Functions and Actions of Retinoids and Carotenoids: Building on the Vision of James Allen Olson

From 1989 to 2001: What Have We Learned About the "Biological Actions of Beta-Carotene"?[1]

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ABSTRACT Dr. James Allen Olson helped us to define the role of beta-carotene in human health by categorizing these as "functions, actions and associations." In the last decade, significant research has shown that beta-carotene acts as an antioxidant in biologically relevant systems, affects several aspects of human immune function and higher intake/serum levels are associated with improvements in certain physiological functions such as lung function. The unexpected findings of increased lung cancer in beta-carotene supplemented smokers in the ATBC and CARET intervention studies have resulted in the need for expanded research efforts to define the mechanism(s) of action of beta-carotene. Recent survey data as well as laboratory animal studies continue to find an inverse association between beta-carotene and cancer risk. Because beta-carotene is the major source of vitamin A for the majority of the world's population, it is critical to define the safe levels of intake from foods and supplements. J. Nutr. 134: 225S–230S, 2004.

KEY WORDS: • *immune function* • *lung function* • *antioxidant* • *cancer* • *lipids*

In May 1988, Jim Olson and I cochaired a FASEB symposium entitled: "Biological Actions of Carotenoids" (1). Jim identified three key criteria for determining the role(s) of carotenoids in human health (2). The first, functions, was defined as essential roles of carotenoids that would result in health impairment if not consumed; the second, actions, was defined as the responses seen following the administration of carotenoids and that are not essential for life; the third, associations, was the result of correlations that may or may not have a causal relationship. The symposium focused on the antioxidant functions of carotenoids, actions on the immune system and associations with cancer prevention. Since the publication of the symposium and our review paper in 1989 (3), new knowledge has been gained about carotenoids in each of these areas.

This review focuses on data published since 1988 that help to elucidate the functions, actions and associations reported for beta-carotene in humans. In vitro, it appears that beta-carotene, as an antioxidant, quenches oxygen-containing free radicals by a number of mechanisms (4); beta-carotene can also regenerate the antioxidant form of vitamin E and possibly other oxidized antioxidants (5–8). With regard to actions on the immune system, beta-carotene has been found to affect certain cell types and responses and not others (9–22). With regard to cancer prevention, the epidemiological evidence continues to show a positive association based upon foods rich in carotenoids (23–28), yet the negative effects of beta-carotene supplementation on lung cancer risk (29,30) were not at all predicable in light of the consistent positive associations reported in survey data. However, it is not clear, based upon the antioxidant and pro-vitamin A functions of beta-carotene, why supplementation caused an increase in lung cancers in smokers in two separate studies. Although the ATBC study (29) was published almost a decade ago, a biologically plausible explanation for the unlikely findings has yet to be found. Perhaps it is time for a new hypothesis. This author suggests that a possible explanation can be coined: "beta-carotene: no good deed goes unpunished." Data indicate that there is an association between beta-carotene and enhanced lung function (31–33). Increased forced expiratory lung volume could translate into deeper breathing of the carcinogens and other oxidants in smokers; that could result in a greater carcinogen burden in smokers supplemented with beta-carotene than those on placebo. At first glance it may seem paradoxical that, in epidemiological studies, increased beta-carotene intake is associated with decreased risk of lung cancer and in intervention studies, the opposite is found even though in both cases immune functions may be enhanced. However, one possible explanation is that epidemiological data represent the true prevention of cancer initiation by beta-carotene via the im-

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mune system or other mechanisms. The intervention studies probably involve individuals who are already initiated and enhancement of immune function may not have the same effects at this stage of cancer. We owe it to Jim and the other carotenoid pioneers to find the biologically plausible explanation for the ATBC results so that this lodestone of uncertainty does not hamper further carotenoid research.

Functions of beta-carotene

For millions of people living in developing countries, beta-carotene is the predominant source of the essential nutrient, vitamin A (retinol and its esters) (3). There are also data that indicate that beta-carotene can bind certain retinoid receptors without being cleaved to form vitamin A (34). Certainly the question remains whether the activity of beta-carotene in lipoproteins is a function or an action (35,36).

