BETA-CAROTENE AND HUMAN HEALTH : A REVIEW OF CURRENT RESEARCH

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ABSTRACT

Beta-carotene research has progressed rapidly in the past few years. New techniques have allowed accurate measurements of beta-carotene concentrations in plasma, tissues, and diet. New animal models, stable isotope techniques, and human depletion and repletion experiments are leading an explosion in the information about beta-carotene utilization in humans. These advances are leading to a better understanding of the possibilities and limitations of the influence of beta-carotene on human health. This review summarizes important new developments in beta-carotene research, and provides an interpretation of how these results reflect the impact of beta-carotene and human health.

Key Words: Beta-Carotene, Human, Health, Antioxidant, Gap-Junction, Immunology.

INTRODUCTION

Beta-carotene is the major source of vitamin A for most of the people in the world.(1-4) Vitamin A is an essential vitamin: necessary for normal development, growth, and eyesight.(1-5) Thus, dietary beta-carotene is essential for most people. But what about the millions of people who obtain enough vitamin A from meat, milk, or other animal products? Is beta-carotene essential, or at least very useful, for people who eat enough preformed vitamin A?

A few years ago, most scientists and physicians would have said yes. In fact, some appeared to believe that beta-carotene was a wonder drug. Beta-carotene was described as an antioxidant that protected against cancer,(6-8) heart disease,(9-11) macular degeneration (12,13) and ageing.(14) It was non-toxic.(15) Several large, well-funded studies were set up to give more beta-carotene to healthy well-fed adults.(16-19) A research project was even conducted which gave beta-carotene
supplements to healthy male physicians living in the United States of America--a group not previously noted to be at-risk for any nutritional deficiency--in the hope that these supplements would delay the onset of cancer or heart disease. (19)

The growth in carotenoid research was explosive. Useful new methods were developed to measure serum and tissue carotenoid concentrations, (20,21) food composition data, (22,23) and dietary intakes. (24) Essential new information was collected about usual dietary intakes (25) and serum concentrations in healthy well-fed individuals, (26) as well as about the mechanisms of beta-carotene action in the antioxidant, (27,28) cell to cell communication, (29,30) and immunology. (31-32) New and better animal models, (33) and new stable isotope methods (34) provided the hope of measuring and understanding crucial aspects of human beta-carotene metabolism for the first time.

However, times have changed. (35,36) Unfortunately, the potential benefits of beta-carotene--or at least beta-carotene supplementation to healthy well-nourished adults--appear to have been over-rated. The results of most of the beta-carotene supplementation trials have been disappointing. Two even suggest that long-term smokers may be harmed by beta-carotene supplements. (16-18) Carotenoids other than beta-carotene are believed to be more relevant to macular degeneration and eye disease. (37) Some investigators suggest that the epidemiological results that appeared to show such strong evidence for beta-carotene are actually related to other carotenoids, (38) other phytochemicals, or even to differences in lifestyle. Studies even suggest that beta-carotene from vegetables is not very useful for preventing vitamin A deficiency. (39,40)

Is beta-carotene a failed wonder drug whose time has come--and gone? Or are these recent set-backs useful corrections in an overly optimistic scenario? What does beta-carotene do? How important is beta-carotene to human health, in general? And how important is it to certain specific populations? How much more money, time, and effort should we spend on beta-carotene studies? And where and how should we spend them? And what has really been accomplished in the past few years?

**What has been accomplished: new methods for beta-carotene research**

Recent progress in carotenoid methodology has been exciting. New, significantly improved methods have been developed to measure carotenoid concentrations in blood. The National Institute of Standards (NIST, USA) led a series of studies to systematically optimize high-performance liquid chromatography methods to measure the six most common carotenoids in human blood (beta-carotene, alpha-carotene, lutein, zeaxanthin, cryptoxanthin, and lycopene, (20,21) New column materials have been manufactured to provide greater specificity and selectivity for the less common carotenoids and their isomers. (41) Potentially of even greater importance, NIST has sponsored a series of round-robin analysis workshops to standardize methods and to compare results from different laboratories. (1) Reference data for the six major carotenoids in human plasma was collected for non-institutionalized population of the United States (26) More data on carotenoid concentrations in serum in other populations is available, though sparse. Representative data is shown in Table 1. This data is consistent. Most studies in the US and Europe show that people have

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# Table 1

**Representative Estimates of Serum or Plasma Concentrations of Beta-Carotene**

<table>
<thead>
<tr>
<th>n</th>
<th>Sex Characteristics</th>
<th>Concentration (μmol/L)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>22949</td>
<td>M/F (USA) 4 + yrs (median)</td>
<td>0.34</td>
<td>Centers for Disease Prevention*</td>
</tr>
<tr>
<td></td>
<td>(5th %)</td>
<td>0.27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(95th %)</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Other population studies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22071</td>
<td>M physicians (USA) 40-84 yrs</td>
<td>0.56</td>
<td>(19)</td>
</tr>
<tr>
<td>29133</td>
<td>M smokers (Finland) 50-69 yrs</td>
<td>0.39</td>
<td>(16)</td>
</tr>
<tr>
<td>72</td>
<td>M/F nonsmoker (USA) 18-65 yrs</td>
<td>0.41</td>
<td>Burri (Communication)</td>
</tr>
<tr>
<td>180</td>
<td>M (USA)</td>
<td>0.21</td>
<td>(42)</td>
</tr>
<tr>
<td>220</td>
<td>/F</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>1196</td>
<td>M (Japan)</td>
<td>0.35</td>
<td>(43)</td>
</tr>
<tr>
<td>618</td>
<td>F</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>M (Europe)</td>
<td>0.32</td>
<td>(44)</td>
</tr>
<tr>
<td>527</td>
<td>M (France)</td>
<td>0.56</td>
<td>(45)</td>
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<tr>
<td>353</td>
<td>F 23-53 yrs</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>330</td>
<td>M/ (USA)</td>
<td>0.26</td>
<td>(46)</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>166</td>
<td>M (Italy)</td>
<td>0.32</td>
<td>(47)</td>
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<tr>
<td>186</td>
<td>F</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>55</td>
<td>M/ (USA)</td>
<td>0.29</td>
<td>(48)</td>
</tr>
<tr>
<td>55</td>
<td>F</td>
<td>0.42</td>
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</tr>
</tbody>
</table>

* Source = Centers for Disease Control and Prevention/National Center for Health Statistics, 1996. Unpublished data presented at EB'96, April 17, 1996 in Washington, DC. Stratified multistage national probability sample for United States of America non-institutionalized population aged 4 yrs and older, with over sampling of children under 6 years, adults over 59 years, black- and Mexican-Americans. Measured by reversed-phase HPLC.

