Review

Lutein and Zeaxanthin and Their Potential Roles in Disease Prevention

Judy D. Ribaya-Mercado, ScD, Jeffrey B. Blumberg, PhD, FACN

Antioxidants Research Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts

Key words: lutein, zeaxanthin, xanthophylls, carotenoids, age-related macular degeneration, cataract, cancer, heart disease, stroke

Lutein and zeaxanthin are xanthophyll carotenoids found particularly in dark-green leafy vegetables and in egg yolks. They are widely distributed in tissues and are the principal carotenoids in the eye lens and macular region of the retina. Epidemiologic studies indicating an inverse relationship between xanthophyll intake or status and both cataract and age-related macular degeneration suggest these compounds can play a protective role in the eye. Some observational studies have also shown these xanthophylls may help reduce the risk of certain types of cancer, particularly those of the breast and lung. Emerging studies suggest as well a potential contribution of lutein and zeaxanthin to the prevention of heart disease and stroke. Even as the evidence for a role of lutein and zeaxanthin in disease prevention continues to evolve, particularly from human studies directed to their bioavailability, metabolism, and dose-response relationships with intermediary biomarkers and clinical outcomes, it is worth noting that recommendations to consume foods rich in xanthophylls are consistent with current dietary guidelines.

Key teaching points:

- Rich dietary sources of lutein and zeaxanthin are dark green leafy vegetables and egg yolks.
- Many epidemiologic studies indicate an inverse relationship between xanthophyll intake and/or status and both cataract and age-related macular degeneration.
- Observational studies and animal model data suggest a potential protective role for xanthophylls against certain kinds of cancers, with promising albeit inconsistent results for breast and lung cancers, and largely a null outcome for bladder cancer.
- A limited body of experimental and epidemiological evidence suggests a potential role for lutein and zeaxanthin in reducing the risk for coronary heart disease and stroke.
- Dietary guidelines for promoting health are consistent with generous intakes of foods rich in lutein and zeaxanthin.

INTRODUCTION

The USDA National Nutrient Database lists the combined content of lutein plus zeaxanthin in foods [1]. These carotenoids are present in a wide variety of plant foods especially dark-green leafy vegetables such as kale, spinach, turnip greens, and collards. Their concentrations in these plant foods, as well as in others such as mustard greens, green peas, summer squash, and broccoli are higher than those of β-carotene concentrations [1] (Table 1). Several studies have reported that lutein bioavailability from green vegetables is higher than that of β-carotene [2–4], although another study [5] suggests otherwise. Lutein and zeaxanthin are also highly concentrated in egg yolks (Table 2) from which they are highly bioavailable due most probably to the lipid matrix in which they reside [6].

Address reprint requests to: Dr. Jeffrey Blumberg, Antioxidants Research Laboratory, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, 711 Washington Street, Boston, MA 02111. E-mail: jeffrey.Blumberg@tufts.edu

Presented, in part, at a meeting sponsored by the American Egg Board and Egg Nutrition Center and held at the U.S. Department of Agriculture, Washington, D.C. on September 23, 2003: *First International Scientific Symposium on Eggs and Human Health: The Transition from Restrictions to Recommendation.*

An honorarium was provided for support of this manuscript by the Egg Nutrition Center. Dr. Blumberg is a member of the Scientific Advisory Panel of the Egg Nutrition Center and the Advisory Board of the Lutein Information Bureau of Kemin Foods, L.C.

Table 1. Content of Lutein plus Zeaxanthin, and β-Carotene in Selected Vegetables[1]

	Lutein plus Zeaxanthin	β-Carotene	Lutein plus Zeaxanthin	β-Carotene
	μg/measure[2]		μg/100g	
Kale	23720	10625	18246	8173
Spinach	20354	11318	11308	6288
Turnip greens	12154	6588	8440	4575
Collards	14619	9147	7694	4814
Mustard greens	8347	5312	5962	3794
Parsley, raw	556	505	5560	5050
Dandelion greens	4944	6248	4709	5950
Peas, green, frozen	3840	2000	2400	1250
Lettuce, romaine, raw	1295	1951	2313	3484
Squash, summer	4048	229	2249	127
Beet greens	2619	6610	1819	4590
Lettuce, green leaf, raw	969	2488	1730	4443
Broccoli	2367	1841	1517	1180
Squash, winter	2901	5726	1415	2793
Brussels sprouts	2012	725	1290	465
Onions, spring or scallions, raw	1137	598	1137	598
Corn, sweet, yellow, canned	2195	69	1045	33
Pumpkin	2484	5135	1014	2096

[1] From the USDA National Nutrient Database [1]. Values are amounts in cooked vegetables unless otherwise stated.
[2] Amounts in 1 cup, except for parsley (10 sprigs weighing 10 g)

.

Table 2. Content of Lutein and Zeaxanthin in Chicken Egg Yolk[1]

	Lutein[2]	Zeaxanthin[2]	Total
μg/yolk	292 ± 117	213 ± 85	505
μg/mg cholesterol	1.19 ± 0.32	0.87 ± 0.23	2.06
μg/100 g yolk	1723 ± 690	1257 ± 502	2980

[1] From Handelman *et al.* [6].
[2] Mean ± SD values.

The structures of lutein and zeaxanthin are characterized by the presence of a hydroxyl group attached to each of the 2 terminal β-ionone rings in the molecule; these xanthophylls are more hydrophilic than other carotenoids found in blood and tissues such as the hydrocarbon carotenoids α- and β-carotene and lycopene, and the monohydroxyl carotenoid, β-cryptoxanthin (Fig. 1). The hydrophilic properties of lutein and zeaxanthin allow them to react with singlet oxygen generated in water phase more efficiently than nonpolar carotenoids [7]. Unlike α- and β-carotene and β-cryptoxanthin, lutein and zeaxanthin are not precursors of vitamin A.

Research involving cell cultures, animal models, and human studies has been directed to the potential role of lutein and zeaxanthin in protecting against several chronic diseases, particularly age-related macular degeneration (AMD) and cataract, cancer at various sites, and heart disease and stroke. An overview of this evidence, stressing principally human studies (especially observational surveys), is summarized in this critical review.

DISEASES OF THE EYE

The xanthophylls are uniquely concentrated in the macular region of the retina [8–10] with zeaxanthin being the dominant component in the central macula and lutein distributed throughout the retina [9–11]. *meso*-Zeaxanthin has been identified in human macula and appears to be a conversion product derived from lutein or zeaxanthin in the retina [12,13]. Bernstein *et al.* [14] and Yemelyanov *et al.* [15] have identified specific xanthophyll-binding proteins in human retina and macula. Lutein and zeaxanthin are the only carotenoids reported to be present in eye lens [16]. There is an inverse relation between macular pigment density and lens density, suggesting that the macular pigment may serve as a marker for xanthophylls in the lens [17,18]. Possible biologic mechanisms of the protective role of lutein and zeaxanthin in the eye have been reviewed by Krinsky *et al.* [13] and include their ability to: [a] filter harmful short-wave blue light, and [b] function as antioxidants. In liposomes, the blue light filtering efficacy of carotenoids has been ranked as lutein > zeaxanthin > β-carotene > lycopene [19]. The identification of oxidation products of lutein and zeaxanthin in human retina [20,21] lens [21], and other ocular tissues [21] lends support for an antioxidant role of xanthophylls in the human eye. Lutein and zeaxanthin have been identified in human rod outer segment membranes where the concentration of long-chain polyunsaturated fatty acids and susceptibility to oxidation is highest [22]. In quails fed supplemental zeaxanthin and exposed to intermittent white light, the number of apoptotic rods and cones in light-damaged eyes were inversely correlated with zeaxanthin concentration in the contralateral retina [23]. In

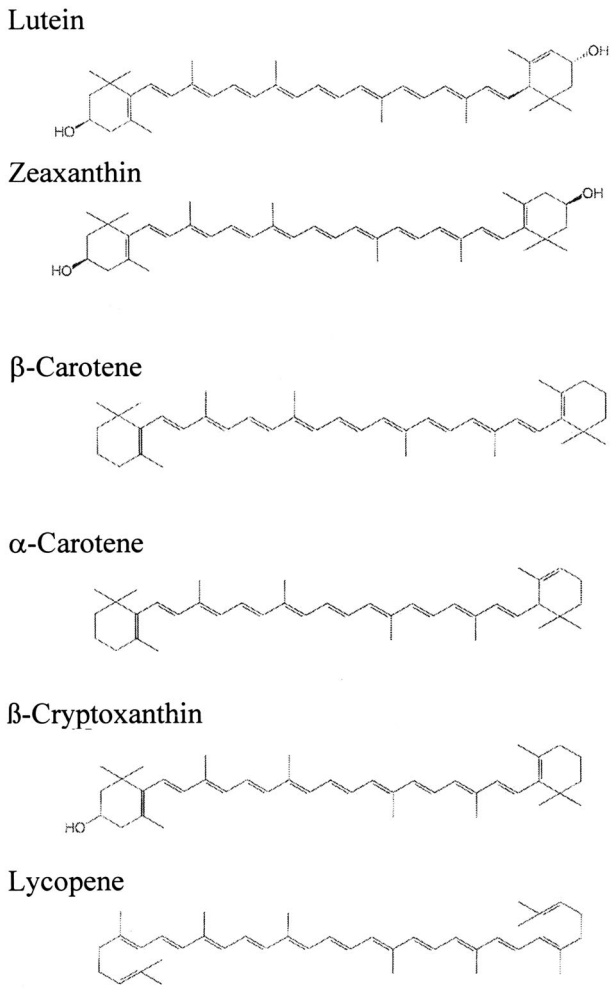

Fig. 1. Structural

retinal pigment epithelial cells exposed to 40% oxygen and photoreceptor outer segments, addition of lutein or zeaxanthin significantly reduced lipofuscin formation [24].

Increased ingestion of foods rich in lutein and zeaxanthin [25,26] or ingestion of lutein supplements [27–29] or zeaxanthin supplements [29] have been reported to increase macular pigment density in healthy adults. In patients with inherited retinal degeneration, lutein supplementation augmented the macular pigment in many but not all patients, though central vision was unchanged after the supplementation [30]. The preservation of visual sensitivity in older people has been associated with macular pigment density [31]. Positive associations have been reported between dietary intakes of lutein plus zeaxanthin *vs.* macular pigment density [32] and between serum concentrations of these carotenoids *vs.* macular pigment density [32–34]. In contrast, Beatty *et al.* [35] found no relationship between intakes of lutein and/or zeaxanthin and macular pigment density.

A. Age-related Macular Degeneration

AMD is the most common cause of visual impairment and irreversible blindness among elderly Americans [36] and a number of investigations have examined the relationship between lutein and zeaxanthin and AMD. In a case-control study, Bone *et al.* [37] examined human autopsy retinas from 56 donors with AMD and from 56 controls without the disease. They found that lutein and zeaxanthin concentrations in three concentric regions centered on the fovea were lower, on average, in AMD donors than controls. Those in the highest quartile of lutein and zeaxanthin content in peripheral retina had an 82% lower odds ratio (OR) for AMD than those in the lowest quartile (OR: 0.18; 95% CI: 0.05–0.64). Bernstein *et al.* [38] reported that compared to non-AMD controls, the average macular pigment levels were 32% lower in AMD patients who did not consume lutein supplements. AMD patients who consumed lutein supplements (≥4 mg/d) after their initial diagnosis had macular pigment levels that were in the normal range.

Data from the Eye Disease Case-Control Study [39] involving 391 cases and 577 controls are consistent with the hypotheses that a reduced risk of neovascular (exudative or wet) AMD is inversely associated with xanthophyll status. Neovascular AMD is less common than atrophic (dry) AMD, but is much more likely to result in severe vision loss [40]. Persons with serum lutein and zeaxanthin concentrations ≥0.668 μmol/L were 70% less likely to have neovascular AMD than those with serum concentrations ≤0.247 μmol/L (OR: 0.3; 95% CI: 0.2–0.6; P-trend = 0.0001). Seddon *et al.* [41] reported that the Eye Disease Case-Control Study also revealed that higher dietary intakes of lutein plus zeaxanthin are strongly associated with a reduced risk for neovascular AMD with an odds ratio of 0.43 for those in the highest quintile of intake compared to those in the lowest quintile (95% CI: 0.2–0.7; P-trend, <0.001). A higher frequency of intake of spinach or collard greens, vegetables that are rich in lutein, was associated with a substantially lower risk for neovascular AMD with an odds ratio of 0.14 for those who ingested these vegetables ≥5 times/wk compared to those who ingested them <1 time/mo (95% CI: 0.01–1.2; P-trend, <0.001) [41].

In a cross-sectional study of 380 elderly men and women in Sheffield, UK, Gale *et al.*. [42] found that plasma zeaxanthin was significantly associated with risk of AMD. Those with plasma zeaxanthin in the lowest third of the distribution had an odds ratio for risk of AMD of 2.0 when compared to those in the highest third (95% CI: 1.0–4.1; P-trend = 0.046). Risk of AMD was also increased in people with lowest plasma lutein and lowest plasma lutein plus zeaxanthin, however, these associations were not statistically significant.

Mares-Perlman *et al.* [43] examined the relationship between dietary and serum status of lutein plus zeaxanthin to photographic evidence of age-related maculopathy among persons >40 y old (n = 8,222) in the third National Health and Nutrition Examination Survey (NHANES III) and found no

inverse correlations. However, when analysis was limited to those in the youngest age groups, higher levels of dietary lutein plus zeaxanthin were related to lower odds for developing pigment abnormalities, an early sign of age-related maculopathy (people in highest *vs.* lowest quintiles of intake OR: 0.1; 95% CI: 0.1–0.3), and lower odds for developing AMD (OR: 0.1; 95% CI: 0.0–0.9). In a study in the Netherlands involving 72 cases and 66 controls, Snellen *et al.* [44] found that the prevalence rate of AMD in subjects with low lutein intake was more than twice that in subjects with high intake (OR: 2.4; 95% CI: 1.1–5.1).

The above studies lend support to the hypothesis that low levels of xanthophylls in the diet, plasma, or macula could represent a risk factor for the development of AMD. However, the clinical implications of low lutein and zeaxanthin status have yet to be clarified. Richter *et al.* [45] reported that among atrophic AMD patients in the Veterans Lutein Antioxidant Supplementation Trial (LAST), ingestion of lutein supplements alone or together with other nutrients, increased macular pigment density and improved visual function (visual acuity, contrast sensitivity, Amsler grid) as compared to ingestion of placebo. Schupp *et al.* [46] reported that in adults with cystic fibrosis, the concentrations of lutein and zeaxanthin in serum and macula are 54–64% lower than those in sex-matched healthy control subjects, and yet there were no significant differences between the 2 groups in visual performance (contrast sensitivity, color discrimination, and retinal function) under conditions of daylight illumination.

