevention — Ongoing			
EUROSCAN	Oral, larynx, lung cancer patients	300,000 IU Vitamin A 600 mg *N*-acetylcysteine	2000	Second cancer	
Mayne et al.	Oral, pharynx, larynx cancer patients	50 mg β-carotene	264	Second cancer (incl. lung)	
Toma et al.	Oral, pharynx, larynx cancer patients	75 mg β-carotene[d]	211	Second cancer (incl. lung)	

Note: OR = odds ratio; CI = 95% confidence interval; RR = relative risk; BC = β-carotene; Vit. E. = vitamin E.

[a] The dose listed was taken every other day. All others are daily doses.
[b] Lung cancer in smokers only (overall RR = 0.93 for β-carotene).
[c] Lung cancer not a primary endpoint of the trial; results based upon relatively few lung cancer cases.
[d] Given in 4-month cycles of three months on medication followed by one month off medication.

based upon relatively small numbers (number of lung cancers not stated). Thus, this finding must be interpreted cautiously.

IV. INTERPRETATION

As detailed above, there is a lack of concordance between the observational studies of antioxidant nutrients and lung cancer and the trials, at least with regard to β-carotene. Even within the trials, there are discordant findings regarding β-carotene and lung cancer. This section will attempt to reconcile and discuss some of these inconsistencies.

Beginning with a comparison of the trials, the CARET and ATBC trial both found an excess of lung cancer in the active intervention group, but it must be noted that the interventions were not the same. The CARET used a combination of β-carotene plus retinol; in contrast the ATBC trial used β-carotene alone or in combination with α-tocopherol in a factorial design, allowing for an evaluation of the independent effect of β-carotene. Since both trials found adverse effects, this has led to the widespread conclusion that the β-carotene must necessarily be the agent responsible for the increased lung cancer incidence. However, as previously noted, the Physicians' Health Study used β-carotene by itself and for a longer duration, and did not see any evidence of an increase in lung carcinogenesis, even among smokers. How does one explain this? Several issues can be considered, including baseline nutrient status, bioavailable dose, smoking interactions, and single nutrients vs. nutrient combinations.

Baseline nutrient status: Plasma β-carotene levels in participants in the Chinese trial, which showed a benefit with antioxidant nutrient supplementation, were low as expected (approximately 0.06 μg/mL).[23] These levels compare to median levels of 0.17 μg/mL in CARET[20] and in ATBC,[19] and even higher levels in the Physicians' Health Study (0.30 μg/mL reported in the placebo group[22]). Based upon the Chinese data, pre-randomization blood nutrient levels could be important in predicting efficacy. Some, but not all, observational studies of dietary β-carotene and lung cancer risk find that the majority of the reduction in lung cancer risk associated with increasing dietary β-carotene intake occurs between the first and second quartiles of intake. For example, in a recent study of lung cancer risk in nonsmokers (where residual confounding by active smoking is not problematic), the odds ratios for lung cancer risk in males dropped from 1.0 for the lowest dietary quartile of raw fruits and vegetables to 0.52 in the second quartile. Improving intake to the third and fourth quartiles had only a marginal additional benefit (odds ratios 0.51 and 0.41, respectively).[8] A recent analysis of total mortality, cancer mortality, and cardiovascular mortality as a function of baseline plasma β-carotene showed notably more deaths in the lowest quartile of plasma β-carotene.[26] For example, 101 persons in the lowest quartile died during the follow-up period, compared to 71, 55, and 58 in the next 3 quartiles of plasma β-carotene. It is thus possible that more moderate doses of supplemental β-carotene (see below), or preferably increasing intake of fruits and vegetables, might have more favorable effects on lung cancer and total mortality if given to populations with low β-carotene intake at baseline.