Approximately 0.2 to 0.6 μmol/L. Smokers tend to have lower concentrations than non-smokers. Women tend to have higher concentrations than men. People known to eat recommended amounts of fruits and vegetables have higher serum beta-carotene concentrations, of about 0.6 to 1.5 μmol/L.
TABLE 2

ESTIMATES OF DIETARY INTAKES OF BETA-CAROTENE

<table>
<thead>
<tr>
<th>n</th>
<th>Sex</th>
<th>Race</th>
<th>Location</th>
<th>Intake (µg/day)</th>
<th>References</th>
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<tr>
<td></td>
<td></td>
<td>Location</td>
<td></td>
<td>mean</td>
<td></td>
</tr>
<tr>
<td>14801</td>
<td>All</td>
<td>National USA</td>
<td>2712</td>
<td>(25)*</td>
<td></td>
</tr>
<tr>
<td>7322</td>
<td>m</td>
<td>Aged 2 mos or older</td>
<td>1038 (median)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7479</td>
<td>f</td>
<td>All</td>
<td>2922</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>1134 (median)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>2514</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>All</td>
<td>960 (median)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1100</td>
<td>f</td>
<td>All (food frequency.)</td>
<td>1950</td>
<td>(49)</td>
<td></td>
</tr>
<tr>
<td>29133</td>
<td>m</td>
<td>Caucasian</td>
<td>2086</td>
<td>(16)</td>
<td></td>
</tr>
<tr>
<td>1556</td>
<td>m</td>
<td>Caucasian</td>
<td>1543 (SD)</td>
<td>(50)</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>both</td>
<td>San Francisco CA</td>
<td>3424</td>
<td>Burri</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>both</td>
<td>Beaver Dam WI USA</td>
<td>1566 (SD)</td>
<td>(42)</td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>m</td>
<td>New Caledonia</td>
<td>8181</td>
<td>(51)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>50-65 yr</td>
<td>7775</td>
<td></td>
<td></td>
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<tr>
<td>102</td>
<td>m</td>
<td>Tahitian</td>
<td>2559</td>
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<td></td>
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<td></td>
<td>f</td>
<td>3612</td>
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</tr>
<tr>
<td>98</td>
<td>m</td>
<td>Cook Islands</td>
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<td></td>
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<tr>
<td></td>
<td>f</td>
<td>13111</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>f</td>
<td>14548</td>
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</tr>
<tr>
<td>37</td>
<td>m</td>
<td>Hawaiian</td>
<td>7281</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>f</td>
<td>6475</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64</td>
<td>m</td>
<td>Hawaiian</td>
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<tr>
<td></td>
<td>f</td>
<td>Caucasian</td>
<td>6952</td>
<td></td>
<td></td>
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<tr>
<td>114</td>
<td>m</td>
<td>Hawaiian</td>
<td>6841</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>Japanese</td>
<td>8677</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>m</td>
<td>Hawaiian</td>
<td>7073</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>f</td>
<td>Chinese</td>
<td>7345</td>
<td></td>
<td></td>
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</tbody>
</table>

*Data from 24 hr recall. Distribution of recalls by day was not uniform, but ranged from a low of 8% of reports for Sunday to a high of 26% of reports from Friday, due to operational procedures allowing for more data collections on Saturday to improve response rates. Reports were collected in English and/or Spanish.
Dietary information was improved by a) measuring the carotenoid content of 120 fruits and vegetables (and about 2500 common foods);(20,21) b) developing food frequency questionnaires for carotenoid intake;(22) and c) collecting reference data on the dietary intake of carotenoids (for the non-institutionalized population of the United States of America, (23) and other populations (Table 2). Mean intakes in the USA and Europe tend to be 2 to 4 mg per day, with median intakes much lower. Some populations, who routinely eat recommended amounts of fruits and vegetables, have intakes of 10 mg per day or more. (Table 2).

New information about carotenoid metabolism is available, too. First, several new and better animal models have been discovered, which might mimic the metabolism of beta-carotene in humans. None of these animal models are entirely similar to humans, but they do absorb beta-carotene intact. These animal models include the ferret,(52) and the preruminant calf.(33,53) Second, stable isotope methods have been developed to estimate the body pool concentrations of both vitamin A and beta-carotene. These isotopes allow investigation of the metabolism, conversion, and utilization of both vitamin A (54) and beta-carotene.(34,55-58)

Finally, there is a wealth of new information on the potential mechanisms of action of beta-carotene in the human body.(27-32) We are fortunate to be in the process of a revolution in carotenoid methodology. At the time of this writing, much of the information about the role of beta-carotene in human health is fragmentary and contradictory. However, these new methods will allow us to answer some important questions soon.

FUNCTIONS AND ACTIONS OF BETA-CAROTENE

Proposed functions and actions of beta-carotene are listed in Table 3. Some of these functions, such as conversion of beta-carotene to vitamin A, have been established beyond a reasonable doubt. Others, such as possible interactions between beta-carotene and skin health or fertility are based on the interpretations of a few studies and are much more speculative.

Beta-carotene as a source of vitamin A

Beta-carotene plays a crucial role in human health, whenever preformed vitamin A is in short supply. It is the major, most active precursor of vitamin A. Vitamin A is essential for human health. It is so critical that it was the first vitamin discovered.(59) Vitamin A deficiency is the leading cause of childhood blindness in the world, affecting approximately 250,000 children a year.(60,61) It is also a leading cause of death. Vitamin A supplementation alone results in a decrease in infant mortality of as much as 60%.(60,61) Meta-analysis suggests an overall decrease in death rate of about 30% from vitamin A supplementation of at risk children alone.(61) However, it is likely that the ranges seen for the efficacy of vitamin A are a more accurate index of the influence of vitamin A supplementation on these populations. Some populations will respond to vitamin A supplementation with a substantial increase in health, while other populations with different nutritional needs will be
TABLE 3

BIOCHEMICAL FUNCTIONS OF BETA-CAROTENE IN HUMANS

1. Vitamin A formation (34,55-58,63-71)
   Beta-carotene is a major source of vitamin A for humans. This interaction is of significant, proven importance to human health.