Associations between lutein and/or zeaxanthin and AMD were not observed in other studies. For example, the Beaver Dam Eye Study in Wisconsin failed to show any significant association of lutein and zeaxanthin with the development of AMD [47–49]. Retrospective analyses found no statistically significant correlations between dietary intakes of lutein and zeaxanthin 10 y before study enrollment *vs.* early- or late-stage age-related maculopathy [48]. In a prospective 5-y follow-up of participants, lutein and zeaxanthin intakes at baseline and 10 y prior to study enrollment were not significantly associated with the development of early signs of maculopathy [49]. No significant difference in non-fasting serum concentrations of lutein and zeaxanthin was observed between a subsample of 167 case-control pairs [47].

Similarly, in northern London, UK, Sanders *et al.* [50] found no significant difference in mean plasma lutein concentrations of 65 elderly patients with age-related maculopathy and 65 controls. In a study in Italy, Simonelli *et al.* [51] also found no significant difference in serum lutein plus zeaxanthin in 48 AMD patients and 46 subjects without AMD. In the Blue Mountains Eye Study in Australia, Flood *et al.* [52] observed no association between baseline intakes of lutein and zeaxanthin and the 5 y incidence of early age-related maculopathy. A recent report of a prospective follow-up study of women in the Nurses' Health Study (n = 77,562 for up to 18 y) and men in the Health Professionals Follow-up Study (n = 40,866 for up to 12 y) by Cho *et al.* [53] showed that fruit intake, but not intakes of vegetables, vitamins A, C, and E, lutein plus zeaxanthin, as well as total carotenoids, were not related to either early or neovascular AMD.

B. Cataract

Oxidative damage to lens cell membranes is considered an important factor in the initiation and progression of age-related cataract [54] and increased lipid peroxidation products have been detected in lens and aqueous humor of patients with cataracts [55,56].

The Nurses' Health Study included an examination of the relation of dietary intake with risk of cataract extraction [57], early age-related nuclear cataract [58], and early age-related cortical and subcapsular cataract [59]. Chasan-Taber *et al.* [57] reported that in a prospective study of this cohort (n = 77,466) with repeated administration of a food-frequency questionnaire during 12 y follow-up, 1,471 cataracts were extracted. Women with the highest intakes of lutein plus zeaxanthin (top 10%) had a 22% reduction in relative risk (RR) of cataract severe enough to require extraction as compared to those with poorest intakes (bottom 20%) (RR: 0.78; 95% CI: 0.63–0.95; P-trend = 0.04). Other carotenoids (β-carotene, α-carotene, lycopene, and β-cryptoxanthin) were not associated with cataract extraction. Increased frequency of intakes of lutein-rich spinach and kale was associated with a moderate decreased risk of cataract extraction. In a sub-sample of nurses without previously diagnosed cataracts and who completed 5 food frequency questionnaires during a 13–15 y period before cataract diagnosis (n = 478), Jacques *et al.* [58] reported that the prevalence of early age-related nuclear lens opacities was significantly lower in those in the highest compared to lowest quintile category of lutein plus zeaxanthin intake (P = 0.03); however, after adjustment for other nutrients, the inverse association was no longer significant (P = 0.08). Taylor *et al.* [59] analyzed data from this same subcohort and reported no significant trends in association between lutein plus zeaxanthin intake and odds of early age-related cortical and posterior subcapsular lens opacities.

In a prospective cohort study of US male health professionals (n = 36,644), Brown *et al.* [60] reported that during 8 years of follow-up, 840 cases of senile cataract extraction were documented. Men with the highest intakes of lutein plus zeaxanthin, but not other carotenoids, had a 19% lower risk of cataract extraction compared to those in the lowest quintile (RR: 0.81; 95% CI: 0.65–1.01; P-trend = 0.03). Among specific foods, broccoli and spinach were consistently associated with decreased risk of cataract.

Associations of lutein and age-related cataract in the Beaver Dam Study were inconsistent. An inverse association between dietary lutein and cataract was seen, but not between serum lutein and cataract. A retrospective study by Mares-Perlman *et al.* [61] in 1,919 subjects showed that women in the highest quintile of lutein intake (median: 949 μg/d) 10 y before dietary

interview had a 27% lower prevalence of severe nuclear sclerosis (OR: 0.73; 95% CI: 0.50–1.06; P-trend = 0.02) compared to women in the lowest quintile of lutein intake (median: 179 μg/d). In men, an inverse association was observed between lutein intake and risk of severe nuclear sclerosis but the relationship did not reach statistical significance. Among the foods evaluated, spinach was significantly associated with decreased risk in women, but not in men.

In a prospective 5 y follow-up of 1,354 persons enrolled in the Beaver Dam Eye Study, Lyle *et al.* [62] reported those in the highest quintile of lutein intake 10 y prior to study enrollment were 50% less likely to have an incident nuclear cataract (OR: 0.5; 95% CI: 0.3–0.8; P-trend = 0.002) as those in the lowest quintile of intake. Similar results were obtained using lutein intake during the year preceding study enrollment (baseline) but only in subjects <65 y old (OR: 0.4; 95% CI: 0.2–0.8; P-trend = 0.06). Thus, longer-term intake of lutein was more strongly associated with nuclear cataract than short-term intake. The stronger association of distant past diet (10 y earlier) with incident cataract as compared with recent past diet (year before baseline) may reflect dietary influences on early biochemical events preceding lens opacification. Intakes of spinach in the highest compared to lowest quintile during the year preceding baseline was related to lower risk for nuclear cataract (OR: 0.6; 95% CI: 0.4–0.9; P-trend = 0.02). Interestingly, in persons <65 y old, intake of eggs in the highest compared to lowest quintile was markedly related to lower risk for nuclear cataract (OR: 0.4; 95% CI: 0.2–0.9; P-trend = 0.004).

In contrast to the findings of an inverse association of dietary lutein and cataract risk, non-fasting lutein in serum obtained at baseline was not significantly associated with the 5 y incidence of severe nuclear cataract in a random subsample of 400 subjects from the Beaver Dam Eye Study cohort [63]. As suggested by the investigators, the lack of an association may have been due to inadequate sample size. A total of 252 persons were eligible for incident cataract, of who only 57 developed nuclear cataract in at least one eye during the 5 y period. The finding of a lack of association between nuclear cataract and serum lutein in this prospective study is in contrast with an earlier report from the Beaver Dam Eye Study in which more severe cataracts were found, particularly in women, with high serum concentrations of lutein when associations were examined cross-sectionally at baseline [64]. The authors speculated that temporal confounding may have influenced the direct associations observed in the cross-sectional study. A cross-sectional study done in Spain among 138 patients with senile cataract and 110 control subjects also reported that higher serum levels of lutein and zeaxanthin were associated with greater risk for cataract [65].

In a cross-sectional study of 372 older adults in England, Gale *et al.* [66] found that the risk of posterior subcapsular cataract (but not nuclear or cortical cataract) was lowest in those with higher concentrations of plasma lutein. The OR of those in the highest tertile of plasma lutein concentration compared to those in the lowest tertile was 0.5 (95% CI: 0.2–1.0; P-trend = 0.012). No association of plasma zeaxanthin and any type of cataract was seen. Interestingly, in a small trial of people with cataract, lutein supplementation (15 mg administered 3 times/wk for 2 y) has been shown to improve visual acuity and glare sensitivity [67].

CANCER

Xanthophylls may possess antimutagenic and anticarcinogenic properties and play a role in the health of body tissues other than the eye as suggested by research studies related to carcinogenesis and the risk for cancer. For example, lutein extracts from Aztec marigold flowers inhibited the mutagenicity of 1-nitropyrene [68] and aflotoxin B1 [69] in a bacterial strain; the effect, however, of lutein on the DNA-repair system of the bacterial strain was either null [68] or modest [69]. Lutein has been identified to be one of three antimutagenic pigments present in edible seaweed [70]. In humans, plasma lutein has been inversely associated with cytochrome CYP1A2 activity, a hepatic enzyme responsible for the metabolic activation of a number of putative human carcinogens [71]. In animal models of colon [72,73] and breast [74,75] cancers, lutein has been demonstrated to exhibit chemopreventive activity. Zeaxanthin, lutein, as well as other carotenoids, have been shown to inhibit, in a dose-dependent manner, the invasive action of rat ascites hepatoma AH109A cells co-cultured with rat mesentery-derived mesothelial cells [76].

The mechanisms for a potential protective role of xanthophylls against carcinogenesis may include selective modulation of apoptosis [74,77,78], inhibition of angiogenesis [74], enhancement of gap junctional intercellular communication [79], induction of cell differentiation [80], prevention of oxidative damage [81], and modulation of the immune system [82–87].

Oxidative metabolites of lutein, thought to arise from lutein's antioxidant mechanism of action, have been isolated and characterized from extracts of human serum and plasma [88]. Haegele *et al.* [81] observed that plasma lutein in women was inversely correlated with indices of oxidative DNA damage [8-hydroxy-2′-deoxyguanosine (8-OHdG) in DNA isolated from peripheral lymphocytes and 8-OHdG excreted in urine] and lipid peroxidation (8-epiprostaglandin F2α in urine). During the course of a dietary intervention with increased fruit and vegetable intakes, they found an inverse correlation between the change in plasma lutein and change in lymphocyte 8-OHdG concentration. However, Collins *et al.* [89] reported that although the basal lutein concentration in serum correlate inversely with oxidized pyrimidines in the DNA of lymphocytes from human volunteers, supplementation with 15 mg lutein/d for 12 wk did not have a significant effect on endogenous oxidative DNA damage in lymphocytes. Similarly, Torbergsen and Collins [90] found that lutein supplementation in healthy

volunteers produced 2-fold increases in mean plasma lutein but had no effect on rejoining of DNA strand breaks in isolated lymphocytes treated with hydrogen peroxide. In a human lymphocyte cell line, there was no evidence that lutein protected cellular DNA from oxidation under basal conditions or after oxidative challenge with hydrogen peroxide; however, lutein enhanced the recovery of cells from oxidative challenge by stimulating DNA strand break repair [91].

The action of carotenoids on the immune system has recently been reviewed by Chew and Park [92]. Protecting the immune system could enhance cell-mediated immune responses and consequently, resistance to tumor formation. The immuno-modulatory action of dietary lutein has been demonstrated in domestic cats [86] and dogs [87]. In mice fed lutein-containing diets, lutein uptake by the spleen suggests a role for lutein in modulating immunity [93]. Lutein has been shown to enhance antibody production in response to T-dependent antigens in spleen cells *in vitro*, as well as in mice *in vivo* [82]. The numbers of immunoglobulin M- and G-secreting cells increased *in vivo* with lutein administration when mice were primed with T-dependent antigens [82]. In cultures of murine T-helper cells and unprimed spleen cells, zeaxanthin augmented the number of immunoglobulin M antibody-secreting cells [83]. Dose-related increases in the expression of the *pim-1* gene, which is involved in early activation of T cells, has been observed in splenic lymphocytes of mice fed lutein, but not β-carotene or astaxanthin [84]. However, in healthy male nonsmokers, lutein supplementation, unlike β-carotene supplementation, had no effect on the percentage of monocytes expressing major histocompatibility complex class II molecules [94], cell surface molecules responsible for presenting antigen to T-helper cells. Further, cell-to-cell adhesion appears to be critical for the initiation of a primary immune response and lutein supplementation, unlike β-carotene supplementation, also had no effect on the expression of intercellular adhesion molecules by monocytes [94].

A. Breast Cancer

The associations of xanthophyll intake or serum levels with breast cancer risk in humans have been investigated in many epidemiologic studies and the results have been inconsistent. Zhang *et al.* [95] reported that during 14 y follow-up (1980–1994) of a large prospective study of 83,234 female nurses who were 34–59 y of age at baseline (Nurses' Health Study), 2,697 developed invasive breast cancer. Prediagnostic intake of lutein plus zeaxanthin was inversely associated with breast cancer risk in premenopausal (n = 784) but not in postmenopausal women (n = 1,913). Among premenopausal women, the RR of those in the highest compared to those in the lowest quintile of intake was 0.79 (95% CI: 0.63–0.99; P-trend = 0.04). The inverse association for increasing quintiles of intake and breast cancer risk was stronger among premenopausal women with a positive family history of breast cancer. The RR of those in the highest compared to those in the lowest quintile of intake was 0.38 (95% CI: 0.18–0.81; P-trend = 0.004). In contrast to this, Cho *et al.* [96] found no association of lutein plus zeaxanthin intakes and breast cancer risk in a prospective cohort of 90,655 female nurses who were 25–42 y of age at baseline (Nurses' Health Study II); during 8 y of follow-up (1991–1999), 714 incident cases of invasive breast cancer were documented.

Freudenheim *et al.* [97] conducted a case-control study (297 cases, 311 control subjects) of premenopausal breast cancer risk and intake of nutrients in western New York. A reduction in premenopausal breast cancer risk was associated with a high intake of lutein plus zeaxanthin during the 2 y prior to dietary interview. The OR of those in the highest quartile compared to those in the lowest quartile of intake was 0.47 (95% CI: 0.28–0.77; P-trend, <0.001).

In a case-control study in northern Sweden (201 cases, 290 controls) from 3 population-based cohorts, Hulten *et al.* [98] found that overall, plasma lutein at baseline was not related to the risk of developing breast cancer. However, results from stratified analysis showed a significant trend of an inverse association between plasma lutein and incident breast cancer risk in premenopausal women from 2 combined cohorts. Ito *et al.* [99] examined 206 breast cancer patients, 150 hospital controls, and 61 healthy controls in India and found that serum xanthophyll levels were significantly lower among cases than controls in post-menopausal but not in premenopausal women.

In a study involving 270 case-control pairs nested within a prospective cohort study in New York with up to 9 y follow-up, Toniolo *et al.* [100] found that the risk of breast cancer was doubled among subjects with prediagnostic serum lutein at the lowest quartile, as compared with those at the highest quartile (OR: 2.08; 95% CI: 1.11–3.90; P-trend = 0.01). No association of serum zeaxanthin with breast cancer risk was observed.

In a case control study (105 cases, 209 controls) nested within a prospective cohort study in Columbia, Missouri, Dorgan *et al.* [101] found no significant protective effect against breast cancer with increased serum lutein plus zeaxanthin overall during up to 9.5 y (median: 2.7 y) follow-up. However, in women that donated blood at least 2 y before diagnosis (60 cases) and 120 matched controls, a marginally significant gradient of decreasing breast cancer risk with increasing serum lutein plus zeaxanthin was observed. Those in the highest quartile had a RR of 0.6 (95% CI: 0.2–1.7; P-trend = 0.08) compared to those in the lowest quartile category. It is thought that the use of blood collected prior to diagnosis may generally be more informative than blood collected after diagnosis, since the disease process could alter the levels of blood constituents.

Rock *et al.* [102] reported that in women newly diagnosed with breast cancer, higher plasma concentration of lutein was associated with an increased likelihood of having estrogen receptor-positive status, a condition that has been linked to improved response to hormone therapy and survival [103]. Kim *et al.* [104] reported the serum concentration of zeaxanthin plus lutein was inversely associated with the risk of breast cancer,

and that the relationship did not differ according to the presence or absence of a p53 mutation.