Actions of beta-carotene

Beta-carotene is a potent quencher of singlet oxygen. However, there have been questions about the relevance of this reaction to human health. There are several conditions in humans in which reactive, metal-containing compounds are generated in sun-exposed skin with the resultant formation of singlet oxygen. The consequent inflammatory response causes painful lesions. There is a very long history of high-dose beta-carotene supplementation of individuals with the genetically inherited condition called erythropoietic protoporphyria (37).

Beta-carotene also acts as an antioxidant that scavenges free radicals deep in human LDL and HDL as well as in cell membranes (36). In vitro studies using radical generating systems have documented the capacity of beta-carotene to quench free radicals by mechanisms that include addition of the radical to the carotenoid, hydrogen abstraction, and/or electron transfer (5-8). Beta-carotene can regenerate the antioxidant form of vitamin E but not vitamin C (5). In lipoproteins, beta-carotene protects vitamin E from oxidation and is oxidized before vitamin E. Of great importance are the data that show the synergistic action of beta-carotene, vitamin E and vitamin C in the protection of lipids in membranes (5,6). The biological relevance of the actions of beta-carotene, and for that matter, any molecule with biological activity, cannot be truly elucidated in the absence of other molecules known to enhance its activity (6).

Questions have also been raised about the relative antioxidant efficacy of beta-carotene at different oxygen pressures. Of importance to effects in humans are data concerning reactions of beta-carotene at pressures seen at sea level, in the lung, blood and within cells. Niki et al. have indicated that the antioxidant activity of beta-carotene is higher at low oxygen pressures found within the human body (5). The cooperative free radical scavenging of beta-carotene and vitamin E reduces the potential for oxidation products to be formed as a result of beta-carotene's scavenging. Needless to say, there are many more dietary antioxidants (including other carotenoids) within human cells, tissues and lipoproteins, and there is still much work to be done to determine the interactions between beta-carotene and these molecules. When all-*trans* beta-carotene acts as an antioxidant, the *cis*-isomer can be formed, and this molecule can be retroconverted to the *trans* form when it dissipates the energy from its reaction with a reactive oxygen species. The conversion of the *cis* isomer to all-*trans* is facilitated by singlet oxygen but not by other reactive species. Further work is required to explain the unique *cis-trans* ratios of beta-carotene in every tissue in the human body, and probably every cell type (8).

I was very fortunate to have been involved in the elucidation of the actions of beta-carotene on immune functions, and showed that it could enhance lymphocyte proliferation independent of its pro-vitamin A function (38). It is entirely possible that all of the subsequent actions described below are a consequence of the antioxidant/singlet oxygen quenching capacity of beta-carotene; however, that specific link has not been made in all studies of immune function. **Table 1** outlines the data from the dozen or so studies that have examined the actions of beta-carotene on several indices of immune function in different population groups (9–22). Dosages ranged from 15 mg/d to 180 mg/d; duration ranged from 3 wk to 12 y; populations included healthy men and women, pregnant and lactating women, male smokers and those exposed to UV light. There appear to be a few consistent findings: healthy men and women, regardless of age, do not show either enhancement of lymphocyte proliferation or interleukin 2 production following supplementation. Unexpectedly, however, there are indications that the mononuclear cells required to stimulate these responses appear to be up-regulated by low-dose supplementation (39). Further, in colon cancer patients, markers of lymphocyte activation were increased with supplementation (40). Delayed type hypersensivity (DTH)[3] skin test responses, a well accepted index of overall immune function, also were unaffected by supplementation in nonimmune stressed populations, but DTH responses were maintained when beta-carotene supplements were taken prior to UV exposure in both young and senior men (20,21). Thus, when UV or cancer stresses the immune system, there may be a role for beta-carotene as an immunoenhancer. There are also three reports of enhanced Natural Killer (NK) cell cytotoxicity in seniors (9,10,22). NK cells are considered to be critical in recognizing and killing malignant cells in the body.

Beta-carotene: "Pro" or anti-carcinogen?

