Beta-Carotene and Other Carotenoids: Promises, Failures, and a New Vision

Jack J. Challem[1]

Abstract

Recent studies have suggested that beta-carotene supplements can increase the risk of lung cancer among current, long-term smokers. However, these studies may have been conceived and executed on a number of faulty assumptions. There was no evidence to suggest that beta-carotene would protect specifically against lung cancer, particularly in high-risk populations. There are significant isomeric and other differences between the synthetic beta-carotene used in these negative studies and natural beta-carotene supplements; these isomeric differences would suggest different behaviors. Researchers ignored the likely roles of other carotenoids in health and the possibility that carotenoids, as a group, function synergistically. The evidence supporting the antioxidant and disease-preventive roles of beta-carotene remains strong, but other associated dietary carotenoids, such as alpha-carotene, lycopene, lutein, zeaxanthin, and cryptoxanthin, also play important and likely complementary roles in health. The use of a single synthetic carotenoid in clinical studies reflects a single-drug, magic-bullet approach, whereas the evidence suggests that nutrients work as a biochemical team.

Introduction

Although a generic "carotene" was first identified in carrots in 1831, the correct chemical formula for beta-carotene was not determined until 1907, and researchers did not discover that the body converted it to vitamin A until 1920. For most of the next 61 years, researchers believed beta-carotene's role in human health was strictly that of a precursor to vitamin A. Until the mid-1980s, relatively little research had been conducted on the nutritional and health roles of other carotenoids, such as alpha-carotene, lycopene, lutein, zeaxanthin, and cryptoxanthin.

Based on epidemiological data, it was hypothesized in 1981 that beta-carotene, apart from its role as provitamin A, might reduce the risk of cancer.[1] Indeed, the evidence seemed strong. Beta-carotene was well established as an antioxidant, and diets high in beta-carotene appeared protective against various types of cancers. As with studies on virtually every nutrient, research suggested a protective role for beta-carotene in some types of cancer and a null effect on other types of cancer.

Confidence in the supplemental and chemopreventive roles of beta-carotene was shaken in 1994 and again in 1996 with the publication of two studies in which current, long-term tobacco smokers or asbestos workers (as well as users of alcoholic beverages) had a slightly increased risk of lung cancer after taking beta-carotene for several years.

Hindsight, as the bromide goes, is always superior to foresight. It now appears that researchers made at least four major errors in embracing beta-carotene as a cancer-preventive agent. First, researchers assumed that synthetic beta-carotene supplements would behave like natural beta-carotene supplements, although the two differ isomerically. Second, researchers made the mistake of studying a population at high-risk for lung cancer when there had been no evidence in published peer-reviewed journals that beta-carotene protected specifically against lung cancer.[2] Third, researchers were unknowingly the victims of their own technical limitations. They could only measure what they could measure. As a consequence, researchers

1. The Nutrition Reporter™ 6782 SW 167th Place, Beaverton, Oregon USA (challem@compuserve.com)

focused on easy-to-measure beta-carotene, while largely ignoring other so-called "mixed" carotenoids found in foods. Fourth, there is evidence that antioxidants, including the carotenoids, function synergistically. Thus, many trials that investigate individual nutrients, as if they were pharmaceuticals, may be doomed to failure. Nutrients generally work best as a team—an idea that, given the nature of biochemistry, should not be at all surprising.

These four errors would misguide many researchers and ultimately lead to reports that beta-carotene supplements increased the risk of lung cancer among current, long-term smokers. This review article will relate some of the evidence supporting beta-carotene's role as an antioxidant, analyze aspects of the studies showing an increased risk of lung cancer associated with synthetic beta-carotene supplements, and suggest a new context in which to interpret these and other carotenoid studies.

Beta-Carotene as an Antioxidant

Beta-carotene is one of approximately 600 fat-soluble carotenoids found in plants. Despite the large number of carotenoids in nature, only about 40-50 are found in the average American diet, and just 14 of these dietary carotenoids appear to play any role in human health, based on their presence in the bloodstream.[3]

In the early to mid-1980s, researchers focused on beta-carotene for a number of reasons. Epidemiological evidence strongly suggested that foods high in beta-carotene, specifically vegetables and fruit, protected against many types of cancer and cardiovascular disease. Nutritional databases at that time noted the presence of beta-carotene but not other carotenoids. The studies were also biased toward beta-carotene in that it could be measured relatively easily in serum, whereas other carotenoids could not.[4] Researchers also felt they had developed a reasonable understanding beta-carotene, in part because it was a well-known precursor to vitamin A. Beta-carotene was recognized as an antioxidant capable of preventing cellular damage, suggesting that supplements could prevent and successful treat precancerous oral leukoplakias.