2. Lipoxygenase (72)
   Co-oxygenation of fatty acids and carotenoids. Lipoxygenases are involved in arachadonic acid metabolism, which is inhibited by beta-carotene. Unknown impact on human health.

3. 3-Hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (EC 1.1.1.34; 73)
   Converts HMG CoA to mevalonate, the rate-limiting enzyme in biosynthesis of cholesterol and other non-steroidal isoprenoids. Carotenoids modify by post-transcriptional regulation. Beta-carotene may exert chemopreventive effects by inhibiting HMG-CoA reductase. Unknown importance to human health.

4. Connexin 43 (29,30,74,75)
   Beta-carotene stimulates connexin 43 to increase gap junction formation; thereby increasing cell to cell communication. This reaction is well-established in vitro, but of unknown importance to human health.

5. Antioxidant/prooxidant (27,28,76-79; but see 80-82)
   Established health benefit to some human and animal populations; of unknown importance to the general human population.

6. Immunological response (31-32,83-88)
   Established importance to animal health; good evidence for benefit to some human populations, but of unknown importance to the general population.

7. Hormone regulation and fertility (89-93, but see 94)
   Numerous reports of beta-carotene supplementation causing fertility improvements in herbivores, but a substantial number of studies have also found no effect. A few reports that carotenoids influence menstrual cycle and thyroid hormones in humans. Unknown importance for human health

less influenced. Although most supplementation programs have used vitamin A as the supplement, the dosage of vitamin A supplied can have toxic or teratogenic effects in some people. (1-5) Therefore, several ongoing supplementation programs are attempting to use either
purified beta-carotene or fruits and vegetables high in carotenoid content instead of purified vitamin A. (39, 62) Beta-carotene capsules have been able to provide the same protection as vitamin A capsules, (2, 3, 39, 62) with fewer toxic side effects. (15)

The conversion of beta-carotene to vitamin A has been studied extensively, (34, 55-58, 63-71) but much of its metabolism remains in doubt. The main difficulty has been that humans do not have typical mechanisms of beta-carotene metabolism and conversion. Most animals metabolize beta-carotene differently than humans do; they do not absorb beta-carotene intact, and low-density lipoprotein is not their main means of beta-carotene transport. (71) Although the importance of some of these differences are unknown, it is clear that research on metabolism done on these common laboratory animals may not be relevant to studies of human metabolism. A second difficulty in studying carotenoid metabolism is that a crucial enzyme involved in carotenoid cleavage (1515-beta-carotene dioxygenase; 65, 66, 70, 71) is unstable and difficult to purify. Other enzymes thought to be involved are nonspecific (alcohol dehydrogenase; 69, 70). So, scientists are still debating whether beta-carotene is cleaved centrally, eccentrically, or by both mechanisms.

There is no doubt, however, that under normal conditions beta-carotene is converted to vitamin A inefficiently. One molecule of beta-carotene, cleaved centrally, should form two molecules of vitamin A (in the form of retinal). One molecule of beta-carotene, cleaved eccentrically, should form one molecule of retinal. This does not seem to happen in vivo. Healthy, vitamin A depleted individuals fed beta-carotene in easily digestible capsules appear to derive one molecule of vitamin A from approximately two molecules of beta-carotene. (67, 68, 95-97) Beta-carotene conversion may depend to some extent on the body stores of vitamin A, (67, 68, 95-99) so depleted individuals typically obtain less vitamin A than depleted individuals do. (34, 97-98)

Worse, beta-carotene conversion to vitamin A appears to be poorest under conditions where it is most necessary. It appears to require fat in the diet. (100, 101) It appears to be influenced unfavorably by infections, fevers, and parasite infestation. (1-5, 100-102) It is less available in vegetables, especially abundant green leafy vegetables, than oils, fruits, or supplements. (39, 40, 97, 102) Thus, it is converted with poor efficiencies by the malnourished people who need it most. In fact, recent field studies have suggested that although beta-carotene supplements can be used to treat vitamin A deficiency effectively (39, 40, 62, 102-104), beta-carotene from green leafy vegetables is a poor source of vitamin A for malnourished people. (39, 100-102) Thus, feeding programs meant to enhance vitamin A status through education, farm production and consumption of green leafy vegetables may not be successful. Alternative strategies, such as supplementation with vitamin A or beta-carotene capsules, may need to be continued until a better overall diet for the population is attained.

Other functions of beta-carotene

Does marginal beta-carotene status cause subtle health problems, even when vitamin A is provided in abundant supply? Some evidence suggests that it does. First, there is an overwhelming consistency in epidemiological studies which point to associations between beta-
carotene consumption or serum concentrations and decreased rates of cancer and heart disease. These epidemiological studies will only be mentioned in this review, because they have been summarized in other recent reviews (6-14, 105-111). Suffice it to say that the evidence is substantial. However, epidemiological studies can only show association; they do not, and cannot show cause and effect. Several mechanisms have been proposed to account for the observed correlations between increased carotenoid consumption or serum concentrations and decreased risk of degenerative diseases. Beta-carotene could function as a redox reagent, an immunological regulator, or by increasing cell to cell communications. There is substantial evidence that beta-carotene is capable of influencing all of these mechanisms, at least in vitro. There is less evidence that beta-carotene functions in vivo by these mechanisms, or that its influence is substantial or significant to human health.

Beta-carotene is an uncommon type of biological redox reagent—one that reduces oxidation products best at low partial pressures of oxygen (less than 150 torr; 27). These low pressures are relevant to many physiological tissues. Beta-carotene is a physiological modulator (111) with the capacity to act as a singlet oxygen quencher (1121). It also can function as an antioxidant or as a reductant, depending on reaction conditions. In vivo, it appears to function most often as an antioxidant and singlet oxygen quencher. As such, it decreases lipoprotein and DNA oxidation. (27, 28, 76-79) Theoretically, these oxidations are detrimental to human health. Lipoprotein oxidation is thought to help initiate atherogenesis (109, 110, 113) DNA and lipoprotein oxidation are also thought to be related to cancer and other degenerative diseases (105-107)

Gap junctions link cells within an organism to form a communicating syncytium, allowing small molecules to pass through cells. Beta-carotene and vitamin A can both stimulate gap-junction formation between cells (29, 30, 74, 75). This function can also be carried out by some other carotenoids, but it is not caused by interconversion of beta-carotene to vitamin A or to these other carotenoids. It is an intrinsic function of beta-carotene itself. The mechanism appears to be a stimulation of connexin 43, a gene coding for a transmembrane protein. Six of these transmembrane proteins form a hexameric array surrounding a water-filled core. Two of these arrays form the structural unit of a gap junction. Beta-carotene, in physiological concentrations (10^4 M range), increases the gap junction, allowing for more cell-cell communication. One mechanism by which carotenoids might prevent cancer is through these gap junctions. Beta-carotene may maintain carcinogen-initiated cells in junctional communication with nontransformed cells, thereby preventing their transformation. This is correlated to ability to inhibit neoplastic transformations. It is unfortunate that connexin 43 activities and gap-junction formations have seldom been studied in humans. Thus, their relative importance to human health is unknown at this time.