Regina Ziegler (41), Richard Moon (42) and Norman Krinsky (43), in 1989, reviewed the literature on epidemiology, in-vitro and laboratory animal studies of beta-carotene in cancer models. At that time, there were relatively few studies, but already an indication that beta-carotene supplementation could inhibit UV-induced tumor formation in the mouse skin cancer model. Epidemiological evidence pointed to an inverse association between carotenoid intake and cancer risk. For vitamin A and the retinoids (synthetic ones in particular), there were consistent data on the therapeutic benefits in patients with cancer of the lung, oral cavity and urinary bladder (44). There were no data on the use of beta-carotene for cancer therapy. The totality of the evidence, therefore, pointed to an anti-carcinogenic action of beta-carotene especially in the early stages of carcinogenesis.

As we are all well aware, in 1994 and again in 1996, well-controlled intervention studies in smokers and in others that were at high risk for lung cancer showed that beta-carotene supplementation unexpectedly increased lung cancer rates (29,30). A number of research strategies have been undertaken to try to explain the findings from these studies, yet it remains unclear whether beta-carotene is a pro-carcinogen or an anti-carcinogen. Animal studies differ in their findings and this may be due to the differences between spe-

[3] Abbreviations used: DTH, delayed type hypersensivity; FEV1, forced expiratory volume in 1 s; NK, natural killer; URT, upper respiratory tract infection.

TABLE 1

Effects of beta-carotene supplementation on human immune responses

Subjects	Beta-carotene dose/duration	Lymphocyte populations	Cytokines	NK[1] cytotoxicity	PHA proliferation	Con A proliferation	DTH	References
Adults, 50+	30, 45, 60 mg/d 2 months	Cells with IL2 and transferrin receptors		Increased NK cells				Watson et al., 1991 (9)
Adult males 50+	50 mg/EOD* 10–12 y		IL2-NE**	Increased				Santos et al., 1996 (10)
Adult males 50+	50 mg/EOD* 10–12 y	NE	NE		NE	NE	NE	Santos et al., 1997 (11)
Healthy male non-smokers	15 mg/day 4 wk	Increased monocytes with HLA-DR, ICAM, LFA-3	Increased TNF alpha					Hughes et al., 1997 (12)
	60 mg/d 44 wk	Increased CD4:CD8						Murata et al., 1994 (13)
Healthy senior males and females	45 mg/d 24 wk			Increased at 3 mo but not at 6 mo				Wood et al., 2000 (14)
Healthy senior females	90 mg/d 3 wk	NE	NE		NE	NE	NE	Santos et al., 1997 (11)
Healthy non- and lactating females	30 mg/d 4 wk				NE			Gossage et al., 2000 (15)
Male smokers	20 mg/d 14 wk	NE			Increased	NE		van Poppel et al., 1993 (16)
Male smokers with leukoplakia	30 mg/d 2 mo			Increased receptors; NK cytotoxicity				Garewal and Shamdas, 1991 (17)
Patients with colon polyps or cancer	30 mg/d 12 wk	Increased IL2R, CD4						Kazi et al., 1997 (18)
HIV patients	180 mg/d 12 wk	NE	NE					Coodley et al., 1996 (19)
	60–120 mg/d 12–28 wk	Decreased CD4:CD8						Silverman et al., 1994 (20)
UV exposed healthy males	30 mg/d 4 wk						Prevented UV-induced suppression	Fuller et al., 1992 (21)
UV exposed senior males	30 mg/d 40 wk						Prevented UV-induced suppression	Herraiz et al., 1998 (22)

[1] Abbreviations: CD4, T cells with cluster of differentiation marker #4; CD8, T cells with cluster of differentiation marker #4; Con A, concavalin A; CVD, cardiovascular disease; DTH, delayed type hypersensitivity; EOD, every other day; HLA-DR, human lymphocyte antigen at the D region; ICAM, intercellular adhesion molecule; IL2, Interleukin 2; IL2R, Interleukin 2 receptor; LFA-3, lymphocyte function; NE, no effect; NK, natural killer cells; PHA, phytohemagglutinin; TNF, tumor necrosis function.

cies. Ponnamperuma et al. (34) reporteded that beta-carotene fed to mice reduced the rate of skin cancer development in a well accepted, validated, two-stage carcinogenesis model. In fact, these researchers had expected that beta-carotene would act as a tumor promoter rather than protector.