Indeed, beta-carotene looked–and *still* looks–extremely promising as a potent antioxidant. The literature supporting beta-carotene's role as an antioxidant is too extensive to cite comprehensively here, except for several brief examples.[5]

One molecule of beta-carotene is capable of quenching up to 1,000 free radicals.[6] In a study that measured exhaled pentane as a marker of oxidative stress, researchers reported that beta-carotene reduced pentane levels in smokers.[7] Another team of researchers found that beta-carotene prevented chromosomal damage in human lymphocytes exposed to x-ray radiation.[8]

Beta-carotene also reduces inflammation and erythema associated with exposure to sunlight. Long-term exposure to ultraviolet-B in sunlight is well established as a source of free radical damage and skin cancer.[9] In addition, studies have found that beta-carotene supplementation (30 mg/d) substantially increases the proliferation of lymphocytes, a marker of immune function and immune cell surveillance.[10]

The strongest evidence of likely anticancer properties comes from research on oral leukoplakia, a precancerous condition often found in long-term smokers and alcoholics. At least five clinical trials have demonstrated that beta-carotene supplements can reverse oral leukoplakias. Laboratory models, animal studies, epidemiological surveys, and clinical trials with human cancer patients all confirm that beta-carotene can prevent oral cancer and even prompt the regression of existing premalignant lesions.[11]

Beta-Carotene Called into Question

Because the whole of beta-carotene research suggested a cancer-preventive role, the negative findings of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention

Study Group (ATBC), published in 1994, were completely unexpected. Known generally as the "Finnish study," ATBC was a placebo-controlled study of 29,133 Finnish male smokers aged 50-69. The experimental group received 20 mg/d of beta-carotene with follow-up for five to eight years. The relative risk (RR) of lung cancer in the beta-carotene group was initially determined to be 1.18, or an 18 percent increase in risk compared with the control group. Smokers taking beta-carotene supplements were also 8 percent more likely to die during the study period. The increase in risk, based on a calculation of person-years, was considered of only marginal statistical significance.[12]

A subsequent analysis of the ATBC data found the RR among smokers taking beta-carotene to be 1.16, and that consumption of about one alcoholic drink daily (11 grams of alcohol, or just under one drink) increased the RR to 1.35.[13] Subjects who smoked less than a pack of cigarettes daily, took beta-carotene, but rarely if ever consumed alcohol had an RR of 1.03, which is a statistically insignificant increase. Other research suggests that the combination of alcohol and vitamin A (to which some beta-carotene is converted in the body) may increase the risk of cancer.[14]

As in the ATBC trial, the Beta-Carotene and Retinol Efficacy Trial (CARET), also found an increased risk of lung cancer among current, long-term smokers taking both beta-carotene and retinol (vitamin A) supplements. The CARET study included 18,314 men and women at risk of lung cancer because they had smoked or had occupational exposure to asbestos, an established lung carcinogen. The experimental group in this trial consumed supplements containing a combination of 30 mg/d of beta-carotene and 25,000 IU/d of retinol.

The premature conclusion of the study and a partial release of data were announced at a press conference on January 18, 1996. The CARET researchers reported that the beta-carotene and retinol supplements resulted in a 28 percent increase in the risk lung cancer and a 17 percent increase in the risk of death, but that these findings were not statistically significant! The RR for lung cancer was 1.28. However, former smokers taking the beta-carotene and retinol had a RR of 0.80, or a 20 percent decrease in lung cancer risk, which was also described as insignificant. Results of the study were eventually published in the *New England Journal of Medicine*.[15]

How could a 28 percent increase in lung cancer risk not be considered significant? Again, it was a statistical risk based on a calculation of person-years. The increased risk was equivalent to six people developing lung cancer instead of an expected five people in every thousand. If the reference point was the five people who would develop lung cancer anyway, one more case of lung cancer would be significant. However, if your reference point was a group of 1,000 people, the increased risk would be only 0.001.