Beta-carotene acts on the immunological system (31, 32, 83-88). The influence of beta-carotene depletion on immunology was discovered decades ago. (84, 85). These early studies found that feeding beta-carotene to depleted animals decreased the number and severity of respiratory infections, and appeared to help overcome infections. (84, 85) Beta-carotene has been implicated in T and B cell proliferation, T helper cell increases, and natural killer cell cytotoxicity, as well as decreased mutagenesis (decreased chromosomal abnormalities and breaks, micronuclei, and
urinary mutagens (31, 83, 86-88). However, the details of these interactions are still sketchy. Interestingly, optimal immunological defense may require more beta-carotene than antioxidant defense. Many immunological changes have been induced by relatively high carotenoid intakes, of over 15 mg per day. (86, 87, 114)

Many reports suggest that beta-carotene supplementation increases fertility in herbivores fed hay (which typically contains only low concentrations of beta-carotene). (89, 92, 93) The influence of beta-carotene supplementation on litter size has been reported for cattle, swine, and rabbits. Furthermore, the corpus luteum of these herbivores sequesters beta-carotene. (Beta-carotene gives is responsible for the coloration of the corpus luteum in these animals, for which the gland was named). These results have been contradicted by other studies finding little or no effect, of beta-carotene on fertility. (94) However, herbivore fertility is a complex process studied under difficult field conditions at different locations with different basal carotenoid intakes. It is not surprising that the evidence is contradictory. Evidence for the influence of beta-carotene on indices of human fertility are suggestive but minimal, resting on a few small controlled human studies. (91)

Beta-carotene may influence thyroid hormone status. (90, 115) Numerous case reports have linked anorexia nervosa, thyroid status, and unusually high serum concentrations of beta-carotene. (115) These case reports are supported by evidence from two small controlled beta-carotene depletion studies. (90) The mechanism for relating beta-carotene to thyroid hormone status is unknown.

How important are these functions to human health? If beta-carotene is not needed for vitamin A formation, is it needed for anything? We can answer this question by examining the influence of carotenoid depletion on human health.

DEPLETION STUDIES

Studies of beta-carotene depletion in humans are rare. Furthermore, they are complicated by other nutrient depletions. In fact, a simple beta-carotene depletion study has never been done. Depleting beta-carotene also means—at the very least—depleting alpha-carotene, cryptoxanthin, lutein, zeaxanthin, lycopene, and a host of carotenoids and flavonoids typically present in minute concentrations in human tissues. There are several reasons for this. First, there are very few known foods that are good sources of carotenoids that do not contain beta-carotene. (Of course, most foods have not been analyzed). Second, most common carotenoids and flavonoids are not approved for use as human supplements. At the present time, only beta-carotene and canthaxanthin, a carotenoid that does not form vitamin A, are approved for human use. Therefore a diet deficient in a single carotenoid cannot usually be supplemented with just this carotenoid. Thus, it is very difficult to invent a diet (at this time) that is depleted of beta-carotene, but not of at least some other carotenoids.

There are a few older, relatively long-term studies that are of interest to carotenoid
researchers. These older studies were not planned as carotenoid depletion studies; they were intended to measure the effects of vitamin A or vitamin C depletion. However, subjects who participated in these studies were fed low carotenoid diets for prolonged periods of time. Thus, they provide information on the limits of the significance of carotenoid depletion to human health. Older studies include the Scorby Research Institute (Sheffield, UK; 95,116) studies of vitamin A and vitamin C depletion in healthy young adults; and the US Army/Iowa State University studies of vitamin A and vitamin C deficiency in adult males. (96,117-120) Newer studies are the free-living study of carotenoid depletion conducted in healthy young men at the University of Illinois at Chicago, (121) and two metabolic unit studies of carotenoid depletion in healthy adult young women conducted at the Western Human Nutrition Research Center in San Francisco, California. (79,88,90,91,98,114,122-125)

Both new and old depletion studies are limited because they have all been conducted in small numbers of relatively similar people (healthy, otherwise well-nourished young or middle-aged adults). They provide almost no information on the effects of beta-carotene depletion and requirements of infants, children, adolescents, and the elderly. They provide little information about psychological status or stress, and none on gap junctions.

Older mixed carotenoid/vitamin depletion studies

Two studies involving beta-carotene depletion (associated with either vitamin A or vitamin C depletion diets) were conducted in Sheffield England during World War II. The first, a vitamin A depletion study, ran for 6 ½ to 25 months during 1942 to 1944. (95) The second, vitamin C depletion study, was conducted for 8 ½ to 22 months in 1944 and 1945. (116) Both were free-living studies, but had more control than is typical for these types of studies because of wartime food rationing and restrictions on movement. Twenty conscientious objectors (17 men and 3 women) participated in the vitamin A depletion study, and twenty (19 men and 1 woman) in the vitamin C depletion study. Half of the subjects participated in both studies. Four subjects were in both the vitamin A and the vitamin C depletion groups. No major deviation from normal developed during the vitamin A depletion study, except that 3 men developed dark adaptation problems on the low vitamin A diet. Scurvy developed in at least 8 of the 10 depleted subjects given the vitamin C deficient diet. All major, defined symptoms of deficiency cleared up with either vitamin A or vitamin C. However, four subjects who participated in both studies developed serious or life-threatening illnesses. (The illnesses were tuberculosis of the spine, probable heart attacks, malaria, impetigo and migraine).