In a prospective, nested case-control study in Washington Country, Maryland of women who participated in a blood collection campaign in 1974 or/and 1989, Sato *et al.* [105] reported that serum lutein was significantly lower (P = 0.02) in 115 subjects from the 1989 cohort (but not the 1974 cohort) who subsequently developed breast cancer compared to their matched controls. However, although an inverse association of serum lutein with breast cancer risk was observed, no significant dose-response association was seen (P-trend = 0.11).

In a large case-cohort analysis (1,452 cases, 5,239 controls) of women enrolled in the Canadian National Breast Screening Study who completed a self-administered dietary questionnaire, Terry *et al.* [106] found no association between dietary intakes of lutein plus zeaxanthin at baseline and breast cancer risk during 11 y follow-up in the overall study population, nor in subgroups defined by menopausal status, family history of breast cancer, smoking status, relative body weight as assessed by body mass index, and intakes of total fat, energy, alcohol, or folic acid. In a population-based case-control study involving 414 incident cases and 429 controls in French Canadians in Montreal, Nkondjock and Ghadirian [107] found that dietary intakes of lutein or zeaxanthin were not associated with breast cancer risk.

In a study of early-onset breast cancer among 568 cases with *in situ* and localized disease and 1,451 population-based controls conducted in 3 centers in the US, Potischman *et al.* [108] reported that dietary intake of lutein during the year before study enrollment was not associated with risk of early-stage breast cancer.

Ronco *et al.* [109] studied 400 women in Uruguay with newly diagnosed breast cancer and 405 controls and found dietary intake of lutein plus zeaxanthin was unrelated to breast cancer risk. Similarly, in a cohort of Finnish women (n = 4697), Jarvinen *et al.* [110] found 88 breast cancer cases diagnosed during 25 y of follow-up, but no significant relationship between lutein intake at baseline and the occurrence of the disease.

In a small case-control study in 46 women whose biopsies revealed invasive or *in situ* breast cancer (cases) and 63 women whose biopsies revealed benign disease (controls), Zhang *et al.* [111] observed an inverse but non-significant association between breast adipose tissue levels of lutein plus zeaxanthin and risk of breast cancer. Yeum *et al.* [112] did not observe any significant differences in the concentrations of lutein and of zeaxanthin in serum and in normal breast adipose tissue of breast cancer patients (n = 44) and benign breast tumor patients (n = 46).

According to Czeczuga-Semeniuk *et al.* [113], the predominant carotenoids in the surrounding fatty tissue in both malignant and benign breast tumor are xanthophyll epoxides, i.e., lutein epoxide (taraxanthin) and zeaxanthin 5,6,5′6′-diepoxide (violaxanthin); and that in neoplastic material, the predominant carotenoids are epoxide carotenoids, as well as other carotenoids including zeaxanthin and lutein. No relationship was found between the severity of neoplasm or lesion diameter and specific carotenoids. The physiological significance of xanthophyll epoxides in breast tissue of patients with malignant or benign breast tumors is not known, but previous studies have shown that β-carotene epoxides were much more active than β-carotene in inducing the differentiation of the leukemic cell line NB4 *in vitro* [114]. However, neither taraxanthin nor violaxanthin were detected in human plasma after the oral ingestion of chemically prepared compounds by 3 volunteers [115]. Xanthophyll epoxides are abundant in green vegetables [116].

Although human studies regarding the relationship of lutein and zeaxanthin with breast cancer risk have been inconclusive, studies in human mammary cells and in animal models support a protective role of xanthophylls. Lutein has been shown to selectively induce apoptosis in transformed cells, but not normal human mammary cells, and protect normal cells, but not transformed cells, from apoptosis induced by the chemotherapy agents etoposide and cisplatin [77]. Low levels of dietary lutein from marigold extract enhanced lymphocyte proliferation, lowered lipid peroxidation, lowered mammary tumor incidence, increased tumor latency, and suppressed tumor growth in susceptible mice [74,75,85] by selectively modulating apoptosis and inhibiting angiogenesis [74], although higher lutein levels were less effective [75].

B. Lung Cancer

The association of xanthophylls and risk of lung cancer was investigated in a large prospective study of male smokers in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study conducted in Finland. Holick *et al.* [117] reported that of 27,084 male smokers who completed a dietary questionnaire at baseline, 1,644 developed lung cancer during 14 y follow-up. Men in the highest quintile of lutein plus zeaxanthin intake at baseline (median: 2,106 μg/d) had a 17% lower risk of lung cancer (OR: 0.83; 95% CI: 0.71–0.99; P-trend = 0.006) compared to men in the lowest quintile of lutein plus zeaxanthin intake (median: 853 μg/d).

De Stefani *et al.* [118] reported that in a case-control study in Uruguay involving 541 cases of lung cancer and 540 hospitalized controls, retrospective dietary information obtained for the year before onset of symptoms (for cases) or the year before study enrollment (for controls) showed that those in the highest quartile of lutein intake had a 43% lower risk of lung cancer (OR: 0.57; 95% CI: 0.39–0.85; P-trend = 0.004) compared to those in the lowest quartile of lutein intake.

In a population based case-control study conducted in Hawaii involving 332 lung cancer cases and 865 controls, Le Marchand *et al.* [119] reported a significant dose-dependent inverse association for dietary lutein and risk of lung cancer. Subjects with a high combined intake of lutein, β-carotene, and

α-carotene had the lowest risk of lung cancer suggesting that a greater protection was afforded by consuming a variety of vegetables as compared to only foods rich in a particular carotenoid.

Goodman *et al.* [120] reported that in a prospective nested study of 276 lung cancer case-control pairs from the Beta-Carotene and Retinol Efficacy Trial high-risk cohort of asbestos-exposed workers and heavy smokers (n = 18,314), the mean serum concentration of lutein was lower for lung cancer cases (121 ng/mL) than controls (137 ng/mL) (P = 0.0008); similarly, the mean serum concentration of zeaxanthin was lower for lung cancer cases (24.1 ng/mL) than controls (27.1 ng/mL) (P = 0.004). Statistically significant trends across quartiles were observed for an inverse association of serum lutein or zeaxanthin with lung cancer risk. The OR for persons in the highest compared to lowest quartile of serum lutein was 0.55 (95% CI: 0.33–0.93; P-trend = 0.02); for serum zeaxanthin, it was 0.56 (95% CI: 0.33–0.95; P-trend = 0.02). The inverse relations were more marked among women than men.

In Washington County, Maryland, Comstock *et al.* [121] found, among subjects who had donated blood for a serum bank in 1989, 258 lung cancer cases through 1993 and matched them with 515 controls; serum/plasma concentrations of lutein plus zeaxanthin were significantly lower by 10.1% among cases than controls. In an ecological study among 20 ethnic groups in several island nations of the South Pacific (Cook Islands, Fiji, Tahiti, and New Caledonia), Le Marchand *et al.* [122] reported that smoking, as expected, explained the majority of the variability (61%) in incidence of lung cancer among the ethnic groups; lutein intake explained 14%, whereas other factors explained only 5–7% each of the remaining variance in incidence. The association of lutein intake with lung cancer was inverse and statistically significant (P = 0.01).

In contrast, examining 2 large cohorts (46,924 men in the Health Professionals Follow-up Study and 77,283 women in the Nurses' Health Study), Michaud *et al.* [123] observed no statistically significant relation between lung cancer risk and intake of lutein. Dietary assessments were derived using food-frequency questionnaires at baseline and during follow-up. Among men, 275 new cases of lung cancer were diagnosed during 10 y follow-up; among women, 519 new cases were diagnosed during 12 y follow-up. In the analyses of individual or pooled cohorts, although those in the highest compared to lowest quintile of intake had lower multivariate relative risks, no significant linear trends were observed across the quintile categories of lutein intake, but the association was stronger and of borderline significance in women (P = 0.09) than in men (P = 0.39).

In a study of 763 lung cancer cases and 564 controls in New Jersey among white male current and recent cigarette smokers, Ziegler *et al.* [124] found that lung cancer risk was increased 62% in those in the lowest quartile (1,727 μg/d) compared to those in the highest quartile (4,196 μg/d) of lutein plus zeaxanthin intake 4 y before diagnosis or study enrollment; the linear trend was borderline but not statistically significant (P-trend = 0.07). In the Netherlands Cohort Study on Diet and Cancer, Voorrips *et al.* [125] reported that in a cohort of 58,279 men, 939 lung cancer cases were registered after 6.3 y follow-up; inverse non-significant association with lutein plus zeaxanthin intake at baseline was limited to small cell and squamous cell carcinomas. In a population-based, case-control study of women in the Missouri Women's Health Study, Wright *et al.* [126] reported that data from 587 incident primary lung cancer cases and 624 controls showed an inverse relation between lung cancer risk and intake of lutein plus zeaxanthin 2 to 3 y prior to interview, but the relation was no longer statistically significant in models adjusted for total vegetable intake.

Yuan *et al.* [127] reported that in a large prospective study of diet and lung cancer in Chinese men and women residing in Singapore (n = 63,257), 482 lung cancer cases occurred during 8 y follow-up; baseline intake of lutein plus zeaxanthin was not associated significantly with lung cancer risk. In the Canadian National Breast Screening Study, Rohan *et al.* [128] reported that a case-cohort study, based on a randomly selected subcohort with 155 incident lung cancer cases and 5,361 non-cases after 8 to 13 y follow-up, showed no association between baseline lutein intake and subsequent lung cancer risk. Kneckt *et al.* [129] reported that during a 25 y follow-up of 4,545 men in the Finnish Mobile Clinic Health Examination Survey, the association of lung cancer incidence (n = 138) with intake of lutein plus zeaxanthin at baseline was not statistically significant. Garcia-Closas *et al.* [130] reported that a study in women in Barcelona, Spain among 103 lung cancer cases and 206 hospital controls showed no association of lutein intake with lung cancer risk.

In a case-control study (209 cases, 622 controls) nested in a large, prospective cohort study among men in Shanghai, China, Yuan *et al.* [131] reported that prediagnostic serum levels of lutein plus zeaxanthin were inversely associated with risk of lung cancer during 12 y follow-up; however, the association was not statistically significant after adjustment for smoking. Ito *et al.* [132] reported that a prospective case-control study (147 cases of lung cancer deaths, 311 controls) nested in the large Japan Collaborative Cohort Study showed an inverse association of serum lutein and zeaxanthin at baseline with risk of lung cancer death among Japanese during 8 y follow-up; however, the risk for those in the highest *vs.* lowest quartile of serum xanthophyll levels were not significantly different.

One prospective study conducted in China in a cohort of tin miners found a positive association of serum lutein plus zeaxanthin with lung cancer risk among alcohol drinkers, but not among non-drinkers [133]. During 6 y follow-up, this case-control study (108 incident lung cancer cases, 216 controls) showed that high serum level of lutein plus zeaxanthin at 2.5 y prior to diagnosis was significantly associated with increased lung cancer risk among alcohol drinkers; the OR of those in the highest compared to lowest tertile of serum xanthophyll level

was 2.3 (95% CI: 1.2–6.6; P-trend = 0.04). Conversely, a high serum level of lutein plus zeaxanthin was associated with decreased lung cancer risk among non-drinkers of alcohol; the OR of those in the highest compared to lowest tertile of serum xanthophyll level was 0.4 (95% CI: 0.2–1.1; P-trend = 0.04).

C. Colorectal Cancer

In a large case-control study, Slattery *et al.* [134] investigated associations of dietary lutein and the risk of colon cancer in 1,993 subjects with first primary incident adenocarcinoma of the colon and 2,410 population-based control subjects in an 8-county area in Utah and in the metropolitan Twin Cities area of Minnesota. An inverse association between dietary lutein intake during the 2 y prior to diagnosis or study admission *vs.* colon cancer risk was detected. The OR for the highest quintile of intake relative to the lowest quintile of intake was 0.83 (95% CI: 0.66–1.04; P-trend = 0.04). A stronger inverse association was observed in persons in whom cancer was diagnosed before they were 67 y of age (OR: 0.66; 95% CI: 0.48–0.92; P-trend = 0.02) and among those with tumors located in the proximal segment of the colon (OR: 0.65; 95% CI: 0.51–0.91; P-trend, <0.01). In this study, lutein intake was the only carotenoid intake that appeared to be inversely associated with colon cancer.

Levi *et al.* [135] reported that in a case-control study in Vaud, Switzerland, involving 223 patients with incident colon or rectal cancer and 491 patients with acute non-neoplastic conditions, inverse associations were observed between lutein plus zeaxanthin intake during the 2 y prior to diagnosis or study admission and risk of colorectal cancer. The OR for persons in highest *vs.* lowest tertile of intake was 0.41 (95% CI: 0.2–0.7). In a study in Washington County, Maryland, in bloods obtained before diagnosis, the median chromatographic peak height for serum lutein was 11% lower for subjects who subsequently developed rectal cancer than for those who did not [136].

McMillan *et al.* [137] reported that in a small study conducted in Glasgow, UK, involving patients with gastrointestinal cancer (10 patients with colon cancer, 2 with liver cancer) and 12 healthy controls, plasma lutein was significantly lower in the cancer group (median: 0.06 μmol/L) than in the control group (median: 0.24 μmol/L) ($P < 0.001$). The cancer patients had an inflammatory response as reflected by an elevated plasma C-reactive protein and anti-inflammatory treatment with ibuprofen resulted in small but significant increases in plasma carotenoid concentrations. These results indicate that in these cancer patients, inflammation plays an important role in the regulation of plasma lutein and other carotenoids, a finding with important implications regarding the use and interpretation of plasma carotenoid levels in gastrointestinal cancer when blood is collected after disease diagnosis. A similar inverse correlation between plasma concentrations of lutein and other carotenoids *vs.* C-reactive protein has been reported in lung cancer patients within 1 mo of diagnosis [138].

Other studies have found inverse associations of the xanthophyll carotenoids with precancerous lesions in colon and rectum. Rumi *et al.* [139] showed that in 59 patients with adenomatous colorectal polyp, the concentration of serum zeaxanthin but not of lutein or other carotenoids was lower than that in a healthy control group. Muhlhofer *et al.* [140] reported that in patients with colorectal adenoma, the concentrations of all carotenoids, including zeaxanthin and lutein, in biopsies of adenomatous tissue were significantly reduced as compared to non-involved mucosa. Nair *et al.* [141] reported that patients with adenomatous polyps have lower levels of lutein in their colon mucosa, but not serum, as compared to control gastrointestinal patients with normal colonic mucosa.