Bioavailable dose: Ano
dosing regimen, formulatior
levels. The Physicians' He
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mean or median).[22] In cont
Hoffmann La Roche formu
to a very high level of 3.0 μ
consisting of 30 mg β-caro
palmitate, elevated media
2.1 μg/mL.[20] Blood levels
to 0.9 μg/mL with the 15
adverse outcomes had the
had been reached. It is po
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Bioavailable dose: Another possibility worthy of consideration surrounds the dosing regimen, formulation, and actual exposure as estimated by plasma β-carotene levels. The Physicians' Health Study gave 50 mg β-carotene (BASF formulation) every other day, a regimen that elevated plasma β-carotene levels only fourfold relative to the placebo group, to a level of 1.2 μg/mL (unclear if this value is for mean or median).[22] In contrast, the ATBC intervention (20 mg β-carotene per day, Hoffmann La Roche formulation) elevated median β-carotene levels nearly 18-fold, to a very high level of 3.0 μg/mL postsupplementation.[19] The CARET intervention, consisting of 30 mg β-carotene/day (Hoffmann La Roche formulation) plus retinyl palmitate, elevated median plasma β-carotene levels approximately 12-fold, to 2.1 μg/mL.[20] Blood levels in participants in the Chinese trial were elevated 15-fold, to 0.9 μg/mL with the 15 mg supplemental β-carotene.[23] Thus, the two trials with adverse outcomes had the highest blood β-carotene levels, suggesting that toxicity had been reached. It is possible that there may be a threshold plasma level below which adverse effects are not seen. In the absence of supporting mechanistic data, future trials of β-carotene, should they be undertaken, would be well-advised to use populations with low β-carotene status at entry and/or select doses/formulations that produce plasma β-carotene levels lower and more physiologic than those achieved in CARET and in the ATBC trial.

Smoking interactions: Smoking status is another potential explanation for the differences in results between the trials. The beneficial results seen in the Chinese trial may reflect a low total prevalence of smoking (30% ever smoking). This is in contrast to 50% ever smoking prevalence in the Physicians' Health Study, nearly 100% in CARET, and 100% in the ATBC trial. CARET smokers given supplemental β-carotene, who had a greater risk for lung cancer than smokers in ATBC given supplemental β-carotene, reported smoking more cigarettes per day than participants in ATBC (24 vs. 20 cigarettes/day). Other trials of β-carotene should examine their data for possible effect modification by smoking status and intensity.

Single nutrients vs. combinations: Alternatively, the beneficial results seen in the Chinese trial may have been due to the selenium, the α-tocopherol, or the combination of the three nutrients, particularly in a micronutrient deficient population. The one trial that used supplemental selenium as a single agent did find beneficial effects on lung cancer,[25] although that was not the primary endpoint of the trial. Another explanation for the beneficial effects observed in the Chinese trial is that a combination of antioxidants may have greater efficacy with regard to cancer prevention than isolated antioxidant nutrients. Experimentally, antioxidant nutrients are known to interact. For example, β-carotene in oil has been shown to be oxidized rapidly in the absence of α-tocopherol; however, it is relatively stable when combined with α-tocopherol.[27] Several ongoing trials (see Table 6.4) are using combinations of antioxidants, the results of which will help clarify the value of combination approaches vs. single agent approaches for cancer prevention. Examples include the British Heart Protection study, a large trial (goal = 20,000) of cholesterol-lowering medication and antioxidant nutrients in a factorial design, the U.S. Women's Antioxidant Cardiovascular Disease Study, and others.

V. CONCLUSIONS AND RECOMMENDATIONS

The vast majority of lung cancer is caused by tobacco, the smoke of which is known to be highly oxidative. Furthermore, individuals who consume more fruits and vegetables, rich sources of many phytochemicals, including antioxidant nutrients, are far less likely to develop lung cancer. Recently, completed trials of antioxidant nutrients and lung cancer prevention, however, indicate that high-dose antioxidant nutrient supplements do not seem to be of value for lung cancer prevention, with the possible exception of selenium, in well-nourished populations. The efficacy of antioxidant nutrient supplementation for lung cancer prevention in micronutrient deficient populations is unknown, as there are too little data available to comment. What, then, is a reasonable course of action for studies of antioxidant nutrients and lung cancer prevention? There are multiple avenues of research that would help to unravel the complexities of this research area. A few suggestions are as follows.

A wealth of new information from additional analyses of completed and ongoing trials will be forthcoming. Some of the subgroup analyses may suggest mechanisms that could then be evaluated for biological plausibility in metabolic or laboratory studies. Such research should help to explain whether or not β-carotene is causally associated with lung cancer risk and the generalizability of the effect. Some mechanisms to explain the adverse effects have been suggested;[28,29] careful evaluation of these hypotheses is needed.

A greater emphasis on the exploration of subgroups in observational studies as well as in trials to determine which subgroups are more likely to benefit from a given intervention is needed. The promising results obtained in the Linxian County, China trial suggest that subgroups with deficiencies of various micronutrients may be of particular interest. The public health approach of identifying populations with nutrient deficiencies and supplementing them to adequacy in order to prevent disease has been used successfully for decades, and should continue to be explored for the purposes of cancer prevention.