U.S. Army and Iowa State University scientists conducted three controlled nutrition studies that contained minimal amounts of beta-carotene during the 1960's. No subjects overlapped in these studies. Two were vitamin C depletion studies, (117,119,120) the last was a vitamin A depletion study. (96,117,118) All participants were healthy middle-aged male prisoners. Nine men participated in the vitamin C deficiency studies, and eight men in the vitamin A depletion study. They ate several diets during these studies; a controlled formula diet that was devoid of vitamin C and carotenoids for most of the experiments; then several solid diets based on casein; then a restricted natural foods diet. The first vitamin C deficiency study ran for 200 days; 113 days depletion and 97 days repletion. The second had 84-92 days of depletion. The details
of the vitamin A depletion study plan, and some of its findings, have never been published in full. (Because guidelines for human nutrition studies became more strict between the time the study was conducted, and the time when attempts were made to get it published). All subjects on the vitamin C depletion diets developed classic signs of scurvy. Most developed problems with dark adaptation (an index of mild vitamin A deficiency) on the vitamin A depletion diet. Again, no significant identifiable symptoms occurred that were not ameliorated by vitamin A or vitamin C repletion.

The bottom line of these mixed depletion studies, and other older studies conducted for shorter terms, is that no specific deficiency symptoms could be related specifically and solely to carotenoid depletion. Either vitamin A alone, or vitamin C alone, was sufficient to ameliorate these severe, identifiable deficiency symptoms. However, only acute symptoms were measured. These depletion studies could not measure increased risks of cancer and heart disease in their small numbers of subjects. They did not measure changes in antioxidant, gap-junction, DNA damage, hormonal status, or fertility. Immunological tests were few, and not sensitive.

Modern carotenoid depletion studies

The first modern carotenoid depletion study was a free-living study of 15 healthy young adult men, who were fed a liquid formula diet containing no carotenoids and a high P/S ratio (to stimulate oxidative damage) for 2 weeks. They were then fed the liquid diet supplemented with either 15 or 120 mg per day of beta-carotene for four weeks. Several indices of oxidative damage and immunological activity were tested. A significant change occurred for thiobarbituric acid reactive substances (TBARS), a widely used but non-specific test of oxidative damage.

The other two carotenoid depletion experiments were carried out on a metabolic unit; with healthy young adult women. In the first study 9 women were fed a low carotenoid diet of natural foods for 68 days, followed by the same diet supplemented with 15 mg per day of beta-carotene for 16 days; then the beta-carotene supplement plus a mixed carotenoid supplement (containing beta-carotene, alpha-carotene, vitamin E, and some lutein, and lycopene) for the final 12 days. The second study was conducted for 120 days. This was a placebo-controlled double-blind study. Twelve women started the study, nine completed. Four volunteers were in the carotenoid depletion group, while five were in a control group that were fed the same low carotenoid diet supplemented with 0.5 mg beta-carotene per day for 60 days. Then both groups were fed the 0.5 mg beta-carotene supplement for the next 40 days, then all were supplemented with 15 mg per day beta-carotene plus 3 mixed carotenoid capsules per day for the last 20 days. Thus, the second study had a control group, but at the cost of having small numbers of subjects in each group.

The first metabolic unit study gave the clearest results. Several indices of oxidative damage (TBARS, oxidized LDL products, ratio of oxidized to reduced glutathione) increased during carotenoid depletion, then reverted to baseline upon repletion. Conjugated dienes, measured in slowly turning over red blood cells, had the same pattern, except with a time lag. Superoxide dismutase, an enzyme involved in oxidative defense) decreased during
depletion and reverted to baseline at repletion. (79) Fewer test results are available at this time from the second study, but fewer of the tests that are available reached statistical significance during the second study (probably because of the small size of the depleted group). However, malondialdehyde (an improved test equivalent to TBARS; 124) and oxidized LDL products (125) increased in the depleted group and reverted to baseline with repletion. Malondialdehyde concentrations in the depleted group were different from the control group during depletion, but not after supplementation with 0.5 mg per day beta-carotene. (124) Thus, there is good, consistent evidence that carotenoid depletion increases oxidative damage. There was no evidence that carotenoid supplementation decreases oxidative damage below baseline levels in these subjects.

The metabolic unit studies also showed numerous indications that carotenoid depletion influences hormone status. In the first study, several thyroid hormones (thyroid stimulating hormone, total thyroxine, free thyroxine) increased during depletion. (90) The increase occurred with approximately a 4 week lag after carotenoid depletion was initiated, and began to normalize during repletion. In the second study, only total thyroxine concentrations changed significantly in the depleted group; thyroid stimulating hormone and free thyroxine showed similar but non-significant trends. Menstrual cycles and luteinizing hormone peak activities were disrupted in both studies. (91) There was no difference between the control and depleted group in the second study, both groups were disrupted according to baseline and post-study records. Acne appeared in seven women during carotenoid depletion in the first study, and was inversely correlated to serum beta-carotene concentrations during depletion and to the body pool of vitamin A. (123) This did not happen on the second study. (However, subjects were excluded from the second study if they had current acne, or a history of skin problems). Thus, the group was selected so that the appearance of acne would have been strong evidence for a role of beta-carotene in skin health, but the absence of its appearance was not good evidence for a lack of effect.

The first study did not show changes in immunology, (88) but the second did. (114) The second study used tests conducted in whole blood, instead of washed cells, and had much less variability. Immunological changes in the second study were the only parameters measured that indicated an effect of beta-carotene supplementation. All other tests were reverted to baseline values after beta-carotene supplementation.

The modern carotenoid depletion studies provide consistent evidence that antioxidant status is influenced by carotenoid depletion and beta-carotene repletion. How important or long-term this interaction is not known. It is also not known whether this decreased antioxidant defense, if sustained, would be sufficient to influence the rates of heart disease and cancer in humans. Furthermore, although serum vitamin C and E concentrations were increased in the carotenoid depletion studies carried out on the metabolic unit, we do not know whether higher dietary intakes of other antioxidants could ameliorate the effects of carotenoid depletion. Depletion studies on the metabolic unit suggest that the carotenoid intake needed to maintain baseline antioxidant status in healthy adults is low, possibly about 600 μg per day (approximately 1/4 the median intake of adults in the United States). Thus, our metabolic unit studies suggest that deleterious changes in antioxidant status caused by low carotenoid dietary intakes would not be common in the US adult population.
The carotenoid depletion studies carried out on the metabolic unit also suggest changes in thyroid and menstrual cycle hormone concentrations. Again, we do not know whether these changes are really significant, or would be prolonged. In fact, many women eating poor diets low in carotenoids do have children, so we speculate that these menstrual hormone changes caused by carotenoid depletion would eventually normalize.