On the positive side, the CARET study reported that subjects with the highest serum beta-carotene levels at the start of the study had a significant 40 percent decrease in lung cancer risk. These initial high beta-carotene levels presumably reflected a high long-term consumption of vegetables and fruit and, it is generally assumed, a high life-long consumption of beta-carotene. It is probable that the baseline beta-carotene levels served as a marker of mixed carotenoid consumption.

In a re-analysis of the data, CARET investigators found a particularly high risk of lung cancer among subjects who smoked, took beta-carotene supplements, and consumed about three alcoholic drinks daily. Subjects in the highest quartile of alcohol intake has an RR of 1.99 for lung cancer–virtually double the risk.[16]

The third study, described at the January 18 press conference and subsequently published, showed a null effect of beta-carotene in a lower risk group of men. The

Physician's Health Study (PHS) involved 22,071 relatively health male physicians. Fifty-one percent of the physicians had smoked at one time, and 11 percent were current smokers. The experimental group received 50 mg of beta-carotene on alternate days for 12 years. There were no statistically significant differences in cancer, heart disease, or mortality between the subjects receiving beta-carotene and those receiving a placebo.[17] Among the current smokers, there was a slight but insignificant decrease in lung cancer incidence (38 vs 41) among subjects taking beta-carotene.[18]

Natural Versus Synthetic Beta-Carotene

Relatively few researchers have noted that each of these disappointing studies used *synthetic* beta-carotene, which may have affected their results. The synthetic beta-carotene (supplied by Hoffman-La Roche) consists of only the all-trans isomer of beta-carotene, which is readily converted by the body to retinol.[19] To be fair, synthetic beta-carotene has been helpful in a number of conditions, including oral leukoplaka. However, a combination of retinol and high alcohol intake may increase the risk of liver disorders and precancerous cell changes.[20]

Why did CARET researchers find a higher risk of lung cancer than did the ATBC researchers? It is conceivable that the combination of all-trans beta-carotene and retinol in the CARET study potentiated the risk of lung cancer in smokers. As much as 70 percent of the all-trans isomer is converted to retinol.[21] CARET subjects received 30 mg of synthetic beta-carotene and 25,000 IU daily of vitamin E, the equivalent of 60,000 IU of vitamin A, an enormous amount that is rarely recommended for any condition. Given the known risks of a combination of high-dose vitamin A and alcohol, this hypothesis is plausible.[22]

In contrast to synthetic all-trans beta-carotene, the most common type of natural beta-carotene supplement (derived primarily from *Dunaliella* species of algae) contains 30-50 percent of the 9-cis isomer in addition to the all-trans. (These isomers are analogous to anagrams, in which the letters of one word can be rearranged to form another, such as "star" and "rats." Just as "star" and "rats" have different meanings, the different isomers appear to have different activities in the body.) There is evidence that the 9-cis isomer is a potent antioxidant. Consistent identification of the 9-cis isomer in serum has been difficult, but researchers recently measured its oxidative degration products. The suggestion was that the 9-cis isomer is rapidly used up quenching free radicals.[23]

The Value of Other Carotenoids

There may be yet another explanation for why synthetic all-trans beta-carotene increased the risk of lung cancer among long-term smokers. The all-trans isomer is but one of many isomers. Natural beta-carotene can, theoretically, form 272 different isomers. Furthermore, all of the 600 known carotenoids could, theoretically, form more than 200,000 different isomers. Experiments using synthetic all-trans isomer of beta-carotene may have suffered from a lack of other isomers.

Dunaliella algae, the principal source of natural beta-carotene supplements, actually contains a mix of carotenoids and carotenoid isomers, include 9-cis. This means that the effects, and apparent benefits, of natural beta-carotene may be due in part to the presence of mixed carotenoids and not just the 9-cis isomer. These other carotenoids, which include alpha-carotene, lutein, zeaxanthin, and cryptoxanthin, could confound experiments meant to study beta-carotene.