No potential protective effect of lutein against colorectal cancer was observed in some large, prospective cohort studies. Terry *et al.* [142] reported that data from a subcohort (295 cases and 5,334 noncases) of the Canadian National Breast Screening Study with 11 y follow-up did not show any association between dietary intakes of lutein (or other carotenoids) and colorectal cancer risk, either overall or for cancers of the colon or rectum when examined separately. Malila *et al.* [143] reported that in middle-aged male smokers who were participants of the ATBC Study and who had complete dietary data and serum samples available from baseline (n = 26,951), 184 colorectal cancer cases were diagnosed during 8 y follow-up; no association was seen between lutein plus zeaxanthin intake or serum levels and risk for colorectal cancer. Similarly, in a case-control study in North Carolina involving 613 colon cancer cases and 996 matched controls, Satia-Abouta *et al.* [144] reported that lutein intake during the year prior to diagnosis or interview was not significantly associated with colon cancer risk in either African-American or white study participants. These null results are consistent with reports from 2 case-control studies which found no association of lutein plus zeaxanthin intake [145] or plasma concentrations [146] with prevalence of colorectal adenomatous polyps.

Animal studies lend support for a chemopreventive role of lutein against colon carcinogenesis. The formation of colonic aberrant crypt foci in rats that received intrarectal doses of N-methylnitrosourea was inhibited by a daily gavage of lutein [72]. A similar protective effect of lutein has been reported against the development of preneoplastic aberrant crypt foci in colons of mice initiated with 1,2-dimethylhydrazine [73].

D. Prostate Cancer

Although lycopene and all-*trans*-β-carotene are the predominant carotenoids in human prostate with mean concentrations of 0.80 and 0.54 nmol/g, respectively, lutein and zeaxanthin are consistently detectable at about half their level, 0.30 and 0.24 nmol/g, respectively, in this tissue [147].

Several large prospective studies have reported that dietary intake [148,149] or circulating levels [120,150–152] of the xanthophylls are unrelated to risk of prostate cancer; however,

other studies have reported a reduction in risk associated with xanthophylls [153,154]. In a population-based case-control study of men <65 y of age in the Seattle area, Cohen *et al.* [153] reported that in 628 men newly diagnosed with prostate cancer and 602 matched controls, lutein plus zeaxanthin intake over the 3 to 5 y period before diagnosis or recruitment was inversely associated with prostate cancer risk. The OR for those with lutein plus zeaxanthin intake ≥2,000 μg/d compared to those with an intake of <800 μg/d was 0.68 (95% CI: 0.45–1.00). In a small hospital-based case-control study of 65 patients with prostate cancer and 132 cancer-free controls, Lu *et al.* [154] found significant inverse associations between prostate cancer risk and plasma concentrations of zeaxanthin and lutein when comparing highest with lowest quartiles. For zeaxanthin, the OR was 0.22 (95% CI: 0.06–0.83; P-trend = 0.003); for lutein, the OR was 0.30 (95% CI: 0.09–1.03; P-trend = 0.006).

In a large, prospective cohort study (Health Professionals Follow-up Study), dietary intakes of 47,894 eligible subjects were assessed at baseline and 3 more times during 6 y follow-up during which 812 new cases of prostate cancer were diagnosed; lutein intake was unrelated to subsequent development of prostate cancer [148]. In the Netherlands Cohort Study (n = 58,279), 642 incident prostate carcinoma cases were seen after 6.3 y follow-up; intake of lutein plus zeaxanthin had no effect on prostate cancer risk [149].

In a study which included 578 men from the Physicians' Health Study cohort who developed prostate cancer within 13 y follow-up and 1,294 matched controls, plasma lutein levels were not associated with prostate cancer risk [150]. A study of 142 case-control pairs in a cohort of Japanese-American men in Hawaii showed that incident cases of prostate cancer over a follow-up period of 20 y was unrelated to prediagnostic serum levels of lutein or zeaxanthin [151]. In two nested case-control studies among men in Washington County, Maryland (with 182 and 142 cases, respectively, each case matched with 2 controls), prediagnostic levels of plasma lutein were unrelated to risk of developing prostate cancer during a median follow-up period of 17 y for the first study and 3.5 y for the second study [152]. Similarly, no statistically significant association between serum lutein or zeaxanthin and prostate cancer risk was found in a prospective nested case-control study of 205 case-control pairs from the Beta-Carotene and Retinol Efficacy Trial cohort [120].

It is worth noting that Hall *et al.* [155] found that lutein and zeaxanthin were less effective than β-carotene, canthaxanthin or lycopene in inhibiting the growth of human DU145 prostate cancer cells.

E. Upper Aerodigestive Tract Cancers

A 5 y case-control study in northern Italy conducted by Franceschi *et al.* [156] involving 304 patients with a first incident squamous cell carcinoma of the esophagus and 743 control patients admitted to hospital for acute illnesses showed that lutein plus zeaxanthin intake was significantly inversely associated with risk of esophageal cancer. In another hospital-based case-control study, Zhang *et al.* [157] examined 95 incident cases of adenocarcinoma of the esophagus and gastric cardia (ACEGC) and 132 controls and found high dietary lutein associated with a decreased risk of ACEGC.

Olmedilla *et al.* [158] have reported that in men with recently diagnosed cancer of the larynx who had undergone total or partial laryngectomy (n = 51), serum concentration of lutein or zeaxanthin was significantly lower than those in a matched control group (n = 51).

In a nested case-control study conducted within a cohort of community volunteers in Washington Country, Maryland, Zheng *et al.* [159] reported that among 25,802 individuals who donated blood in 1974, 28 were diagnosed with primary oral and pharyngeal cancer during 1975 to 1990; 4 controls were selected for each case. Although serum lutein was lower in cases compared to controls (mean: 18.96 and 23.28 μg/dL, respectively) the difference was not statistically significant (P = 0.10). The OR of developing oral and pharyngeal cancer was 0.61 in persons in the highest compared to those in the lowest tertile of serum lutein, but the P-trend was not statistically significant, possibly due to the small sample size.

Nomura *et al.* [160] reported that in 69 cancer cases identified among 6,832 American men of Japanese ancestry during a surveillance period of 20 y and 138 matched controls, prediagnostic serum lutein or zeaxanthin concentrations were not significantly related to risk of subsequent cancer of the upper aerodigestive tract taken together or separated into esophageal, laryngeal, and oral-pharyngeal cancer. Further, in patients with upper aerodigestive tract cancer, lutein intake was not correlated with mutagen sensitivity as measured by an *in vitro* assay based on the quantification of bleomycin-induced chromatid breaks in cultured lymphocytes [161].

F. Ovarian Cancer

Dietary intake of lutein plus zeaxanthin has been inversely associated with ovarian cancer risk. A case-control study in 5 Italian areas conducted by Bidoli *et al.* [162] among 1,031 patients with confirmed incident epithelial ovarian cancer and 2,411 patients admitted to area hospitals for acute, non-neoplastic diseases (controls) showed that those in the highest quintile of lutein plus zeaxanthin intake had a 40% lower risk of developing ovarian cancer than those in the lowest quintile of intake (OR: 0.6; 95% CI: 0.5–0.8).

Similarly, Bertone *et al.* [163] reported that in a population-based, case-control study conducted in Massachusetts and Wisconsin, an inverse relationship between intake of lutein plus zeaxanthin and risk of ovarian cancer was observed. Incident cases were identified through statewide tumor registries (n = 327); controls were randomly selected from lists of licensed drivers and Medicare recipients (n = 3,129). Dietary intake

data were collected by telephone interview. Participants with the highest dietary intake of lutein plus zeaxanthin at 5 y prior to diagnosis had a 40% lower RR of ovarian cancer (95% CI: 0.36–0.99) compared to those with lowest intakes.

G. Endometrial Cancer

McCann *et al.* [164] reported that in women in the Western New York Diet Study, data collected from 232 endometrial cancer cases and 639 matched controls showed an inverse association of dietary lutein plus zeaxanthin intake during the 2 y prior to interview and risk of endometrial cancer (OR: 0.3; 95% CI: 0.2–0.5). In contrast, Jain *et al.* [165] reported no association between baseline lutein intake and subsequent risk of endometrial cancer in a randomly selected subcohort (n = 5,684) of the Canadian National Breast Screening Study of which 221 women developed endometrial cancer after 8 to 13 y follow-up.

It is interesting to note that Kucuk *et al.* [166] found that plasma cholesterol β-epoxides, oxidation products of cholesterol, are elevated in endometrial cancer patients and are inversely related to plasma *cis*-lutein plus zeaxanthin, but not other carotenoids. This data suggests there may be a preferential depletion of xanthophylls under oxidant stress in these cancer patients.

H. Kidney Cancer

In a study conducted in Los Angeles involving 1,204 renal cell carcinoma patients matched to the same number of neighborhood controls, Yuan *et al.* [167] found strong inverse associations between the intake of cruciferous and dark green leafy vegetables and cancer risk; a significant inverse association of lutein intake with cancer risk was also observed. Of potential relevance to renal cancer, lutein was found to protect against ultraviolet A light-induced oxidative stress in cultured rat kidney fibroblasts by restoring the activities of the antioxidant enzymes catalase and superoxide dismutase and decreasing the production of lipid peroxidation products [168].

I. Bladder Cancer

A study of 111 case-control pairs in a cohort of Japanese-American men showed that prediagnostic serum levels of lutein or of zeaxanthin were inversely associated with risk of developing bladder cancer over 20 y follow-up; however after adjusting for cigarette smoking, the inverse relation was no longer statistically significant [169]. Similarly, other studies failed to show a significant association between lutein intake with risk of bladder cancer [170–173]. An investigation of the Health Professionals Follow-up Study found that high cruciferous vegetable consumption from 1 mo to 10 y before cancer diagnosis may reduce bladder cancer risk [170]. Among individual lutein-rich cruciferous vegetables, a significant inverse association with risk of bladder cancer was observed for broccoli, but not for kale nor Brussels sprouts. No association was observed between lutein intake and bladder cancer risk [170]. In another prospective cohort study among male smokers who were initially enrolled in the ATBC Study and followed over a median of 11 y, dietary intakes of lutein plus zeaxanthin were not related to bladder cancer risk [171]. The prospective Netherlands Cohort Study also showed that dietary lutein plus zeaxanthin intake are not associated with bladder cancer risk during 6.3 y follow-up [172]. Similarly, a case-control study in Spain did not support the hypothesis that lutein intake is protective against bladder cancer risk [173].

J. Gastric Cancer

In the Netherlands Cohort Study (n = 120,852), a prospective case-cohort study conducted by Botterweck *et al.* [174] showed that in 282 incident cases of gastric carcinoma during 6.3 y follow-up and 3,123 controls, intake of lutein plus zeaxanthin at baseline was not associated with the development of gastric carcinoma. Similarly, in a case-control study in Spain that included 354 gastric cancer cases and 354 controls, Garcia-Closas *et al.* [175] found no association between lutein intake and risk of gastric cancer. Also, an ecological study by Tsubono *et al.* [176] conducted in 5 regions of Japan with different mortality rates from gastric cancer failed to observe an association between mean plasma lutein and zeaxanthin concentrations in men who were sampled randomly from the general populations *vs.* the mortality rates from gastric cancer in the 5 regions.

Interestingly, one study suggests a potential adverse association between xanthophylls and gastric cancer. Abneth *et al.* [177], in a prospective case-cohort study in Linxian, China involving 590 esophageal, 395 gastric cardia, and 87 gastric non-cardia case subjects and 1,053 controls, found high baseline serum concentrations of lutein plus zeaxanthin were directly associated with incident gastric non-cardia adenocarcinoma.

K. Cervical Cancer

Batieha *et al.* [178] employed a population-based, nested case-control study of women in Washington County, Maryland to compare 50 cases who developed invasive cervical cancer or *in situ* carcinoma with 100 matched controls and found prediagnostic serum lutein was not related to subsequent risk of cervical cancer after 16 y follow-up. Similarly, in surveying 4 Latin American countries, Potischman *et al.* [179] found no association between mean serum lutein among 387 cases of invasive cervical cancer stages I and II and 670 controls.

In Hawaii, Goodman *et al.* [180] examined 147 multiethnic women with squamous intraepithelial lesions of the cervix and 191 clinic controls and plasma lutein was not significantly associated with risk of cervical dysplasia. In Japan, Nagata *et al.* [181] matched 156 women diagnosed with cervical dysplasia to women attending the same clinic but with normal cervical

cytology and found a small but non-statistically significant inverse association between serum lutein plus zeaxanthin and risk of cervical dysplasia. In a case-control study in Chicago involving 102 women with cervical intraepithelial neoplasia and 102 women with normal Pap smears, VanEenwyk *et al.* [182] reported that cervical neoplasia was not associated with dietary lutein intakes or serum lutein concentrations.

In contrast to those investigations which showed no association of xanthophylls and cervical neoplasia and carcinoma, in a study of Southwestern American Indian women involving 81 cases with cervical intraepithelial neoplasia (CIN) stages II and III and 160 controls from the same clinics, Schiff *et al.* [183] found a higher status of serum lutein plus zeaxanthin was associated with a lower risk for CIN stages II and III with an OR for those in the highest *vs.* lowest tertile of 0.40 (95% CI: 0.17–0.95; P = 0.03). Peng *et al.* [184] measured the concentrations of lutein and zeaxanthin in paired plasma and cervical tissue of cervical cancer (n = 27), precancer (n = 33), and non-cancer (n = 27) patients in Arizona, and found that plasma zeaxanthin was lowest among cancer patients, and highest among precancer patients; similar results were obtained for plasma lutein. Zeaxanthin in cancerous tissue was higher than in precancerous or non-cancerous tissue. The relevance of this relationship to cervical carcinogenesis is not known.

The association between serum antioxidant nutrients and persistent human papillomavirus (HPV) infection, an established risk factor for cervical cancer, was studied by Giuliano *et al.* [185] in a group of 123 low-income Hispanic women. Individual serum carotenoids including lutein were about 24% lower among women found to be HPV positive at two time points 3 mo apart, as compared with women found to be HPV negative twice or HPV positive once. In another study, persistent HPV infection was found lower among women who reported intake of lutein plus zeaxanthin in the upper 2 quartiles compared with those in the lowest quartile [186].

L. Skin Cancer

Animal studies have shown that lutein may have a protective role against light-induced skin damage. For example, Gonzalez *et al.* [187] reported that in hairless mice, dietary lutein plus zeaxanthin supplementation diminished the effects of ultraviolet B irradiation by reducing acute inflammatory responses and epidermal hyperproliferation. However, in humans, no evidence has been found that xanthophylls play a protective role against incident squamous cell carcinoma of the skin. Fung *et al.* [188] reported that data from the Nurses' Health Study and Health Professionals Follow-up Study showed no association between dietary lutein plus zeaxanthin intake measured every 2–4 y and risk of skin cancer during 14 and 10 y follow-up, respectively. It is worth noting that only very low amounts of xanthophyll esters are present in human skin compared to the concentrations of β-carotene and lycopene in this tissue [189].