**BETA-CAROTENE SUPPLEMENTATION**

Results of supplementation studies are relatively difficult to interpret. There is little information at this time about the typical dose-response curve of beta-carotene to indices of oxidative damage in healthy adult humans, and less of the usual range of response. There is essentially no information on the dose-response curve of beta-carotene on immunological, cell to cell.

### TABLE 4

<table>
<thead>
<tr>
<th>Dose of β-carotene (mg/day)</th>
<th>Duration (days)</th>
<th>n</th>
<th>sex</th>
<th>Baseline β-carotene (µmol/L)</th>
<th>End β-carotene (µmol/L)</th>
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<tr>
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Table 4 (continued)

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<th>Dose of β-carotene (mg/day)</th>
<th>Duration (days)</th>
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<th>Sex</th>
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<td>6</td>
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<td>28</td>
<td>8</td>
<td>m/f</td>
<td>0.47 (all 35) 1.2</td>
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<td>(127)</td>
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<tr>
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<td>28</td>
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<td>M/f</td>
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<td>4380 ~11036</td>
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<td>2.2</td>
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<td>(19)</td>
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<tr>
<td>30</td>
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<td>30</td>
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<td>5</td>
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<td>0.42</td>
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<td></td>
</tr>
<tr>
<td>30</td>
<td>70</td>
<td>8</td>
<td>F</td>
<td>0.49</td>
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</tr>
<tr>
<td>40 14 days</td>
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</tr>
<tr>
<td>20</td>
<td>84</td>
<td>98</td>
<td>M</td>
<td>0.31</td>
<td>5.0</td>
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<tr>
<td>50</td>
<td>2993 ~900</td>
<td>M/f</td>
<td>0.61</td>
<td>6.1</td>
<td></td>
<td>(135)</td>
</tr>
<tr>
<td>60</td>
<td>270</td>
<td>8</td>
<td>M/f</td>
<td>1.4</td>
<td>28^3</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>21</td>
<td>10</td>
<td>F</td>
<td>0.48</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>180-300 Life long Erythropoietic protoporphryia</td>
<td></td>
<td></td>
<td></td>
<td>133</td>
<td>M/f</td>
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</table>

^1Representative samples  ^2Placebo group  ^3in lipoproteins
cell communication, or hormonal status. There is essentially no information on dose-response, normal, and dangerously abnormal variations in response for people with diseases, or disease causing habits such as smoking. There is also little or no information on the appropriate duration of beta-carotene supplementation; or when supplementation should be started for greatest effect; or what types and what stages of heart disease and cancer might be treatable. Thus, current supplementation trials have tried a wide variety of dosages of beta-carotene, for various lengths of time, for a variety of peoples, with a variety of risk factors and diseases. In fact, little attention has been paid to the baseline beta-carotene concentrations of the populations studied. However, information on baseline beta-carotene concentrations and of the effects of beta-carotene supplementation on these concentrations is accumulating. This information is summarized in Table 4. Table 4 suggests that daily supplements of 6 (to 10?) mg of beta-carotene should increase serum beta-carotene concentrations in most individuals to the concentrations that have been associated with reduced risks of cancer and heart disease in epidemiological studies. These serum concentrations are also attainable from eating a well-rounded diet. Unfortunately, very few supplementation studies have been conducted with these relatively low levels of supplementation.

Results from beta-carotene supplementation trials have been variable. Some have suggested a benefit for beta-carotene supplementation, others have suggested neither benefit nor harm, and a few have suggested that beta-carotene supplementation has been harmful (at least to heavy smokers and alcohol consumers). This is not surprising, because few of the participants in supplementation trials have had low serum concentrations of beta-carotene. Most have average concentrations, while some participants have unusually high levels. Subjects with normal to high serum concentrations of beta-carotene are not likely to have significant responses, unless the amounts of beta-carotene needed by the body are typically much higher than the amounts provided by typical healthy diets.

**Treatment of putative precancerous states**

Several studies of the effects of beta-carotene supplementation have treated putative precancerous lesions such as the recurrence of oral leukoplakia and micronucleated cells in smokers or betel nut chewers. Oral leukoplakia and micronucleated cells are not cancer. Oral leukoplakia is the presence of irregular white patches of cells in the oral cavity that are not painful, nor associated with infections such as herpes. Micronucleated cells are just an increased percentage of cells with small nuclei. Neither essential precursors to cancer, nor does their presence invariably lead to cancer. Most of these lesions have benign outcomes—the risk of cancer is closer to 30 than to 100%. However, they are associated with higher than normal risks for relatively rapid progression of oral cancers.

Most of these trials have investigated the influence of short-term (less than 6 months) supplementation with moderate pharmacological doses of beta-carotene (30 to 90 mg per day). These amounts of beta-carotene are very difficult, or impossible, to obtain from a normal, healthy diet (Table 2). They are not associated with toxic symptoms. However, large minorities (15% or more) of participants report skin-yellowing, suggesting that much of the beta-carotene is not being metabolized by normal metabolic pathways. Most of these studies
have reported favorable outcomes. The average response rate appears to be 50 to 60% (44,140-143).

Studies measuring antioxidant status, immunology or adenomas have given more mixed results. Several have shown that beta-carotene supplementation was associated with a significant decrease in oxidative damage; (77,78,83,86,144,145) other equally well-designed studies showed no effect. (80,82,132,146) Of course, even if beta-carotene supplementation were associated with clear and consistent decreases in putative midpoints markers of cancer and disease, that would not be the same as showing an actual reduction in the incidence of cancer or heart disease. They are only midpoint indicators. Furthermore, there is no information on the duration of the effects of carotenoid supplementation or depletion. Evidence from depletion studies suggests that at least some of the deleterious effects of carotenoid depletion are not permanent.