One of the hypotheses about the carcinogenicity of beta-carotene suggests that it is the carotenoid oxidative products formed when beta-carotene is exposed to smoke that are the carcinogens (45). However, when Torbergsen and Collins (46) exposed lymphocytes from individuals supplemented with beta-carotene to H_2O_2, a potent oxidant, the DNA in the lymphocytes was better able to repair itself following the oxidative damage than the DNA from the same individuals before supplementation and following a wash-out period.

Following the publication of the ATBC (29) and CARET (30) trials, a number of ongoing chemoprevention trials in smokers (and nonsmokers) were stopped. One study continued, although there were significant dropouts. Mayne et al. (47) recently published their results using 50 mg/d of beta-carotene in patients who had been treated for early stage head and neck cancer. About half of the cohort were current smokers. After >4 y of intervention, there was no effect of the supplementation on risk of developing a second primary or recurrent cancer. However, the beta-carotene group had a nonsignificantly increased rate of survival in both smokers and nonsmokers. There was also a nonsignificant increase in lung cancers.

In addition to the Mayne et al. study, Correa et al. (48) published the results of their study in Colombian men and women with precancerous gastric lesions. The objective of this placebo-controlled, long-term (6 y) intervention trial was to determine if supplementation with beta-carotene (30 mg/d),

vitamin C (2 g/d) or both, with or without anti-H. pylori treatment, reduced the progression of these lesions from atrophy to metaplasia to dysplasia. Of the 976 subjects who were entered into the trial, 631 subjects completed the trial. Compared to the placebo group, all interventions resulted in significant regression of lesions. The greatest benefit was seen in the group given beta-carotene and vitamin C, with an eightfold increase in regression. There was no indication of either the initial vitamin A status or the prevalence of smokers in this cohort; such follow-up information is important in determining the totality of evidence of the risk:benefit of beta-carotene supplementation.

It has been very interesting to watch the string of publications, and the lack of media (and scientific) attention given to the subsequent data from the ATBC cohort. Many reports have been published following the initial paper from the ATBC study (49–56). Albanes et al. reported that there was no increased risk of lung cancer in those who smoked 5–19 cigarettes/d, and/or consumed less than one alcoholic drink/d. In another paper, beta-carotene supplementation decreased the risk of prostate cancer by 32% in nondrinkers and increased the risk in drinkers (49). Whether or not the ATBC participants smoked or drank alcohol, if they were in the beta-carotene supplemented groups, they had a lower rate of prostatic hyperplasia and made fewer visits to physicians related to prostate complaints (51).

The Women's Health Study, involving about 20,000 US women, included several subgroups that received 50 mg of beta-carotene every other day for 2 y. The women were followed for another 2 y after the supplementation ceased. The investigators reported no significant differences in the incidence of cancer, CVD, or total mortality; in the 13% who were smokers, there were no differences in the number of cancers or CVD in the supplemented vs. the placebo group (57).

Beta-carotene and lung function

Several epidemiological studies have found positive associations between intakes of carotenoid-containing fruits and vegetables and lung function (32,33). Results from the NHANES III survey (32) showed that the higher the intake of foods with antioxidant activity the better the lung function in smokers. In another study, high tomato intake was associated with higher lung function as measured by forced expiratory volume in 1 s (FEV1) (33). In other studies, but not all studies, high plasma beta-carotene levels were associated with higher FEV1 and forced vital capacity. These associations were not consistently seen for vitamin E (31).

Beta-carotene: an example of no good deed goes unpunished

As mentioned above, several epidemiological studies have found that there is an inverse relationship between serum beta-carotene levels and lung function. One report showed an increase in lung function in smokers consuming carotenoid-rich diets (33). Based upon the associations reported, it is hypothesized that beta-carotene supplementation in smokers in the ATBC and CARET trials caused an increase in lung function. It is possible that even a modest increase in lung function over the 5 or more years of supplementation provided a greater exposure to tobacco carcinogens and other oxidative compounds. This hypothesis would also presume that there would be a dose:response relationship resulting in a greater increase in lung cancer in those who were the heaviest smokers and took beta-carotene compared to those who smoked the least or were former smokers. In fact, these are the findings from the ATBC study. There is even a small indication that the former smokers had a lower risk of lung cancer if they were in the beta-carotene group (56).