CORONARY HEART DISEASE AND STROKE

A growing body of experimental evidence and observational studies suggest that lutein and zeaxanthin may play a role in the prevention of coronary heart disease and stroke. In a coculture model of the arterial wall, Dwyer *et al.* [190] found lutein to be highly effective in reducing oxidation of low-density lipoproteins (LDL) and inhibiting the inflammatory response of monocytes to LDL trapped in the artery wall. They also found that dietary supplementation with lutein in two mouse models (apoE-null mice and LDL receptor-null mice) reduced plasma lipid hydroperoxides and the size of aortic lesions [190]. With *in vitro* experiments of human LDL, lutein and zeaxanthin have been shown to act as scavengers of peroxynitrite radicals [191], the product of the reaction between nitric oxide and superoxide. Further, plasma lutein has been found inversely related with soluble E-selectin, a biomarker of vascular endothelial activation, in an Australian population with a very high mortality from coronary heart disease [192], although in this study and another [193], no association was noted between plasma xanthophylls and C-reactive protein, a marker of chronic inflammation and a risk factor for coronary heart disease. In contrast, other investigators have reported an inverse association between plasma levels of high-sensitivity C-reactive protein and lutein [194].

A. Coronary Heart Disease

That lutein may have a protective effect against the progression of early atherosclerosis is supported by epidemiologic data from 2 studies which related circulating xanthophylls with carotid artery intima-media thickness (IMT). In a prospective study of a random sample of 480 participants from the Los Angeles Atherosclerosis Study cohort who had no history of heart attack, angina, revascularization or stroke, Dwyer *et al.* [190,194] found the progression of intima-media thickness of the common carotid arteries over a period of 18 mo was close to null (0.004 mm) among those in the highest quintile of plasma lutein and zeaxanthin concentrations at baseline, whereas it was increased by 0.021 mm among those in the lowest quintile (P = 0.01). On average, for every 1 μmol/L increase of plasma lutein or zeaxanthin, IMT progression was reduced by 3.2 or 4.7 μm/18 mo, respectively [194]. Significant inverse associations of IMT progression with plasma β-cryptoxanthin or α-carotene were also observed, but not with plasma lycopene or α- or γ-tocopherols [194].

In a study of 231 asymptomatic case-control pairs selected from the Atherosclerosis Risk in Communities (ARIC) study cohort, Iribarren *et al.* [195] reported that mean serum lutein plus zeaxanthin concentrations were lower in cases than controls (mean: 0.35 and 0.37 IU, respectively; P = 0.05). A modest inverse association was also found between serum lutein plus zeaxanthin concentrations and carotid intima-media

thickness, although ambiguity may have been introduced by measurement of the xanthophylls 3 y after carotid examination.

In a nested case-control study from the Washington County, Maryland cohort (n = 25,802) in 123 persons newly diagnosed with myocardial infarction and their matched controls, Street *et al.* [196] found a suggestive trend of an inverse association between first myocardial infarction and serum lutein (but not zeaxanthin) in bloods collected 7 to 14 y before the onset of the disease (P-trend = 0.09). When the results were stratified by smoking status, low serum levels of both xanthophylls were associated with an increased risk of subsequent myocardial infarction among smokers, but not non-smokers.

In contrast, Osganian *et al.* [197] examined 12 y follow-up data from the Nurses' Health Study cohort (n = 73,286) and found intake of lutein plus zeaxanthin was not significantly associated with risk of development of incident coronary artery disease (both nonfatal myocardial infarction and fatal coronary artery disease), regardless of smoking status. Similarly, Hak *et al.* [198] found no relationship between any plasma carotenoids, including lutein, and risk of myocardial infarct in a nested case-control analysis of the Physicians' Health Study with 13 y follow-up. Sesso *et al.* [199] also observed no relationship between quartiles of plasma lutein plus zeaxanthin and risk of cardiovascular disease (including cardiovascular disease death, nonfatal myocardial infarction, nonfatal stroke, percutaneous transluminal coronary angioplasty, coronary artery bypass graft, and angina pectoris) in 483 case-control pairs of participants in the Women's Health Study during 4.8 y follow-up.

From the Brubeck Study in northern Italy, D'Odorico *et al.* [200] found high plasma levels of α- and β-carotene, but not lutein, zeaxanthin, or other carotenoids, were inversely associated with the prevalence of atherosclerosis in the carotid and femoral arteries and with the 5 y incidence of atherosclerotic lesions in the carotid arteries. Similarly, in a case-control subsample from the elderly population of the Rotterdam Study, Klipstein-Grobusch *et al.* [201] found no association between aortic atherosclerosis and serum xanthophylls in samples collected at baseline and preserved for 5.8 y from 108 subjects with moderate/severe atherosclerosis and 109 controls. A case-control and follow-up study in Spain found no significant association of risk of myocardial infarction *vs.* plasma lutein plus zeaxanthin measured within the first 24 h after disease onset and 1 y later [202].

Another approach to determining the relationship between xanthophylls and risk for heart disease employs measures of oxidative stress as intermediary biomarkers. In the plasma of 30 patients with congestive heart failure, Polidori *et al.* [203] found lutein was significantly lower and malondialdehyde, a product of lipid peroxidation, was significantly higher as compared to 55 controls. Patients with more severe congestive heart failure had lower plasma lutein and higher malondialdehyde levels compared to patients with less severe disease condition. Further, ejection fraction, evaluated by echocardiography, was inversely correlated with malondialdehyde and directly correlated with lutein plasma levels in the patients [203]. In a comparative study of 5 European countries by Wright *et al.* [204], fasting LDL lutein concentration was found highest in France where cardiovascular disease death rate was lowest, but no relationship was observed between total antioxidant content of LDL and *ex vivo* resistance of LDL to Cu^{2+}-induced oxidation in healthy subjects from the 5 sites with different cardiovascular mortality rates. Hininger *et al.* [205] supplemented healthy male volunteers daily with 15 mg lutein for 3 mo and found significant increases in plasma and LDL lutein content but no change in the resistance of LDL to Cu^{2+}-induced oxidation or modification of the LDL polyunsaturated:saturated fatty acid ratio. Similarly, no changes were found in plasma reduced or oxidized glutathione, protein-SH groups or the activities of erythrocyte antioxidant enzymes glutathione peroxidase and superoxide dismutase.

B. Stroke

Investigating the Health Professionals Follow-up Study (n = 43,738), Ascherio *et al.* [206] found, among men who did not have cardiovascular disease or diabetes, an inverse relation of borderline statistical significance between lutein intake at baseline and incident stroke (210 ischemic, 70 hemorrhagic, and 48 unclassified stroke) after 8 y follow-up. The RR of those in the highest *vs.* lowest quintile of lutein intake was 0.70 after multivariate analysis (95% CI: 0.49–1.01; P-trend = 0.06). Similarly, among the ATBC cohort (n = 26,593) of male smokers without a history of stroke, Hirvonen *et al.* [207] observed a significant inverse relation between intake of lutein plus zeaxanthin and risk for subarachnoid hemorrhage after 6.1 y follow-up; however, after simultaneous modeling for other dietary antioxidants (vitamins C and E, β-carotene, lycopene, and flavonoids), the association was no longer evident. In plasma, an inverse correlation between lutein and malondialdehyde in 28 ischemic stroke patients in comparison to matched controls was found by Polidori *et al.* [208]. Further, lower lutein and higher malondialdehyde concentrations in plasma were observed in patients with poor early phase recoveries relative to those who were functionally stable, suggesting lutein may modulate clinical outcomes following ischemic stroke.

CONCLUSIONS

A continuously growing body of evidence suggests that lutein and zeaxanthin may contribute to the protection against several age-related diseases, including cataract, AMD, heart disease, and some forms of cancer. Though the data, especially for cataract and AMD, is compelling, there are inconsistencies in the epidemiological results, perhaps due partly to differences in the range of xanthophyll intakes and status or in the severity

of the lesions or disease state in the cohorts being examined. Of course, observational studies always present the potential for confounding from unknown dietary or other lifestyle factors not considered in the assessment instrument or for which the xanthophylls may merely serve as markers. If some of the putative benefits indicated by the results of these investigations are substantiated, they may also reflect less a simple and independent action of the xanthophylls than a synergistic role of lutein and zeaxanthin in combination with other antioxidants, including the hydrocarbon carotenoids and related phytochemicals. However, such complex defense networks are not readily determined, in part, because of the complicating influences of physiological and genetic factors affecting the bioavailability and metabolism of the constituent nutrients as well as the pathogenic process.

While consistency of results across studies is an important criterion for demonstrating a relationship between nutrients and chronic disease, so is biological plausibility. Thus, the evidence showing the xanthophylls possess antioxidant activity, filter blue light, stabilize membranes, and bind selectively to retinal transport proteins all support a distinct role for them in the eye. While there are a limited number of animal models for AMD, principally monkeys and quail, available to test some of these hypotheses, preliminary experimental studies are beginning to suggest an inverse relationship between xanthophylls and retinal disease. Similarly, mouse models of carcinogenesis indicate that lutein can inhibit tumor incidence, growth, and latency via effects on angiogenesis and apoptosis, though further research is clearly required. Experiments testing the mechanisms of lutein and zeaxanthin during the development of atherosclerosis are still very limited, but it is promising to note these carotenoids do increase the resistance of LDL to oxidation *in vitro* and reduce lipid peroxidation and aortic lesions in mice. Even as the evidence for a role of lutein and zeaxanthin in disease prevention continues to evolve from basic research as well as from human studies directed to their bioavailability, metabolism, and dose-response relationships with intermediary biomarkers and clinical outcomes, it is worth noting that recommendations to consume foods rich in xanthophylls are consistent with current dietary guidelines.

REFERENCES

1. USDA National Nutrient Database for Standard Reference, Release 16, Reports by Single Nutrients. USDA Nutrient Data Laboratory, Agricultural Research Service. http://www.nal.usda.gov/fnic/foodcomp/Data/SR16 Accessed November 21, 2003.
2. Gartner C, Stahl W, Sies H: Preferential increase in chylomicron levels of the xanthophylls lutein and zeaxanthin compared to beta-carotene in the human. Int J Vitam Nutr Res 66:119–125, 1996.
3. van het Hof KH, Brouwer IA, West CE, Haddeman E, Steegers-Theunissen RPM, van Dusseldorp M, Weststrate JA, Eskes TKAB, Hautvast JGAJ: Bioavailability of lutein from vegetables is 5 times higher than that of β-carotene. Am J Clin Nutr 70:261–268, 1999.
4. Castenmiller JJ, West CE, Linssen JP, van het Hof KH, Voragen AG: The food matrix of spinach is a limiting factor in determining the bioavailability of beta-carotene and to a lesser extent of lutein in humans. J Nutr 129:349–355, 1999.
5. Chopra M, O'Neill ME, Keogh N, Wortley G, Southon S, Thurnham DI: Influence of increased fruit and vegetable intake on plasma and lipoprotein carotenoids and LDL oxidation in smokers and nonsmokers. Clin Chem 46:1818–1829, 2000.
6. Handelman GJ, Nightingale ZD, Lichtenstein AH, Schaefer EJ, Blumberg JB: Lutein and zeaxanthin concentrations in plasma after dietary supplementation with egg yolk. Am J Clin Nutr 70:247–251, 1999.
7. Ojima F, Sakamoto H, Ishiguro Y, Terao J: Consumption of carotenoids in photosensitized oxidation of human plasma and plasma low-density lipoprotein. Free Rad Biol Med 15:377–384, 1993.
8. Bone RA, Landrum JT, Tarsis SL: Preliminary identification of the human macular pigment. Vis Res 25:1531–1535, 1985.
9. Bone RA, Landrum JT, Fernandez L, Tarsis SL: Analysis of the macular pigment by HPLC: retinal distribution and age study. Invest Ophthalmol Vis Sci 29:843–849, 1988.
10. Handelman GJ, Dratz EA, Reay CC, van Kuijk FJGM: Carotenoids in the human macula and whole retina. Invest Ophthalmol Vis Sci 29:850–855, 1988.
11. Bone RA, Landrum JT, Friedes LM, Gomez CM, Kilburn MD, Menendez E, Vidal I, Wang W: Distribution of lutein and zeaxanthin stereoisomers in the human retina. Exp Eye Res 64:211–218, 1997.
12. Bone RA, Landrum JT, Hime GW, Cains A, Zamor J: Stereochemistry of the human macular carotenoids. Invest Ophthalmol Vis Sci 34:2033–2040, 1993.
13. Krinsky NI, Landrum JT, Bone RA: Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr 23:171–201, 2003.
14. Bernstein PS, Balashov NA, Tsong ED, Rando RR: Retinal tubulin binds macular carotenoids. Invest Ophthalmol Vis Sci 38:167–175, 1997.
15. Yemelyanov AY, Katz NB, Bernstein PS: Ligand-binding characterization of xanthophyll carotenoids to solubilized membrane proteins derived from human retina. Exp Eye Res 72:381–392, 2001.
16. Yeum KJ, Taylor A, Tang G, Russell RM: Measurement of carotenoids, retinoids, and tocopherols in human lenses. Invest Ophthalmol Vis Sci 36:2756–2761, 1995.
17. Hammond BR, Wooten BR, Snodderly DM: Density of the human crystalline lens is related to the macular pigment carotenoids, lutein and zeaxanthin. Optom Vis Sci 74:499–504, 1997.
18. Berendschot TTJM, Broekmans WMR, Klopping-Ketelaars IAA, Kardinaal AFM, Van Poppel G, Van Norren D: Lens aging in relation to nutritional determinants and possible risk factors for age-related cataract. Arch Ophthalmol 120:1732–1737, 2002.
19. Junghans A, Sies H, Stahl W: Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. Arch Biochem Biophys 391:160–164, 2001.
20. Khachik F, Bernstein PS, Garland DL: Identification of lutein and