**CANCER AND HEART DISEASE PHASE III TRIALS**

Results from four Phase III trials which used beta-carotene (usually coupled with other antioxidants) in attempts to prevent cancer and heart disease have been reported. Phase III trials were originally designed to test the effectiveness of a drug intervention. They work best for testing new drugs that are unavailable to the general public, because they compare outcomes in large numbers of people without the ability to restrict intakes in the untreated groups. In nutritional interventions it is generally not possible to find a group that has not, and will not, consume the nutrient being studied. Furthermore, it is difficult to restrict the dosages taken by the control group. Thus, nutritional intervention trials typically are restricted to comparing high, pharmacological intakes of a nutrient to a variable, high and low physiological intake of that same nutrient in the control group. They are by far the best method available for testing the outcome of consuming high, pharmacological intakes of a nutrient. However, they are likely to be ineffective in testing for responses that occur at low physiological intakes. (For example, a Phase III trial of the influence of vitamins A or C in the general population of the United States would almost certainly show no benefit of such supplementation. In fact, unless dosages were picked carefully, a vitamin A trial would probably show an unfavorable, toxic response in the general population. However, it is well established that these are both essential nutrients, in small doses).

These Phase III trials administered doses of beta-carotene to healthy middle-aged and elderly adults, in an attempt to decrease the incidence of cancer or heart disease. All were double-blind, placebo-controlled, randomized trials. Unfortunately, these trials only measured cancer, heart-disease, and death. They did not investigate or report on oxidative damage, immunological or precancerous midpoint markers. Thus, we know the final outcomes of the trials, but do not know the mechanisms or changes involved that resulted in these endpoints. These trials are summarized in Table 5.

Overall, the Phase III trials suggest that beta-carotene supplementation is of little or no value to the general population, with the possible exceptions of populations that are at risk for low nutrient intakes of antioxidant vitamins. (129,130) In the case of the ATBC and CARET
<table>
<thead>
<tr>
<th>Trial (References)</th>
<th>Description</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linxian (129-130)</td>
<td>29584, m/f, Oriental, Chinese, 40-69 years, low consumption of nutrients, region of highest stomach cancer incidence in China; 15 mg, plus 50 mg selenium and 30 mg a-tocopherol; 5.25 year duration</td>
<td>positive: 18% decrease in stomach and esophageal cancers; 8% decrease in overall mortality. (note: mixed supplement)</td>
</tr>
<tr>
<td>Physicians' Health USA, (19)</td>
<td>22071, m, &gt;90% Caucasian, 40-84 yrs, none, 25 mg, average 12 year duration</td>
<td>no effect on cancer, heart disease, or death rate. no obvious positive or negative trends. Skin yellowing in 17% of supplemented group</td>
</tr>
<tr>
<td>ATBC (Finnish) (16,17)</td>
<td>29133, m, Caucasian, Finland 50-69 yrs, heavy smokers (and drinkers*), 30 mg; average 5 ½ yrs</td>
<td>negative: 17% increase in lung cancer, 7% increase in overall mortality increased mortality in heavy drinkers, (personal communication, Albanes). Skin yellowing in 24% of supplemented group. No effect on coronary heart disease</td>
</tr>
<tr>
<td>CARET (18)</td>
<td>18158, m/f, &gt;90% Caucasian USA, 50-69 yrs, heavy smokers and/or asbestos exposed workers; 30 mg plus 25000 IU vitamin A, average 4 ½ yr</td>
<td>negative (not significant) trends toward increased cancer and death rates; trial ended early because of trends similar to ATBC results. Note: mixed supplement, dose of vitamin A occasionally toxic). Skin yellowing in 25%of supplemented group</td>
</tr>
</tbody>
</table>
trials, the average serum concentrations of beta-carotene were identical to the average concentration of beta-carotene in the general US population (compare Table 5 to Table 1). Initial serum beta-carotene concentrations were higher than average in the Physicians' Health Trial. Further trials may be useful in populations where carotenoid consumption and serum concentrations are well below average intakes of well fed populations.

However, great caution is essential. There is no evidence that suggests that even the subset of people with low initial beta-carotene concentrations benefited from the high-dosage supplementation provided by the ATBC, CARET, or Physician's Health trials (16-19). At this point, the only favorable outcome was reported in the Linxian trial; and that trial used a mixed antioxidant supplement. The favorable outcome may have resulted from a combination of selecting a population that had low beta-carotene consumption and status combined with administration of physiological dosages of beta-carotene. However, the benefit observed might also have been coincidental, or caused by another of the nutrients provided. In any case, we should obtain better understanding of the mechanisms of action of beta-carotene and its dose-response curves before attempting further supplementation trials. Further investigations into the toxicity of long-term supplementation with beta-carotene should also be done.

TOXICITY

It was previously believed that beta-carotene was not toxic (15,137,138). In fact, acute toxicity--with well-defined symptoms--is obviously rare. Carotodermia, a yellow discoloration of the skin, has been sporadically reported in the medical literature for decades (97,137,138). It is an easily recognized, visible physical symptom that can occur with over-consumption of carotenoid-rich foods (especially carrots, but also citrus fruits), carotenoid supplements. Carotodermia appears in some, but not all, people when they eat large amounts of beta-carotene. Typically, the amount consumed must be at least 15 mg of beta-carotene per day; as supplements or as fruits and vegetables. The percentage of people with carotodermia increases as the daily intake of carotene increases. Carotodermia appears in approximately one-quarter of people taking supplements of 30 mg per day, (15-19) and increases to well over half of the population taking extremely high pharmacological intakes (82,137,138). Most carotodermia is caused by high carotenoid intakes, but it can occur through other mechanisms. It appears frequently in anoerexia (115). It has also been described in a case report of a brain tumor (147). The etiology of these influences are unknown. This discoloration appears to be benign. Despite being a visible, easily investigated abnormality, no serious medical complaints have been associated with carotodermia; nor has it been associated with increased genotoxicity or birth defects (15,137,138). Studies feeding very high doses to rodents have shown few abnormalities. In fact, beta-carotene is approved for human consumption, and has been used as a food colorant and in medical treatments of skin conditions for over 25 years without reports of major side effects. Thus, there is substantial evidence that beta-carotene, even given in very high pharmacological doses, is not acutely toxic.

Almost nothing is known about the effects of chronic pharmacological supplementation on
BETA-CAROTENE AND HUMAN HEALTH

human health. These effects, if present, are ill-defined and difficult to recognize and differentiate. The Physician's Health study, possibly the longest nutrient supplementation trial of a large healthy population in history, showed no evidence of chronic toxicity. However, the ATBC trial and possibly the CARET trial suggest that prolonged pharmacological treatment with beta-carotene might be toxic in heavy smokers (especially if they are also heavy drinkers).