It is also possible that beta-carotene supplementation enhanced the depressed immune responses normally seen in smokers, and consequently reduced the number of days that the smokers suffered from upper respiratory tract infections (URT). As an antioxidant, beta-carotene could also reduce the ongoing low-level inflammatory responses seen in smokers' lungs. The hypothesis is that the supplemented group would have more days of exposure to smoke than the smokers who got URT infections, again increasing the potential for increased carcinogen burden. Unfortunately, there are no data currently available on the sick days experienced by smokers in the two chemoprevention studies. It seems possible to develop animal models to assess the validity of these hypotheses. Certainly, the ongoing clinical trials that are still administering beta-carotene may provide further clarification.

Benefits seen with beta-carotene supplementation

As indicated in the functions section, beta-carotene is the major source of vitamin A for the vast majority of the world's population. Beta-carotene is unique in that it is the only carotenoid out of the 500 carotenoids identified that can theoretically form two molecules of retinol; less than a dozen other carotenoids can form at most one molecule of retinol (vitamin A) (3). Beta-carotene supplements have been provided in clinical studies to thousands of study participants with no indication of harm and indications of benefit. With regards to chemoprevention specifically, Correa et al. (48) showed that beta-carotene supplementation reduced the progression of precancerous lesions of gastric cancer. It is of great importance to clearly determine the actions of beta-carotene as these may provide a rationale for the perplexing data on the increased risk of lung cancer in smokers who used beta-carotene supplements in some studies and not in others. Beta-carotene supplementation may be of value to individuals exposed to sunlight (or other sources of UV) as a protector of immune responses (21,22). More research is required to determine the full value of beta-carotene for immune functions and the dose levels for efficacy are not completely known.

Ringer et al. (36) showed a decade ago that beta-carotene supplementation over a broad range of supplemental intakes (15–300 mg/d for one month) resulted in a significant increase in HDL, an important indicator of cardiovascular health. The associative data from NHANES III suggests that the highest serum levels are associated with a lowered risk of angina (58).

On-going studies

The Women's Health Initiative is the largest observation/intervention study ever undertaken globally. Over 150,000 postmenopausal women have been enrolled since 1993. Baseline information on their use of supplements indicates that 44% take a supplement that contains beta-carotene and 5% use a single supplement containing only beta-carotene. Antioxidant use has increased with age, in white or Asian women and in former smokers. Use of antioxidants was least prevalent in smokers (59).

Probably the largest intervention study in the US that still provides beta-carotene supplements is the Physician's Health Study II. About 15,000 male physicians over 55 y of age are enrolled in this 5-y, placebo-controlled, double blind study. Beta-carotene supplementation is 50 mg every other day.

Outcomes include all cancers, prostate cancer, new myocardial infarcts, nonfatal stroke, all CVD deaths, cataracts and age-related macular degeneration (60). Although there may not be sufficient smokers enrolled to determine an effect of beta-carotene supplementation on lung cancer, it would certainly be of interest to examine lung function in this cohort.

The Olson legacy

Dr. James Allen Olson devoted his scientific career to understanding the functions and actions of vitamin A and beta-carotene. We owe it to him to determine the cause(s) of the increased lung cancers in the smokers who were supplemented with beta-carotene because Jim was a strong advocate of optimizing human vitamin A status. He had worked in and visited numerous developing countries where the populations rely on food and supplemental sources of beta-carotene as the source of vitamin A. Many studies reviewed here and elsewhere document the beneficial actions of beta-carotene on health parameters in different population groups. Moreover, the epidemiological data continue to find beneficial associations between high intakes of dietary carotenoids and health outcomes. It is critical that we elucidate the mechanism(s) of action of beta-carotene as this will enable us to understand the findings from studies with perplexing, unexpected results.

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