zeaxanthin oxidation products in human and monkey retinas. Invest Ophthalmol Vis Sci 38:1802–1811, 1997.
21. Bernstein PS, Khachik F, Carvalho LS, Muir GJ, Zhao DY, Katz NB: Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. Exp Eye Res 72:215–223, 2001.
22. Rapp LM, Maple SS, Choi JH: Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. Invest Ophthalmol Vis Sci 41:1200–1209, 2000.
23. Thomson LR, Toyoda Y, Langner A, Delori FC, Garnett KM, Craft N, Nichols CR, Cheng KM, Dorey CK: Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. Invest Ophthalmol Vis Sci 43:3538–3549, 2002.
24. Sundelin SP, Nilsson SE: Lipofuscin-formation in retinal pigment epithelial cells is reduced by antioxidants. Free Radic Biol Med 31:217–225, 2001.
25. Hammond Jr BR, Johnson EJ, Russell RM, Krinsky NI, Yeum KJ, Edwards RB, Snodderly DM: Dietary modification of human macular pigment density. Invest Ophthalmol Vis Sci 38:1795–1801, 1997.
26. Johnson EJ, Hammond BR, Yeum KJ, Qin J, Wang XD, Castaneda C, Snodderly DM, Russell RM: Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. Am J Clin Nutr 71:1555–1562, 2000.
27. Landrum JT, Bone RA, Joa H, Kilburn MD, Moore LL, Sprague KE: A one year study of the macular pigment: the effect of 140 days of lutein supplement. Exp Eye Res 65:57–62, 1997.
28. Berendschot TTJM, Goldbohm RA, Klopping WAA, van de Kraats J, van Norel J, van Norren D: Influence of lutein supplementation on macular pigment, assessed with two objective techniques. Invest Ophthalmol Vis Sci 41:3322–3326, 2000.
29. Bone RA, Landrum JT, Guerra LH, Ruiz CA: Lutein and zeaxanthin dietary supplements raise macular pigment density and serum concentrations of these carotenoids in humans. J Nutr 133:992–998, 2003.
30. Aleman TS, Duncan JL, Bieber ML, de Castro E, Marks DA, Gardner LM, Steinberg JD, Cideciyan AV, Maguire MG, Jacobson SG: Macular pigment and lutein supplementation in retinitis pigmentosa and Usher syndrome. Invest Ophthalmol Vis Sci 42:1873–1881, 2001.
31. Hammond BR, Wooten BR, Snodderly DM: Preservation of visual sensitivity of older subjects: association with macular pigment density. Invest Ophthalmol Vis Sci 39:397–406, 1998.
32. Curran-Celentano J, Hammond Jr BR, Ciulla TA, Cooper DA, Pratt LM, Danis RB: Relation between dietary intake, serum concentrations, and retinal concentrations of lutein and zeaxanthin in adults in a Midwest population. Am J Clin Nutr 74:796–802, 2001.
33. Bone RA, Landrum JT, Dixon Z, Chen Y, Llerena CM: Lutein and zeaxanthin in the eyes, serum and diet of human subjects. Exp Eye Res 71:239–245, 2000.
34. Broekmans WMR, Berendschot TTJM, Klopping-Ketelaars IAA, de Vries AJ, Goldbohm RA, Tijburg LBM, Kardinaal AFM, van Poppel G: Macular pigment density in relation to serum and adipose tissue concentrations of lutein and serum concentrations of zeaxanthin. Am J Clin Nutr 76:595–603, 2002.
35. Beatty S, Murray IJ, Henson DB, Carden D, Koh HH, Boulton ME: Macular pigment and risk for age-related macular degeneration in subjects from a Northern European population. Invest Ophthalmol Vis Sci 42:439–446, 2001.
36. National Advisory Eye Council: Vision Research—A National Plan: 1999–2003. US Dept of Health and Human Services, National Eye Institute, National Institutes of Health. NIH Publication No. 98-4120, Bethesda, MD, 1998.
37. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE: Macular pigment in donor eyes with and without AMD: a case control study. Invest Ophthalmol Vis Sci 42:235–240, 2001.
38. Bernstein PS, Zhao DY, Wintch SW, Ermakov IV, McClane RW, Gellermann W: Resonance Raman measurement of macular carotenoids in normal subjects and in age-related macular degeneration patients. Ophthalmol 109:1780–1787, 2002.
39. Eye Disease Case-Control Study Group: Antioxidant status and neovascular age-related macular degeneration. Arch Ophthalmol 111:104–109, 1993.
40. Ferris FL III, Fine SL, Hyman L: Age-related macular degeneration and blindness due to neovascular maculopathy: the Blue Mountains Eye Study. Arch Ophthalmol 102:1649–1642, 1984.
41. Seddon JM, Ajani UA, Sperduto RD, Hiller R, Blair N, Burton TC, Farber MD, Gragoudas ES, Haller J, Miller DT, Yannuzzi LA, Willett W, for the Eye Disease Case-Control Study Group: Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. J Am Med Assoc 272:1413–1420, 1994.
42. Gale CR, Hall NF, Phillips DIW, Martyn CN: Lutein and zeaxanthin status and risk of age-related macular degeneration. Invest Ophthalmol Vis Sci 44:2461–2465, 2003.
43. Mares-Perlman JA, Fisher AI, Klein R, Palta M, Block G, Millen AE, Wright JD: Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the Third National Health and Nutrition Examination Survey. Am J Epidemiol 153: 424–432, 2001.
44. Snellen ELM, Verbeek ALM, Van den Hoogen GWP, Cruysberg JRM, Hoyng CB: Neovascular age-related macular degeneration and its relationship to antioxidant intake. Acta Ophthalmol Scand 80:368–371, 2002.
45. Richter S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D, Pei K, Tsipursky M, Nyland J: Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). Optometry 75:216–230, 2004.
46. Schupp C, Olano-Martin E, Gerth C, Morrissey BM, Cross CE, Werner JS: Lutein, zeaxanthin, macular pigment, and visual function in adult cystic fibrosis patients. Am J Clin Nutr 79:1045–1052, 2004.
47. Mares-Perlman JA, Brady WE, Klein R, Klein BEK, Bowen P, Stacewicz-Sapuntzakis M, Palta M: Serum antioxidants and age-related macular degeneration in a population based case control study. Arch Ophthalmol 113:1518–1523, 1995.
48. Mares-Perlman JA, Klein R, Klein BEK, Greger JL, Brady WE, Palta M, Ritter LL: Association of zinc and antioxidant nutrients with age-related maculopathy. Arch Ophthalmol 114:991–997, 1996.
49. VandenLangenberg GM, Mares-Perlman JA, Klein R, Klein

BEK, Brady WE, Palta W: Associations between antioxidant and zinc intake and the 5 year incidence of early age-related maculopathy in the Beaver Dam Eye Study. Am J Epidemiol 148:204–214, 1998.

50. Sanders TAB, Haines AP, Wormald R, Wright LA, Obeid O: Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. Am J Clin Nutr 57:428–433, 1993.
51. Simonelli F, Zarrilli F, Mazzeo S, Verde V, Romano N, Savoia M, Testa F, Vitale DF, Rinaldi M, Sacchetti L: Serum oxidative and antioxidant parameters in a group of Italian patients with age-related maculopathy. Clin Chim Acta 320:111–115, 2002.
52. Flood V, Smith W, Wang JJ, Manzi F, Webb K, Mitchell P: Dietary antioxidant intake and incidence of early age-related maculopathy: The Blue Mountains Eye Study. Ophthalmology 109:2272–2278, 2002.
53. Cho E, Seddon JM, Rosner B, Willett WC, Hankinson SE: Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related maculopathy. Arch Ophthalmol 122:883–892, 2004.
54. Taylor A, Jacques PF, Epstein EM: Relations among aging, antioxidant status, and cataract. Am J Clin Nutr 62:1439S–1447S, 1995.
55. Babizhayev MA: Failure to withstand oxidative stress induced by phospholipid hydroperoxides as a possible cause of the lens opacities in systemic diseases and ageing. Biochim Biophys Acta 1315:87–99, 1996.
56. Bhuyan KC, Bhuyan DK: Molecular mechanisms of cataractogenesis: III. Toxic metabolites of oxygen as initiators of lipid peroxidation and cataract. Curr Eye Res 3:67–81, 1984.
57. Chasan-Taber L, Willett WC, Seddon JM, Stampfer MJ, Rosner B, Colditz GA, Speizer FE, Hankinson SE: A prospective study of carotenoid and vitamin A intakes and risk of cataract extraction in US women. Am J Clin Nutr 70:509–516, 1999.
58. Jacques PF, Chylack Jr LT, Hankinson SE, Khu PM, Rogers G, Friend J, Tung W, Wolfe JK, Padhye N, Willett WC, Taylor A: Long-term nutrient intake and early age-related nuclear lens opacities. Arch Ophthalmol 119:1009–1019, 2001.
59. Taylor A, Jacques PF, Chylack Jr LT, Hankinson SE, Khu PM, Rogers G, Friend J, Tung W, Wolfe JK, Padhye N, Willett WC: Long-term intake of vitamins and carotenoids and odds of early age-related cortical and posterior subcapsular lens opacities. Am J Clin Nutr 75:540–549, 2002.
60. Brown L, Rimm EB, Seddon JM, Giovannucci EL, Chasan-Taber L, Spiegelman D, Willett WC, Hankinson SE: A prospective study of carotenoid intake and risk of cataract extraction in US men. Am J Clin Nutr 70:517–524, 1999.
61. Mares-Perlman JA, Brady WE, Klein BEK, Klein R, Haus GJ, Palta M, Ritter LL, Shoff SM: Diet and nuclear lens opacities. Am J Epidemiol 141:322–334, 1995.
62. Lyle BJ, Mares-Perlman JA, Klein BEK, Klein R, Greger JL: Antioxidant intake and risk of incident age-related nuclear cataracts in the Beaver Dam Eye Study. Am J Epidemiol 149:801–809, 1999.
63. Lyle BJ, Mares-Perlman JA, Klein BEK, Klein R, Palta M, Bowen PE, Greger JL: Serum carotenoids and tocopherols and incidence of age-related nuclear cataract. Am J Clin Nutr 69:272–277, 1999.
64. Mares-Perlman JA, Brady WE, Klein BEK, Klein R, Palta M, Bowen P, Stacewicz-Sapuntzakis M: Serum carotenoids and tocopherols and severity of nuclear and cortical opacities. Invest Ophthalmol Vis Sci 36:276–288, 1995.
65. Olmedilla B, Granado F, Blanco I, Herrero C, Vaquero M, Millan I: Serum status of carotenoids and tocopherols in patients with age-related cataracts: a case-control study. J Nutr Health Aging 6:66–68, 2002.
66. Gale CR, Hall NF, Phillips DIW, Martyn CN: Plasma antioxidant vitamins and carotenoids and age-related cataract. Ophthalmology 108:1992–1998, 2001.
67. Olmedilla B, Granado F, Blanco I, Vaquero M: Lutein but not α-tocopherol supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. Nutrition 19:21–24, 2003.
68. Gonzalez de Mejia EG, Loarca-Pina G, Ramos-Gomez M: Antimutagenicity of xanthophylls present in Aztec marigold (*Tagetes erecta*) against 1-nitroprene. Mutat Res 389:219–226, 1997.
69. Gonzalez de Mejia EG, Ramos-Gomez M, Loarca-Pina G: Antimutagenic activity of natural xanthophylls against aflatoxin B1 in *Salmonella typhimurium*. Environ Mol Mutagen 30:346–353, 1997.
70. Okai Y, Higashi-Okai K, Yano Y, Otani S: Identification of antimutagenic substances in an extract of edible red alga, Porphyra tenera (Asakusa-nori). Cancer Lett 100:235–240, 1996.
71. Le Marchand L, Franke AA, Custer L, Wilkens LR, Cooney RV: Lifestyle and nutritional correlates of cytochrome CYP1A2 activity. Inverse associations with plasma lutein and alpha-tocopherol. Pharmacogenetics 7:11–19, 1997.
72. Narisawa T, Fukaura Y, Hasebe M, Ito M, Aizawa R, Murakoshi M, Uemura S, Khachik F, Nishino H: Inhibitory effects of natural carotenoids, alpha-carotene, beta-carotene, lycopene and lutein, on colonic aberrant crypt foci formation in rats. Cancer Lett 107:137–142, 1996.
73. Kim JM, Araki S, Kim DJ, Park CB, Takasuka N, Baba-Toriyama H, Ota T, Nir Z, Khachik F, Shimidzu N, Tanaka Y, Osawa T, Uraji T, Murakoshi M, Nishino H, Tsuda H: Chemopreventive effects of carotenoids and curcumins on mouse colon carcinogenesis after 1,2-dimethylhydrazine initiation. Carcinogenesis 19: 81–85, 1998.
74. Chew BP, Brown CM, Park JS, Mixter PF: Dietary lutein inhibits mouse mammary tumor growth by regulating angiogenesis and apoptosis. Anticancer Res 23:3333–3339, 2003.
75. Park JS, Chew BP, Wong TS: Dietary lutein from marigold extract inhibits mammary tumor development in BALB/c mice. J Nutr 128:1650–1656, 1998.
76. Kozuki Y, Miura Y, Yagasaki K: Inhibitory effects of carotenoids on the invasion of rat ascites hepatoma cells in culture. Cancer Lett 151:111–115, 2000.
77. Sumantran VN, Zhang R, Lee DS, Wicha MS: Differential regulation of apoptosis in normal versus transformed mammary epithelium by lutein and retinoic acid. Cancer Epidemiol Biomarkers Prev 9:257–263, 2000.
78. Muller K, Carpenter KI, Challis IR, Skepper JN, Arends MJ: Carotenoids induce apoptosis in the T-lymphoblast cell line Jurkat E6.1. Free Radic Res 36:791–802, 2002.
79. Zhang LX, Cooney RV, Bertram JS: Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H/