Most epidemiological studies in the 1980s assumed beta-carotene was the principal active carotenoid, whereas in retrospect most of these studies are better interpreted as "mixed carotenoid" studies.

For example, in one epidemiological study, researchers reported that beta-carotene protected against lung cancer. A reanalysis, using a more comprehensive carotenoid database, showed that alpha-carotene appeared to exert a more protective effect than did beta-carotene.[24]

Similarly, diets high in beta-carotene have been associated with a reduced risk of lung cancer among Hawaiian men and women. Several years later, using an expanded database of carotenoid levels in foods, it became clear that beta-carotene, alpha-carotene, and lutein were the most protective carotenoids.[25]

The carotenoid picture could be further complicated by the chemically kaleidoscopic nature of the carotenoids: there may be several hundred isomers formed by the five carotenoids found in *Dunaliella*. Biochemically, multiple carotenoids and isomers probably provide a toolbox of molecules and may be more advantageous biologically than any single isomer.

This situation is illustrated by comparing several studies on the roles of carotenoids and coronary heart disease. One study found no benefit from 50 mg/d of beta-carotene, and another reported a slight increase in the risk of angina with 20 mg/d.[26,27] In contrast, a study that measured total blood carotenoid levels reported an overall 36 percent risk reduction in myocardial infarction and 72 percent risk reduction among nonsmokers.[28]

Given these findings, it is worthwhile to briefly review some of the research on other common dietary carotenoids.

Alpha-carotene

In cell-culture studies, alpha-carotene inhibits the growth of neuroblastoma cells, with suppression of cell growth occurring in 18 hours.[29] In mice, alpha-carotene substantially reduces the incidence of liver, lung, and skins cancers. Tumor numbers and sizes are smaller among animals receiving supplemental alpha-carotene, and in terms of tumor suppression, alpha-carotene appears substantially more potent than beta-carotene.[30]

Lycopene

A red-pigmented carotenoid, lycopene may be the most potent free radical scavenger among the carotenoids.[31,32] Recent research has focused on lycopene's role in preventing prostate cancer. However, it may play a protective role in preventing breast and stomach cancers as well.

The richest dietary source of lycopene is tomatoes and tomato products, particularly tomato sauces. Cooked tomatoes provide more lycopene than raw tomatoes, probably because heat breaks down the fruit's cellular matrix and releases more of the carotenoid. Furthermore, tomatoes are often cooked with oil (e.g. spaghetti or pizza sauce), which would increase the bioavailability of the fat-soluble carotenoid.[33]

Researchers have also found that men eating diets high in tomato sauces were 45 percent less likely to develop prostate cancer than were men eating few or no tomato products. Tomato juice, which is neither cooked nor prepared with oil, provided no reduction in risk.[34] Two additional analyses showed the same reduction in prostate cancer risk among men eating large quantities of tomatoes.[35] Another study found that people eating diets high in tomatoes, and therefore lycopene, had about one-half the risk of stomach cancer, compared with people eating few tomatoes.[36]

In animal experiments, researchers have reported that lycopene-supplemented diet significantly suppressed the development of breast cancers. Mice consuming supplemental lycopene benefited from an increase in the number of T4 helper cells and improving immune function.[37]

Lutein and Zeaxanthin

Lutein is often associated with zeaxanthin in food, and the body readily converts some lutein to zeaxanthin. These carotenoids are often referred to as the yellowish "macular pigment." Thin macular pigment appears to be a major contribut-

ing factor in macular degeneration, a leading cause of blindness. The macula is the central part of the retina in primates and humans and is responsible for sharp and detailed vision.

In comparing the diets of 356 patients with macular degeneration to 520 controls, researchers found that high dietary consumption of carotenoids was associated with a 43 percent reduction in the risk of disease. A further analysis of the diet found that lutein and zeaxanthin were the principal carotenoids associated with this lower risk of macular degeneration. The scientific rationale for a protective role of lutein and zeaxanthin is very strong. The retina is rich in polyunsaturated fatty acids (PUFAs), which are susceptible for free radical damage. These PUFAs may be protected by lutein and zeaxanthin. Furthermore, as yellow pigments, lutein and zeaxanthin, would filter out visible blue light, a principal source of free-radical damage in the eye.[38]