The beneficial effects observed in the Linxian County trial with the combination of three antioxidant nutrients — β-carotene, α-tocopherol, and selenium — suggest that further attention should also be given to combinations of nutrients, both in the observational studies as well as in the trials. Several observational studies of antioxidant nutrients and chronic disease have attempted to create an "antioxidant index," to take into account a person's dietary intake and/or blood nutrient status of several antioxidant nutrients, including β-carotene, vitamin C, vitamin E, and selenium. While intuitively appealing, there is no consistent approach used for generating antioxidant indices, nor have any of the indices been validated as being predictive of some meaningful measure of total antioxidant capacity. A challenge for the antioxidant/chronic disease field is to develop and validate meaningful biomarkers of oxidative damage and antioxidant status. This is perhaps more difficult than what might seem at first glance, given the fact that antioxidant capacity has been found to be a function of the initiating (reactive oxygen) species involved.[30] Since we do not know at this point what reactive oxygen species are involved in the pathogenesis of various diseases such as lung cancer, the predictive value of any measure of

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1. Kabat, G. C., Recent d
73, 1993.
2. American Cancer Soci
3. Shopland, D. R., Eyre,
is lung cancer now th
Cancer Inst., 83, 1142
4. U.S. Department of H
Report of the Surgeo
Chronic Disease Prev
MD, 1990, 110.
5. Church, D. F. and Pr
implications, *Environ*
6. Ziegler, R. G., Mayn
Control, 7, 157, 199
7. Dragsted, L. O., Stru
Biochemical and bio
8. Mayne, S. T., Janeric
beta carotene and lu
9. Olson, J. A., Needs
10. Guthrie, H. A., *Intro*
11. Micozzi, M. S., Bee
raw and cooked foo
12. Khachik, F., Goli, M
of food preparation
of tomatoes and se
13. Mangels, A. R., Ho
of fruits and veget
14. Le Marchand, L., I
Intake of specific c
1993.
15. Ziegler, R. G., Col.
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antioxidant capacity is thus unknown. The reactive oxygen species involved in the pathogenesis of some diseases are better characterized; for example, erythropoietic protoporphyria is known to involve singlet oxygen.[31] β-Carotene's well-known therapeutic value in the treatment of this disease[31] most likely relates to the fact that β-carotene is one of the most efficient quenchers of singlet oxygen. This also implies that measures of resistance to singlet oxygen-induced oxidation would be predictive with regard to that disease. Cancer, in contrast, is far more complex, likely involving multiple reactive oxygen species, potentially at multiple steps in the disease process (initiation, promotion, progression). This must be considered by those attempting to develop/validate antioxidant measures for observational studies of cancer etiology, as well as those designing antioxidant-based interventions for cancer prevention clinical trials.

REFERENCES

1. Kabat, G. C., Recent developments in the epidemiology of lung cancer. *Semin. Surg. Oncol.*, 9, 73, 1993.
2. American Cancer Society, *Cancer Facts and Figures 1996*, Atlanta, GA.
3. Shopland, D. R., Eyre, H. J., and Pechacek, T. F., Smoking-attributable cancer mortality in 1991: is lung cancer now the leading cause of death among smokers in the United States? *J. Natl. Cancer Inst.*, 83, 1142, 1991.
4. U.S. Department of Health and Human Services, *The Health Benefits of Smoking Cessation: A Report of the Surgeon General*, Public Health Service, Centers for Disease Control, Center for Chronic Disease Prevention and Health Promotion, Office of Smoking and Health, Rockville, MD, 1990, 110.
5. Church, D. F. and Pryor, A. W., Free radical chemistry of cigarette smoke and its toxicological implications, *Environ. Health Perspect.*, 64, 111, 1985.
6. Ziegler, R. G., Mayne, S. T., and Swanson, C. A., Nutrition and lung cancer, *Cancer Causes and Control*, 7, 157, 1996.
7. Dragsted, L. O., Strube, M., and Larsen, J. C., Cancer protective factors in fruits and vegetables: Biochemical and biological background, *Pharmacol. Toxicol.*, 72(suppl), s116, 1993.
8. Mayne, S. T., Janerich, D. T., Greenwald, P., Chorost, S., Tucci, C., Zaman, M. B., et al., Dietary beta carotene and lung cancer risk in U.S. nonsmokers, *J. Natl. Cancer Inst.*, 86, 33, 1994.
9. Olson, J. A., Needs and sources of carotenoids and vitamin A, *Nutr. Rev.*, 52, 67, 1994.
10. Guthrie, H. A., *Introductory Nutrition*, 4th Edition, C. V. Mosby, St. Louis, 1979, 279.
11. Micozzi, M. S., Beecher, G. R., Taylor, P. R., and Khachik, F., Carotenoid analyses of selected raw and cooked foods associated with a lower risk for cancer, *J. Natl. Cancer Inst.*, 82, 282, 1990.
12. Khachik, F., Goli, M. B., Beecher, G. R., Holden, J. H., Lusby, W. R., Tenorio, M. D., et al., Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables, *J. Agric. Food Chem.*, 40, 390, 1992.
13. Mangels, A. R., Holden, J. M., Beecher, G. R., Forman, M. R., and Lanza, E., Carotenoid content of fruits and vegetables: an evaluation of analytic data, *J. Am. Diet. Assoc.*, 93, 284, 1993.
14. Le Marchand, L., Hankin, J. H., Kolonel L. N., Beecher, G. R., Wilkens, L. R., and Zhao, L. P., Intake of specific carotenoids and lung cancer risk, *Cancer Epidemiol., Biomarkers Prev.*, 2, 183, 1993.
15. Ziegler, R. G., Colavito, E. A., Hartge, P., McAdams, M. J., Schoenberg, J. B., Mason, T. J., et al., The importance of alpha-carotene, beta-carotene, and other phytochemicals in the etiology of lung cancer, *J. Natl. Cancer Inst.*, 88, 612, 1996.