10T1/2 cells: relationship to their cancer chemopreventive action. Carcinogenesis 12:2109–2114, 1991.
80. Gross MD, Bishop TD, Belcher JD, Jacobs Jr DR: Induction of HL-60 cell differentiation by carotenoids. Nutr Cancer 27:169–173, 1997.
81. Haegele AD, Gillette C, O'Neill C, Wolfe P, Heimendinger J, Sedlacek S, Thompson HJ: Plasma xanthophyll carotenoids correlate inversely with indices of oxidative DNA damage and lipid peroxidation. Cancer Epidemiol Biomark Prev 9:421–425, 2000.
82. Jyonouchi H, Zhang L, Gross MD, Tomita Y: Immunomodulating actions of carotenoids: enhancement of *in vivo* and *in vitro* antibody production to T-dependent antigens. Nutr Cancer 21: 47–58, 1994.
83. Jyonouchi H, Sun S, Mizokami M, Gross MD: Effects of various carotenoids on cloned, effector-stage T-helper cell activity. Nutr Cancer 26:313–324, 1996.
84. Park JS, Chew BP, Wong TS, Zhang JX, Magnuson NS: Dietary lutein but not astaxanthin or beta-carotene increases *pim-1* gene expression in murine lymphocytes. Nutr Cancer 33:206–212, 1999.
85. Chew BP, Wong MW, Wong TS: Effects of lutein from marigold extract on immunity and growth of mammary tumors in mice. Anticancer Res 16:3689–3694, 1996.
86. Kim HW, Chew BP, Wong TS, Park JS, Weng BBC, Byrne KM, Hayek MG, Reinhart GA: Modulation of humoral and cell-mediated immune responses by dietary lutein in cats. Vet Immunol Immunopath 73:331–341, 2000.
87. Kim HW, Chew BP, Wong TS, Park JS, Weng BBC, Byrne KM, Hayek MG, Reinhart GA: Dietary lutein stimulates immune response in the canine. Vet Immunol Immunopath 74:315–327, 2000.
88. Khachik F, Beecher GR, Smith Jr JC: Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. J Cell Biochem 22:236–246, 1995.
89. Collins AR, Olmedilla B, Southon S, Granado F, Duthie SJ: Serum carotenoids and oxidative DNA damage in human lymphocytes. Carcinogenesis 19:2159–2162, 1998.
90. Torbergsen AC, Collins AR: Recovery of human lymphocytes from oxidative DNA damage. The apparent enhancement of DNA repair by carotenoids is probably simply an antioxidant effect. Eur J Nutr 39:80–85, 2000.
91. Astley SB, Elliott RM, Archer DB, Southon S: Increased cellular carotenoid levels reduce the persistence of DNA single-strand breaks after oxidative challenge. Nutr Cancer 43:202–213, 2002.
92. Chew BP, Park JS: Carotenoid action on the immune response. J Nutr 134:257S–261S, 2004.
93. Park JS, Chew BP, Wong TS: Dietary lutein absorption from marigold extract is rapid in BALB/c mice. J Nutr 128:1802–1806, 1998.
94. Hughes DA, Wright AJA, Finglas PM, Polley ACJ, Bailey AL, Astley SB, Southon S: Effects of lycopene and lutein supplementation on the expression of functionally associated surface molecules on blood monocytes from healthy male nonsmokers. J Infect Dis 182:S11–S15, 2000.
95. Zhang S, Hunter DJ, Forman MR, Rosner BA, Speizer FE, Colditz GA, Manson JE, Hankinson SE, Willett WC: Dietary carotenoids and vitamins A, C, and E and risk of breast cancer. J Natl Cancer Inst 91:547–556, 1999.
96. Cho E, Spiegelman D, Hunter DJ, Chen WY, Zhang SM, Colditz GA, Willett WC: Premenopausal intakes of vitamins A, C, and E, folate, and carotenoids, and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 12:713–720, 2003.
97. Freudenheim JL, Marshall JR, Vena JE, Laughlin R, Brasure JR, Swanson MK, Nemoto T, Graham S: Premenopausal breast cancer risk and intake of vegetables, fruits, and related nutrients. J Natl Cancer Inst 88:340–348, 1996.
98. Hulten K, Van Kappel AL, Winkvist A, Kaaks R, Hallmans G, Lenner P, Riboli E: Carotenoids, alpha-tocopherols, and retinol in plasma and breast cancer risk in northern Sweden. Cancer Causes Control 12:529–537, 2001.
99. Ito Y, Gajalakshmi KC, Sasaki R, Suzuki K, Shanta V: A study on serum carotenoid levels in breast cancer patients of Indian women in Chennai (Madras), India. J Epidemiol 9:306–314, 1999.
100. Toniolo P, Van Kappel AL, Akhmedkhanov A, Ferrari P, Kato I, Shore RE, Riboli E: Serum carotenoids and breast cancer. Am J Epidemiol 153:1142–1147, 2001.
101. Dorgan JF, Sowell A, Swanson CA, Potischman N, Miller R, Schussler N, Stephenson Jr HE: Relationships of serum carotenoids, retinol, a-tocopherol, and selenium with breast cancer risk: results from a prospective study in Columbia, Missouri (United States). Cancer Causes and Control 9:89–97, 1998.
102. Rock CL, Saxe GA, Ruffin MT, August DA, Schottenfeld D: Carotenoids, vitamin A, and estrogen receptor status in breast cancer. Nutr Cancer 25:281–296, 1996.
103. Rutqvist LE: The significance of hormone receptors to predict the endocrine responsiveness of human breast cancer. Acta Oncol 29:371–377, 1990.
104. Kim MK, Park YG, Gong G, Ahn SH: Breast cancer, serum antioxidant vitamins, and p53 protein overexpression. Nutr Cancer 43:159–166, 2002.
105. Sato R, Helzlsouer KJ, Alberg AJ, Hoffman SC, Norkus EP, Comstock GW: Prospective study of carotenoids, tocopherols, and retinoid concentrations and the risk of breast cancer. Cancer Epidemiol Biomarkers Prev 11:451–457, 2002.
106. Terry P, Jain M, Miller AB, Howe GR, Rohan TE: Dietary carotenoids and risk of breast cancer. Am J Clin Nutr 76:883–888, 2002.
107. Nkondjock A, Ghadirian P: Intake of specific carotenoids and essential fatty acids and breast cancer risk in Montreal, Canada. Am J Clin Nutr 79:857–864, 2004.
108. Potischman N, Swanson CA, Coates RJ, Gammon MD, Brogan DR, Curtin J, Brinton LA: Intake of food groups and associated micronutrients in relation to risk of early-stage breast cancer. Int J Cancer 82:315–321, 1999.
109. Ronco A, De Stafani E, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, LeBorgne F: Vegetables, fruits, and related nutrients and risk of breast cancer: a case-control study in Uruguay. Nutr Cancer 35:111–119, 1999.
110. Jarvinen R, Knekt P, Seppanen R, Teppo L: Diet and breast cancer risk in a cohort of Finnish women. Cancer Lett 114:251–253, 1997.
111. Zhang S, Tang G, Russell RM, Mayzel KA, Stampfer MJ, Willett WC, Hunter DJ: Measurement of retinoids and carotenoids in breast adipose tissue and a comparison of concentrations in breast

cancer cases and control subjects. Am J Clin Nutr 66:626–632, 1997.
112. Yeum KJ, Ahn SH, de Paiva SAR, Lee-Kim YC, Krinsky NI, Russell RM: Correlation between carotenoid concentrations in serum and normal breast adipose tissue of women with benign breast tumor or breast cancer. J Nutr 128:1920–1926, 1998.
113. Czeczuga-Semeniuk E, Wolczynski S, Markewicz W: Preliminary identification of carotenoids in malignant and benign neoplasms of the breast and surrounding fatty tissue. Neoplasma 50:280–286, 2003.
114. Duitsman PK, Barua AB, Becker B, Olson JA: Effects of epoxycarotenoids, β-carotene and retinoic acid on the differentiation and viability of the leukemic cell line NB4 in vitro. Int J Vitam Nutr Res 69:303–308, 1999.
115. Barua AB, Olson JA: Xanthophyll epoxides, unlike β-carotene monoepoxides, are not detectibly absorbed by humans. J Nutr 131:3212–3215, 2001.
116. Khachik F, Beecher GR, Goli MB, Lusby WR: Separation, identification, and quantification of carotenoids in fruits, vegetables, and human plasma by high-performance liquid chromatography. Pure Appl Chem 63:71–80, 1991.
117. Holick CN, Michaud DS, Stolzenberg-Solomon R, Mayne ST, Pietinen P, Taylor PR, Virtamo J, Albanes D: Dietary carotenoids, serum β-carotene, and retinol and risk of lung cancer in the Alpha-Tocopherol, Beta-Carotene cohort study. Am J Epidemiol 156:536–547, 2002.
118. De Stefani E, Boffetta P, Deneo-Pellegrini H, Mendilaharsu M, Carzoglio JC, Ronco A, Olivera L: Dietary antioxidants and lung cancer risk: a case-control study in Uruguay. Nutr Cancer 34: 100–110, 1999.
119. Le Marchand L, Hankin JH, Kolonel LN, Beecher GR, Wilkens LR, Zhao LP: Intake of specific carotenoids and lung cancer risk. Cancer Epidemiol Biomarkers Prev 2:183–187, 1993.
120. Goodman GE, Schaffer S, Omenn GS, Chen C, King I: The association between lung and prostate cancer risk, and serum micronutrients: results and lessons from Beta-Carotene and Retinol Efficacy Trial. Cancer Epidemiol Biomarkers Prev 12:518–526, 2003.
121. Comstock GW, Alberg AJ, Huang HY, Wu K, Burke AE, Hoffman SC, Norkus EP, Gross M, Cutler RG, Morris JS, Spate VL, Helzlsouer KJ: The risk of developing lung cancer associated with antioxidants in the blood: ascorbic acid, carotenoids, alpha-tocopherol, selenium, and total peroxyl radical absorbing capacity. Cancer Epidemiol Biomarkers Prev 6:907–916, 1997.
122. Le Marchand L, Hankin JH, Bach F, Kolonel LN, Wilkens LR, Stacewicz-Sapuntzakis M, Bowen PE, Beecher GR, Laudon F, Baque P: An ecological study of diet and lung cancer in the South Pacific. Int J Cancer 63:18–23, 1995.
123. Michaud DS, Feskanich D, Rimm EB, Colditz GA, Speizer FE, Willett WC, Giovannucci E: Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. Am J Clin Nutr 72:990–997, 2000.
124. Ziegler RG, Colavito EA, Hartge P, McAdams MJ, Schoenberg JB, Mason TJ, Fraumeni Jr JF: Importance of α-carotene, β-carotene, and other phytochemicals in the etiology of lung cancer. J Natl Cancer Inst 88:612–615, 1996.
125. Voorrips LE, Goldbohm RA, Brants HA, van Poppel GA, Sturmans F, Hermus RJ, van den Brandt PA: A prospective cohort study on antioxidant and folate intake and male lung cancer risk. Cancer Epidemiol Biomarkers Prev 9:357–365, 2000.
126. Wright ME, Mayne ST, Swanson CA, Sinha R, Alavanja MC: Dietary carotenoids, vegetables, and lung cancer risk in women: the Missouri women's health study (United States). Cancer Causes Control 14:85–96, 2003.
127. Yuan JM, Stram DO, Arakawa K, Lee HP, Yu MC: Dietary cryptoxanthin and reduced risk of lung cancer: the Singapore Chinese Health Study. Cancer Epidemiol Biomarkers Prev 12: 890–898, 2003.
128. Rohan TE, Jain M, Howe GR, Miller AB: A cohort study of dietary carotenoids and lung cancer risk in women (Canada). Cancer Causes Control 13:231–237, 2002.
129. Knekt P, Jarvinen R, Teppo L, Aromaa A, Seppanen R: Role of various carotenoids in lung cancer prevention. J Natl Cancer Inst 91:182–184, 1999.
130. Garcia-Closas R, Agudo A, Gonzalez CA, Riboli E: Intake of specific carotenoids and flavonoids and the risk of lung cancer in women in Barcelona, Spain. Nutr Cancer 32:154–158, 1998.
131. Yuan JM, Ross RK, Chu XD, Gao YT, Yu MC: Prediagnostic levels of serum beta-cryptoxanthin and retinol predict smoking-related lung cancer in Shanghai, China. Cancer Epidemiol Biomarkers Prev 10:767–773, 2001.
132. Ito Y, Wakai K, Suzuki K, Tamakoshi A, Seki N, Ando M, Nishino Y, Kondo T, Watanabe Y, Ozasa K, Ohno Y, JACC Study Group: Serum carotenoids and mortality from lung cancer: a case-control study nested in the Japan Collaborative Cohort (JACC) study. Cancer Sci 94:57–63, 2003.
133. Ratnasinghe D, Forman MR, Tangre JA, Qiao Y, Yao SX, Gunter EW, Barrett MJ, Giffen CA, Erozan Y, Tockman MS, Taylor PR: Serum carotenoids are associated with increased lung cancer risk among alcohol drinkers, but not among non-drinkers in a cohort of tin miners. Alcohol Alcoholism 35:355–360, 2000.
134. Slattery ML, Benson J, Curtin K, Ma K, Schaeffer D, Potte RJD: Carotenoids and colon cancer. Am J Clin Nutr 71:575–582, 2000.
135. Levi F, Pasche C, Lucchini F, La Vecchia C: Selected micronutrients and colorectal cancer. A case-control study from the canton of Vaud, Switzerland. Eur J Cancer 36:2115–2119, 2000.
136. Comstock GW, Helzlsouer KJ, Bush TL: Prediagnostic serum levels of carotenoids and vitamin E as related to subsequent cancer in Washington County, Maryland. Am J Clin Nutr 53: 260S–264S, 1991.
137. McMillan DC, Sattar N, Talwar D, O'Reilly DSJ, McArdle CS: Changes in micronutrient concentrations following anti-inflammatory treatment in patients with gastrointestinal cancer. Nutrition 16:425–428, 2000.
138. Talwar D, Ha TKK, Scott HR, Cooney J, Fell GS, O'Reilly DS, Lean MEJ, McMillan DC: Effect of inflammation on measures of antioxidant status in patients with non-small cell lung cancer. Am J Clin Nutr 66:1283–1285, 1997.
139. Rumi Jr G, Szabo I, Vincze A, Matus Z, Toth G, Rumi G, Mozsik G: Decrease in serum levels of vitamin A and zeaxanthin in patients with colorectal polyp. Eur J Gastroenterol Hepatol 11: 305–308, 1999.
140. Muhlhofer A, Buhler-Ritter B, Frank J, Zoller WG, Merkle P, Bosse A, Heinrich F, Biesalski HK: Carotenoids are decreased in biopsies from colorectal adenomas. Clin Nutr 22:65–70, 2003.
141. Nair S, Norkus EP, Hertan H, Pitchumoni CS: Serum and colon