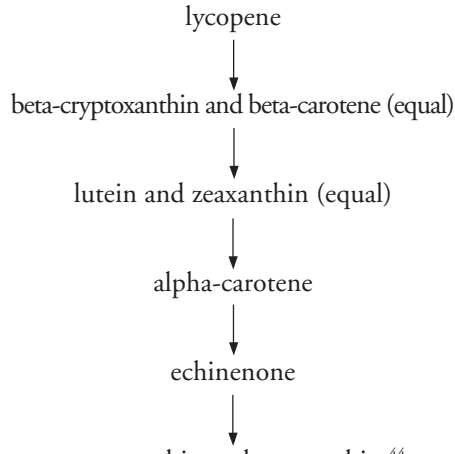
In studies of identical twins, researchers noted that differences in the thickness of the macular pigment was strongly influenced by diet.[39] Monkeys fed carotenoid-free diets lose the yellow color of the macular and suffer degenerative changes to the eyes.[40] Diets high in lutein and lutein supplements can thicken the macular pigment.[41]

Cryptoxanthin

Cryptoxanthin, one of the more obscure dietary carotenoids, also appears to possess some anti-cancer properties. Although research on this carotenoid is limited, cryptoxanthin may reduce the risk of cervical cancer and may inhibit growth of the Epstein-Barr virus.[42,43]

Carotenoid Antioxidant Hierarchy and Synergism

A recent experiment comparing the antioxidant properties of carotenoids found that lycopene had the greatest ability to scavenge free radicals. The complete antioxidant hierarchy of carotenoids was:



Evidence suggests that antioxidants in general, and carotenoids specifically, function synergistically and that a diversity of antioxidants potentiates their benefits. For example, a diverse combination of antioxidants, including beta-carotene, is far more effective in quenching free radicals than are only one of two antioxidants.[45] The synergistic effect of carotenoids is suggested by animal studies showing that beta-carotene supplements increase blood levels of both beta- and alpha-carotene.[46]

Noting such synergism among nutrients, a leading nutritional epidemiologist recently argued that clinical studies may not the ideal method for assessing their health benefits: "If anything is well established in biochemistry, it is that nutrients interact with one another...Probably no single agent exists that is completely sufficient; rather, nutrients act optimally in conjunction with other agents. Furthermore, it is possible, even probably, that the effective amount of an agent is different for different conditions. For example, a dose or an agent that is ineffective at preventing lung cancer may be effective at preventing cataracts."[47]

This sentiment has been echoed by other researchers, who have written that "not all antioxidants are created equal. A variety of nutrients have antioxidant ac-

tivity or are integral to the function of antioxidant enzymes. However, the substantial differences in the structure and biologic properties of specific antioxidants are likely to influence their ability to prevent a specific disease...Therefore, a null finding for one antioxidant on disease risk does not mean that other antioxidants could not be effective."[48]

Conclusion

In retrospect, researchers invested considerable resources to demonstrate that beta-carotene supplements could prevent lung cancer when no clear evidence had suggested that it would be of value in preventing this condition. They compounded this error with others, including the assumption that synthetic beta-carotene was equivalent to natural beta-carotene supplements, that other carotenoids had no significant biological value, and that carotenoid synergism played no role in health. In one instance, researchers also rushed to judgment, presenting their findings at a press conference rather than in a scientific journal and causing a panic among beta-carotene users.

However, the negative findings related to beta-carotene may have a positive outcome and lead to a new vision of the carotenoids as a family of beneficial nutrients. These studies appear to be fueling studies on other carotenoids and carotenoid isomers. Further experiments and evaluation of the ATBC and CARET studies have suggested that the increased risk of lung cancer with beta-carotene supplementation may be related to the use of a synthetic beta-carotene isomer and alcohol.

This review has focused on the antioxidant and some of the disease-preventing properties of beta-carotene and other carotenoids, as well as questions raised about the safety of beta-carotene in recent studies. It has not attempted to discuss the provitamin A properties of carotenoids. Nor has it discussed the potential interactions between carotenoids and steroids, which could also influence the risk of cancer, coronary heart disease, and other diseases.