16. McLarty, J. W., Holiday, D. B., Girard, W. M., Yanagihara, R. H., Kummet, T. D., and Greenberg, S. D., Beta-carotene, vitamin A and lung cancer chemoprevention: results of an intermediate endpoint study, *Am. J. Clin. Nutr.*, 62(Suppl), 1431S, 1995.
17. Van Poppel, G., Kok, F. J., and Hermus, R. J., Beta-carotene supplementation in smokers reduces the frequency of micronuclei in sputum, *Br. J. Cancer*, 66, 1164, 1992.
18. Van Poppel, G., Poulsen, H., Loft, S., and Verhagen, H., No influence of beta-carotene on oxidative DNA damage in male smokers, *J. Natl. Cancer Inst.*, 87, 310, 1995.
19. 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.*, 330, 1029, 1994.
20. Omenn, G. S., Goodman, G. E., Thornquist, M. D., Balmes, J., Cullen, M. R., Glass, A., et al., Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease, *N. Engl. J. Med.*, 334, 1150, 1996.
21. Pocock, S. J., *Clinical Trials: A Practical Approach*, John Wiley and Sons, New York, 1983.
22. Hennekens, C. H., Buring, J. E., Manson, J. E., Stampfer, M., Rosner, B., Cook, N. R., 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.*, 334, 1145, 1996.
23. Blot, W. J., Li, J.-Y., Taylor, P. R., Guo, W., Dawsey, S., Wang, G.-Q., et al., Nutrition intervention trials in Linxian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population, *J. Natl. Cancer Inst.*, 85, 1483, 1993.
24. Blot, W. J., Li, J-Y., Taylor, P. R., and Li, B., Lung cancer and vitamin supplementation, Letter to the editor, *N. Engl. J. Med.*, 331, 614, 1994.
25. Combs, G. F., Jr., Selenium and cancer prevention, in *Antioxidants and Disease Prevention*, ch 8 of this volume.
26. Greenberg, E. R., Baron, J. A., Karagas, M. R., Stukel, T. A., Nierenberg, D. W., Stevens, M. M., et al., Mortality associated with low plasma concentration of beta carotene and the effect of oral supplementation, *J. Am. Med. Assoc.*, 275, 699, 1996.
27. Terao, J., Yamauchi, R., Murakami, H., and Matsushita, S., Inhibitory effects of tocopherols and beta-carotene on singlet oxygen-initiated photooxidation of methyl linoleate and soybean oil, *J. Food Process. Preserv.*, 4, 79, 1980.
28. Mayne, S. T., Handelman, G. J., and Beecher, G., Beta-carotene and lung cancer promotion: A plausible relationship?, *J. Natl. Cancer Inst.*, 88, 1513, 1996.
29. Olson, J. A., Benefits and liabilities of vitamin A and carotenoids, *J. Nutr.*, 126(suppl.), 1208S, 1996.
30. Halliwell, B. and Gutteridge, J. M. C., The definition and measurement of antioxidants in biological systems, Letter to the Editor, *Free Rad. Biol. Med.*, 18, 125, 1995.
31. Mathews-Roth, M. M., Carotenoids in erythropoietic protoporphyria and other photosensitivity diseases, *Ann. N.Y. Acad. Sci.*, 691, 127, 1993.

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