mucosa micronutrient antioxidants: differences between adenomatous polyp patients and controls. Am J Gastroenterol 96:3400–3405, 2001.
142. Terry P, Jain M, Miller AB, Howe GR, Rohan TE: Dietary carotenoid intake and colorectal cancer risk. Nutr Cancer 42:167–172, 2002.
143. Malila N, Virtamo J, Virtanen M, Pietinen P, Albanes D, Teppo L: Dietary and serum alpha-tocopherol, beta-carotene and retinol, and risk for colorectal cancer in male smokers. Eur J Clin Nutr 56:615–621, 2002.
144. Satia-Abouta J, Galanko JA, Martin CF, Potter JD, Ammerman A, Sandler RS: Associations of micronutrients with colon cancer risk in African Americans and whites: results from the North Carolina Colon Cancer Study. Cancer Epidemiol Biomarkers Prev 12:747–754, 2003.
145. Enger SM, Longnecker MP, Chen MJ, Harper JM, Lee ER, Frankl HD, Haile RW: Dietary intake of specific carotenoids and vitamins A, C, and E, and prevalence of colorectal adenomas. Cancer Epidemiol Biomarkers Prev 5:147–153, 1996.
146. Shikany JM, Witte JS, Henning SM, Swendseid ME, Bird LC, Frankl HD, Lee ER, Haile RW: Plasma carotenoids and the prevalence of adenomatous polyps of the distal colon and rectum. Am J Epidemiol 145:662–557, 1997.
147. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ, Erdman Jr JW: *Cis-trans* lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol Biomarkers Prev 5:823–833, 1996.
148. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC: Intake of carotenoids and retinol in relation to risk of prostate cancer. J Natl Cancer Inst 87:1767–1776, 1995.
149. Schuurman AG, Goldbohm RA, Brants HA, van den Brandt PA: A prospective cohort study on intake of retinol, vitamins C and E, and carotenoids and prostate cancer risk. Cancer Causes Control 13:573–582, 2002.
150. Gann PH, Ma J, Giovannucci E, Willett W, Sacks FM, Hennekens CH, Stampfer MJ: Lower prostate cancer risk in men with elevated plasma lycopene levels: results of a prospective analysis. Cancer Res 59:1225–1230, 1999.
151. Nomura AMY, Stemmermann GN, Lee J, Craft NE: Serum micronutrients and prostate cancer in Japanese Americans in Hawaii. Cancer Epidemiol Biomarkers Prev 6:487–491, 1997.
152. Huang HY, Alberg AJ, Norkus EP, Hoffman SC, Comstock GW, Helzlsouer KJ: Prospective study of antioxidant micronutrients in the blood and the risk of developing prostate cancer. Am J Epidemiol 157:335–344, 2003.
153. Cohen JH, Kristal AR, Stanford JL: Fruit and vegetable intakes and prostate cancer risk. J Natl Cancer Inst 92:61–68, 2000.
154. Lu QY, Hung JC, Heber D, Go VLW, Reuter VE, Cordon-Cardo C, Scher HI, Marshall JR, Zhang ZF: Inverse associations between plasma lycopene and other carotenoids and prostate cancer. Cancer Epidemiol Biomarkers Prev 10:749–756, 2001.
155. Hall AK: Liarozole amplifies retinoid-induced apoptosis in human prostate cancer cells. Anticancer Drugs 7:312–320, 1996.
156. Franceschi S, Bidoli E, Negri E, Zambon P, Talamini R, Ruol A, Parpinel M, Levi F, Simonato L, La Vecchia C: Role of micronutrients, vitamins and minerals in the aetiology of squamous-cell carcinoma of the oesophagus. Int J Cancer 86:626–631, 2000.
157. Zhang ZF, Kurtz RC, Yu GP, Sun M, Gargon N, Karpeh Jr M, Fein JS, Harlap S: Adenocarcinomas of the esophagus and gastric cardia: the role of diet. Nutr Cancer 27:298–309, 1997.
158. Olmedilla B, Granado F, Blanco I, Rojas-Hidalgo E: Evaluation of retinol, alpha-tocopherol, and carotenoids in serum of men with cancer of the larynx before and after commercial enteral formula feeding. J Parenter Enteral Nutr 20:145–149, 1996.
159. Zheng W, Blot WJ, Diamond EL, Norkus EP, Spate V, Morris JS, Comstock GW: Serum micronutrients and the subsequent risk of oral and pharyngeal cancer. Cancer Res 53:795–798, 1993.
160. Nomura AM, Ziegler RG, Stemmermann GN, Chyou PH, Craft N: Serum micronutrients and upper aerodigestive tract cancer. Cancer Epidemiol Biomarkers Prev 6:407–412, 1997.
161. Spitz MR, McPherson RS, Jiang H, Hsu TC, Trizna Z, Lee JJ, Lipman SM, Khuri FR, Steffen-Batey L, Chamberlain RM, Schantz SP, Hong WK: Correlates of mutagen sensitivity in patients with upper aerodigestive tract cancer. Cancer Epidemiol Biomarkers Prev 6:687–692, 1997.
162. Bidoli E, La Vecchia C, Talamini R, Negri E, Parpinel M, Conti E, Montella M, Carbone MA, Franceschi S: Micronutrients and ovarian cancer: a case-control study in Italy. Ann Oncol 12:1589–1593, 2001.
163. Bertone ER, Hankinson SE, Newcomb PA, Rosner B, Willett WC, Stampfer MJ, Egan KM: A population-based case-control study of carotenoid and vitamin A intake and ovarian cancer (United States). Cancer Causes Control 12:83–90, 2001.
164. McCann SE, Freudenheim JL, Marshall JR, Brasure JR, Swanson MK, Graham S: Diet in the epidemiology of endometrial cancer in western New York (United States). Cancer Causes Control 11:965–974, 2000.
165. Jain MG, Rohan TE, Howe GR, Miller AB: A cohort study of nutritional factors and endometrial cancer. Eur J Epidemiol 16: 889–905, 2000.
166. Kucuk O, Churley M, Goodman MT, Franke A, Custer L, Wilkens LR, St. Pyrek J: Increased plasma level of cholesterol-5β,6β-epoxide in endometrial cancer patients. Cancer Epidemiol Biomarkers Prev 3:571–574, 1994.
167. Yuan JM, Gago-Dominguez M, Castelao JE, Hankin JH, Ross RK, Yu M: Cruciferous vegetables in relation to renal cell carcinoma. Int J Cancer 77:211–216, 1998.
168. O'Connor I, O'Brien N: Modulation of UVA light-induced oxidative stress by β-carotene, lutein and astaxanthin in cultured fibroblasts. J Dermatol Sci 16:226–230, 1998.
169. Nomura AM, Lee J, Stemmermann GN, Franke AA: Serum vitamins and the subsequent risk of bladder cancer. J Urol 170: 1146–1150, 2003.
170. Michaud DS, Spiegelman D, Clinton SK, Rimm EB, Willett WC, Giovannucci E: Fruit and vegetable intake and incidence of bladder cancer in a male prospective cohort. J Natl Cancer Inst 91:605–613, 1999.
171. Michaud DS, Pietinen P, Taylor PR, Virtanen M, Virtamo J, Albanes D: Intake of fruits and vegetables, carotenoids and vitamins A, E, C in relation to the risk of bladder cancer in the ATBC cohort study. Br J Cancer 87:960–965, 2002.
172. Zeegers MP, Goldbohm RA, van den Brandt P: Are retinol, vitamin C, vitamin E, folate and carotenoids intake associated with bladder cancer risk? Results from the Netherlands cohort Study. Br J Cancer 85:977–983, 2001.
173. Garcia R, Gonzalez CA, Agudo A, Riboli E: High intake of

specific carotenoids and flavonoids does not reduce the risk of bladder cancer. Nutr Cancer 35:212–214, 1999.
174. Botterweck AA, van den Brandt PA, Goldbohm RA: Vitamins, carotenoids, dietary fiber, and the risk of gastric carcinoma: results from a prospective study after 6.3 years of follow-up. Cancer 88:737–748, 2000.
175. Garcia-Closas R, Gonzalez CA, Agudo A, Riboli E: Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain. Cancer Causes Control 10:71–75, 1999.
176. Tsubono Y, Tsugane S, Gey KF: Plasma antioxidant vitamins and carotenoids in five Japanese populations with varied mortality from gastric cancer. Nutr Cancer 34:56–61, 1999.
177. Abneth CC, Qiao YL, Dawsey SM, Buckman DW, Yang CS, Blot WJ, Dong ZW, Taylor PR, Mark SD: Prospective study of serum retinol, beta-carotene, beta-cryptoxanthin, and lutein/zeaxanthin and esophageal and gastric cancers in China. Cancer Causes Control 14:645–655, 2003.
178. Batieha AM, Armenian HK, Norkus EP, Morris JS, Spate VE, Comstock GW: Serum micronutrients and the subsequent risk of cervical cancer in a population-based nested case-control study. Cancer Epidemiol Biomarkers Prev 2:335–339, 1993.
179. Potischman N, Herrero R, Brinton LA, Reeves WC, Stacewicz-Sapuntzakis M, Jones CJ, Brenes MM, Tenorio F, de Britton RC, Gaitan E: A case-control study of nutrient status and invasive cervical cancer. II. Serologic indicators. Am J Epidemiol 134: 1347–1355, 1991.
180. Goodman MT, Kiviat N, McDuffie K, Hankin JH, Hernandez B, Wilkens LR, Franke A, Kuypers J, Kolonel LN, Nakamura J, Ing G, Branch B, Bertram CC, Kamemoto L, Sharma S, Killeen J: The association of plasma micronutrients with the risk of cervical dysplasia in Hawaii. Cancer Epidemiol Biomarkers Prev 7:537–544, 1998.
181. Nagata C, Shimizu H, Yoshikawa H, Noda K, Nozawa S, Yajima A, Sekiya S, Sugimori H, Hirai Y, Kanazawa K, Sugase M, Kawana T: Serum carotenoids and vitamins and risk of cervical dysplasia from a case-control study in Japan. Br J Cancer 81: 1234–1237, 1999.
182. VanEenwyk J, Davis FG, Bowen PE: Dietary and serum carotenoids and cervical intraepithelial neoplasia. Int J Cancer 48:34–38, 1991.
183. Schiff MA, Patterson RE, Baumgartner RN, Masuk M, van Asselt-King L, Wheeler CM, Becker TM: Serum carotenoids and risk of cervical intraepithelial neoplasia in southwestern American Indian women. Cancer Epidemiol Biomarkers Prev 10:1219–1222, 2001.
184. Peng YM, Peng YS, Childers JM, Hatch KD, Roe DJ, Lin Y, Lin P: Concentrations of carotenoids, tocopherols, and retinol in paired plasma and cervical tissue of patients with cervical cancer, precancer, and noncancerous diseases. Cancer Epidemiol Biomarkers Prev 7:347–350, 1998.
185. Giuliano AR, Papenfuss M, Nour M, Canfield LM, Schneider A, Hatch K: Antioxidant nutrients: associations with persistent human papillomavirus infection. Cancer Epidemiol Biomarkers Prev 6:917–923, 1997.
186. Giuliano AR, Siegel EM, Roe DJ, Ferreira S, Baggio ML, Galan L, Duarte-Franco E, Villa LL, Rohan TE, Marshall JR, Franco EL: Dietary intake and risk of persistent human papillomavirus (HPV) infection: The Ludwig-McGill HPV Natural History Study. J Infect Dis 188:1508–1516, 2003.
187. Gonzalez S, Astner S, An W, Goukassian D, Pathak MA: Dietary lutein/zeaxanthin decreased ultraviolet B-induced epidermal hyperproliferation and acute inflammation in hairless mice. J Invest Dermatol 121:399–405, 2003.
188. Fung TT, Spiegelman D, Egan KM, Giovannucci E, Hunter DJ, Willett WC: Vitamin and carotenoid intake and risk of squamous cell carcinoma of the skin. Int J Cancer 103:110–115, 2003.
189. Wingerath T, Sies H, Stahl W: Xanthophyll esters in human skin. Arch Biochem Biophys 355:271–274, 1998.
190. Dwyer JH, Navab M, Dwyer KM, Hassan K, Sun P, Shircore A, Hama-Levy S, Hough G, Wang X, Drake T, Merz NB, Fogelman AM: Oxygenated carotenoid lutein and progression of early atherosclerosis. The Los Angeles Atherosclerosis Study. Circulation 103:2922–2927, 2001.
191. Panasenko OM, Sharov VS, Briviba K, Sies H: Interaction of peroxynitrite with carotenoids in human low density lipoproteins. Arch Biochem Biophys 373:302–305, 2000.
192. Rowley K, Walker KZ, Cohen J, Jenkins AJ, O'Neal D, Su Q, Best JD, O'Dea K: Inflammation and vascular endothelial activation in an Aboriginal population: relationships to coronary disease risk factors and nutritional markers. Med J Aust 178:495–500, 2003.
193. Boosalis MG, Snowdon DA, Tully CL, Gross MD: Acute phase response and plasma carotenoid concentrations in older women: findings from the Nun Study. Nutrition 12:475–478, 1996.
194. Dwyer JH, Paul-Labrador MJ, Fan J, Shircore AM, Merz CNB, Dwyer KM: Progression of carotid intima-media thickness and plasma antioxidants: The Los Angeles Atherosclerosis Study. Arterioscler Thromb Vasc Biol 24:313–319, 2004.
195. Iribarren C, Folsom AR, Jacobs Jr DR, Gross MD, Belcher JD, Eckfeldt JH, for the ARIC Study Investigators: Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis. A case-control study. Arterioscler Thromb Vasc Biol 17:1171–1177, 1997.
196. Street DA, Comstock GW, Salkeld RM, Schuep W, Klag MJ: Serum antioxidants and myocardial infarction. Are low levels of carotenoids and alpha-tocopherol risk factors for myocardial infarction? Circulation 90:1154–1161, 1994.
197. Osganian SK, Stampfer MJ, Rimm E, Spiegelman D, Manson JE, Willett WC: Dietary carotenoids and risk of coronary artery disease in women. Am J Clin Nutr 77:1390–1399, 2003.
198. Hak AE, Stampfer MJ, Campos H, Sesso HD, Gaziano JM, Willett W, Ma J: Plasma carotenoids and tocopherols and risk of myocardial infarction in a low-risk population of US male physicians. Circulation 108:802–807, 2003.
199. Sesso HD, Buring JE, Norkus EP, Gaziano JM: Plasma lycopene, other carotenoids, and retinol and the risk of cardiovascular disease in women. Am J Clin Nutr 79:47–53, 2004.
200. D'Odorico A, Martines D, Kiechl S, Egger G, Oberhollenzer F, Bonvicini P, Sturniolo GC, Naccarato R, Willeit J: High plasma levels of α- and β-carotene are associated with a lower risk of atherosclerosis. Results from the Bruneck study. Atherosclerosis 153:231–239, 2000.

201. Klipstein-Grobusch K, Launer LJ, Geleijnse JM, Boeing H, Hofman A, Witteman JCM: Serum carotenoids and atherosclerosis. The Rotterdam Study. Atherosclerosis 148:49–56, 2000.
202. Ruiz Rejon F, Martin-Pena G, Granado F, Ruiz-Galiana J, Blanco I, Olmedilla B: Plasma status of retinol, alpha- and gamma-tocopherols, and main carotenoids to first myocardial infarction: case control and follow-up study. Nutrition 18:26–31, 2002.
203. Polidori MC, Savino K, Alunni G, Freddio M, Senin U, Sies H, Stahl W, Mecocci P: Plasma lipophilic antioxidants and malondialdehyde in congestive heart failure patients: relationship to disease severity. Free Radic Biol Med 32:148–152, 2002.
204. Wright AJA, Southon S, Chopra M, Meyer-Wenger A, Moser U, Granado F, Olmedilla B, Corridan B, Hinninger I, Roussel AM, van den Berg H, Thurnham DI: Comparison of LDL fatty acid and carotenoid concentrations and oxidative resistance to LDL in volunteers from countries with different rates of cardiovascular disease. Brit J Nutr 87:21–29, 2002.
205. Hininger IA, Meyer-Wenger A, Moser U, Wright A, Southon S, Thurnham D, Chopra M, van den Berg H, Olmedilla B, Favier AE, Roussel AM: No significant effects of lutein, lycopene or β-carotene supplementation on biological markers of oxidative stress and LDL oxidizability in healthy adult subjects. J Am Coll Nutr 20:232–238, 2001.
206. Ascherio A, Rimm EB, Hernan MA, Giovannucci E, Kawachi I, Stampfer MJ, Willett WC: Relation of consumption of vitamin E, vitamin C and carotenoids to risk for stroke among men in the United States. Ann Intern Med 130:963–970, 1999.
207. Hirvonen T, Virtamo J, Korhonen P, Albanes D, Pietinen P: Intake of flavonoids, carotenoids, vitamins C and E, and risk of stroke in male smokers. Stroke 31:2301–2306, 2000.
208. Polidori MC, Cherubini A, Stahl W, Senin U, Sies H, Mecocci P: Plasma carotenoid and malondialdehyde levels in ischemic stroke patients: relationship to early outcome. Free Radic Res 36:265–268, 2002.

Received September 13, 2004.