In conclusion, evidence supports the idea that natural beta-carotene and mixed carotenoid supplements are antioxidants with health-preserving properties, but that synthetic beta-carotene may have both risks as well as benefits. It also appears that antioxidants and carotenoids likely function in a synergistic manner, and a larger number of antioxidants are superior to a smaller number. In essence, the sum of carotenoids and other antioxidants is greater than their parts.

References

1. Peto R, Doll R, Buckley JD, et al: Can dietary beta-carotene materially reduce human cancer rates? *Nature*, 1981;280:201-208.
2. De Luca LM and Ross SH: Beta-carotene increases lung cancer incidence in cigarette smokers. *Nutr Rev*, 1995;54:178-180.
3. Khachik F, Beecher GR, and Smith Jr JC: Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *J of Cellular Biol,* 1995;22 (Suppl):236-246.
4. Ziegler RG, Subar AF, Craft NE, et al.: Does beta-carotene explain why reduced cancer risk is associated with vegetable and fruit intake? *Canc Res,* 1992;52 (Suppl):2060-2066.
5. See Handelman GJ: Carotenoids as scavengers of active oxygen species. In eds. Cadenas E and Packer L, eds:Handbook of Antioxidants, Marcel Dekker, New York, 1996.
6. Di Mascio P, Murphy ME. Sies H: Antioxidant defense systems: the role of carotenoids, tocopherols, and thiols. *Am J Clin Nutr,* 1991;53:194S-200S.
7. Allard JP, Royall D, Kurian R, et al.: Effects of beta-carotene supplementation on lipid peroxidation in humans. *Am J Clin Nutr*, 1994;59:884-90.
8. Umegaki K, Ikegami S, Inoue K, et al.: Beta-carotene prevents x-ray induction of micronuclei in human lymphocytes. *Am J Clin Nutr,* 1994;59:409-412.
9. Gollnick HPM, Hopfenmüller, Hemmes C: Systemic beta carotene plus topical UV-sunscreen are an optimal protection against harmful effects of natural UV-sunlight: results of the Berline-Eilath study. *Euro J Dermatol,* 1996;6:200-205.
10. Moriguchi S, Okishima N, Sumida S, et al.: Beta-carotene supplementation enhances

lymphocyte proliferation with mitogens in human peripheral blood lymphocytes. *Nutr Reseach,* 1996;16:211-218.
11. Garewal HS and Schantz S: Emerging role of beta-carotene and antioxidant nutrients in prevention of oral cancer. *Archives Otolaryngology–Head and Neck Surgery,* 1995; 121:141-144.
12. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group: The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med,* 1994;330:1029-1035.
13. Albanes D, Heinonen OP, Taylor PR, et al.: Alpha-tocopherol and beta-carotene supplements and lung cancer incidence in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention study: effects of baseline characteristics and study compliance. *J Natl Canc Inst,* 1996;88:1560-1570.
14. Bland J: The beta-carotene controversy in perspective. *J of Appl Nutr,* 1996;48:42-45.
15. Omenn GS, Goodman GE, Thornquist MD, et al.: Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N Engl J Med,* 1996;334:1150-1155.
16. Omenn GS, Goodman GE, Thornquist MD, et al.: Risk factors for lung cancer and for intervention effects in CARET, the beta-carotene and retinol efficacy trial. *J Natl Canc Inst,* 1996;88:1550-1559.
17. Hennekens CH, Buring JE, Manson JE, et al.: Lack of effect of long-term supplementation with beta-carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Eng J Med*, 1996;334:1145-1149.
18. Erdman JW Jr, Russell RM, Rock CL, et al.: Beta-carotene and the carotenoids: beyond the intervention trials. Nutr Rev, 1996;54:185-188.
19. Ben-Amotz A and Levy Y: Bioavailability of a natural isomer mixture compared with synthetic all-trans beta-carotene in human serum. *Am J Clin Nutr*, 1996;63:729-34.
20. Bland J, op. cit.
21. Ben-Amotz A and Levy Y, op. cit.
22. Challem JJ: Re: Risk factors for lung cancer and for intervention effects in CARET, the beta-carotene and retinol efficacy trial. *J Natl Canc Inst,* Feb 19, 1997;89: in press.
23. Ben-Amotz A and Levy Y, op. cit.
24. Ziegler RG, Colavito EA, Hartge P, et al.: Importance of alpha-carotene, beta-carotene, and other phytochemicals in the etiology of lung cancer. *J Natl Canc Inst,* 1996;88:612-615.
25. Le Marchand L, et al., Cancer Epidemiology, Biomarkers & Prevention, 1993; 2:183-7.
26. Greenberg ER, Baron JA, Karagas MR, et al.: Mortality associated with low plasma concentration of beta carotene and the effect of oral supplementation. *JAMA,* 1996;275:699-703.
27. Rapola JM, Virtamo J, Haukka JK, et al.: Effect of vitamin E and beta carotene on the incidence of angina pectoris. *JAMA,* 1996;275:693-698.
28. Morris DL, Kritchevsky SB, and Davis CE: Serum carotenoids and coronary heart disease. *JAMA,* 1994;272:1439-1441.
29. Murakoshi M, Takayasu j, Kimura O, et al.: Inhibitory effects of alpha-carotene on proliferation of the human neuroblastoma cell line GOTA. *J Nat Canc Inst* 1989;81:1649-52.
30. Murakoshi M, Nishino H, Satomi Y, et al.: Potent preventive action of alpha-carotene against carcinogenesis: spontaneous liver carcinogenesis and promoting state of lung and skin carcinogenesis in mice are suppressed more effectively by alpha-carotene than by beta-carotene. *Canc Res*, 1992;52:6583-7.
31. Di Masco P, Kaiser S, Sies H: Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch Biochem Biophys,* 1989;274:532-538.
32. Miller NJ, Sampson J Candeias LP, et al.: Antioxidant activity of carotenes and xanthophylls. *FEBS Letters*, 1996;384:240-2.
33. Giovannucci E, Ascherio A, Rimm EB et al.: *J Natl Canc Inst,* 1995;87:1767-76.
34. Ibid.
35. Personal communication with E. Giovannucci, Harvard University, June 11, 1996.
36. Franceschi S, Bidoli E, La Vecchia C, et al.: Tomatoes and risk of digestive-tract cancers. Intl J Cancer, 1994;59:181-4
37. Kobayashi T, Iijima K, Mitamura T, et al.: Effects of lycopene, a carotenoid, on intrathymic T cell differentiation and peripheral CD4/CD8 ratio in a high mammary tumor strain of SHN retired mice. *Anti-Cancer Drugs*, 1996;7:195-8.
38. Seddon JM, Ajani UA, Sperduto RD, et al.: Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *JAMA,* 1994;272:1413-20
39. Hammond Jr BR, Fuld K, and Curran-Celentano J: Macular pigment in monozygotic twins. Invest Ophthal & Vis Sci, 1995;36:2431-41
40. Stampfer MJ and Willett WC: Correspondence. *NEJM,* 1996;335:669.
41. Personal communication, Jun 11, 1996.
42. Batieha AM, Armenian HK, Norkus EP, et al.: Serum micronutrients and the subsequent risk of cervical cancer in a population-based

nested case-control study. Cancer Epidemiology, Biomarkers & Prevention, 1993;2:335-9
43. Tsushima M, Maoka T, Katsuyama M, et al.: Inhibitory effect of natural carotenoids on Epstein-Barr virus activation activity of a tumor promoter in Raji cells. A screening study for anti-tumor promoters. *Biological & Pharmaceutical Bulletin,* 1995;18:227-33
44. Miller NJ, op. cit.
45. Chen H and Tappel AL: Protection of vitamin E, selenium, trolox C, ascorbic acid palmitate, acetylcysteine, coenzyme Q10, beta-carotene, canthaxanthin, and (+)-catechin against oxidative damage to rat blood and tissues in vivo. *Free Rad Biol & Med,* 1995;18:949-53.
46. Zhou JR. Gugger ET. Erdman JW Jr: The crystalline form of carotenes and the food matrix in carrot root decrease the relative bioavailability of beta- and alpha-carotene in the ferret model. J *Am Coll of Nutr,* 1996;15:84-91.
47. Block G: Are clinical trials really the answer? *Am J Clin Nutr,* 1995;62S:1517S-20S.
48. Hankinson SE and Stampfer MJ: All that glitters is not beta carotene. *JAMA,* 1994;272:1455-6.