Thus, few studies suggest that beta-carotene supplementation will help or hurt most well-fed, healthy populations. Does beta-carotene supplementation benefit smaller, restricted populations of people with certain diseases? The answer to this question is yes.

BETA-CAROTENE AND DISEASE STATES

Beta-carotene supplementation has proven beneficial to people with erythropoietic protoporphyria (EPP). In fact, it has been an effective and standard medical treatment for this condition for decades. Beta-carotene is used to treat this disease to such an extent that the long term effects of beta-carotene supplementation in these individuals have never been studied, for the simple reason that there is no control group of people with EPP that is not treated with high doses of beta-carotene. Thus, beta-carotene is used in one of the only known beneficial nutritional treatments of well-fed individuals.

Beta-carotene supplementation has also been attempted in diseases which have a known defect in antioxidant or immunological status. These populations, unlike the general US or Western European population, have diseases which result in unusually low antioxidant or immunological defense status. Thus, supplementation of these groups is not to increase antioxidant or immunological activity to greater than average activity (which may or may not be beneficial). Instead, supplements are given in an attempt to raise antioxidant or immunological activities toward the average levels of activity found in healthy people. Research results have been promising, but are very preliminary. They are based on only a few short-term experiments in small numbers of patients. None have been able to investigate long-term effects or supplementation on antioxidant or immunological response. Little information is available on effects of beta-carotene supplementation on overall health status or death of people with illnesses (except EPP).

Erythropoietic protoporphyria

Beta-carotene has been used to treat EPP for over 25 years. This represents one of the few markedly successful nutritional treatments of a human disease. EPP is a fairly common genetic disease (of unknown incidence). It is thought to be inherited as an autosomal dominant trait with partial penetrance, but might possibly be an autosomal recessive disease. It is not especially associated with any race or ethnic origin. Symptoms usually appear shortly after birth, but sometimes years later. The primary defect of the disease is a mutation of ferrochelatase (protoheme ferrolyase, EC 4.99.1.1), the last enzyme in the heme biosynthetic pathway. Ferrochelatase synthesizes the insertion of ferrous iron into protoporphyrin IX to form protoheme. In EPP, the activity of ferrochelatase is decreased to 10 to
50% of normal. This results in increased concentrations of protoporphyrin in tissues, mainly red blood cells. The main symptom of the disease is extreme sensitivity to sunlight. Even a few minutes of sunlight can cause erythema, puritis, pain and burning. Untreated EPP results in scarring, 'orange peel' skin, and hysteria and psychotic episodes. This symptom is thought to be caused by excess porphyrins in the skin (though measurements of porphyrin concentrations in the skin have been equivocal). These porphyrins can be excited by sunlight to form a highly reactive triplet state, which catalyses oxygen free radical formation.

The main treatment for EPP is high doses of beta-carotene.(137,148,149) Treatment of children begins with 60 mg per day, and increases to typical doses of 180 mg per day, and even to 300 mg per day or more. Protection from sunlight usually results when blood concentrations reach at least 7.45 µmole/L (400 mg/dL). Most subjects benefit from this treatment, and can in fact lead normal, active lives. Some do not. Factors affecting the degree of response are not understood. However, they are not related to serum beta-carotene concentrations or to the degree of skin discoloration achieved. Most subjects have no serious side effects, and acute toxicity has not been observed.

Unfortunately, little is known about the effect of this treatment on antioxidant, immunological, or gap-junction activity. Little is known about the long-term toxicity or genotoxicity of beta-carotene in patients with EPP. The difficulty lies mainly in finding an appropriate control group that has not been treated with beta-carotene. Beta-carotene therapy has been attempted in several other skin disorders and photosensitivity disorders (Gunther's disease, actinic reticuloid, solar urticaria, hydroa aestivale, porphyria varigata) but with mixed results.(137,150) Pharmacological doses of beta-carotene also are effective protection against sunburn, but the effect is too mild to use it as sole protection.(151)

Cystic Fibrosis

Several studies have shown that beta-carotene concentrations are depressed severely in cystic fibrosis, a genetic disease that produces chronic lung infection and inflammation in children (152-154). Beta-carotene concentrations in these children are often one-quarter normal or less (152-155). Oxidative damage appears to be abnormally high in cystic fibrosis.(154-156) There are several probable reasons for this depression: first, the chronic lung inflammation may cause increased oxidative stress. Second, the intestinal malabsorption associated with the disease may reduce the patients' ability to absorb antioxidants from the diet. Two small therapeutic studies of cystic fibrosis patients have shown a marked increase in serum beta-carotene concentration and decreases in two indices of lipid peroxidation compared to that seen in normal control subjects (lag time of conjugated diene formation and decrease in plasma malondialdehyde formation) after feeding either 0.5 mg beta-carotene per kg body weight per day for 3 months (155); or 13.3 mg per day for two months.(156)

AIDS/HIV Infection

Plasma beta-carotene concentrations are decreased during HIV infection.(157-160) Scientists have found that short-term supplemental beta-carotene appears to increase CD4 cells.
Subjects using 180 mg per day beta-carotene had increased white blood cells, lymphocytes, B-lymphocytes, t-helper (CD4) cells, and CD4/CD8 (t-helper/suppressor) ratio (158). Another study, giving 60 mg per day for 4 months to 11 HIV-infected patients, showed no changes in these parameters, but did show an increase in natural killer cells and activated lymphocytes. (159) The mechanism for this benefit is unknown. It may be related to vitamin A formation, with vitamin A in turn stimulating lymphocytes and macrophages. It might be a direct effect of beta-carotene. Beta-carotene might stimulate bone marrow to produce white blood cells, or might block destruction of CD4 or other white cells, or might affect cytokine formation. Alternatively, beta-carotene might be acting as an antioxidant to enhance T-lymphocyte response by decreasing free radicals. However, all these studies are in their infancy. They are on small numbers of people, fed high doses of beta-carotene for short lengths of time. The long-term influence of beta-carotene, if any, is unknown.

CONCLUSIONS AND FUTURE AREAS OF RESEARCH

The explosion of research and information on carotenoids has led to rapid progress in this field, and has produced welcome--and sometimes surprising--information about a nutrient that had been neglected in the past. This has been an interesting time for carotenoid researchers, and will continue to be interesting as the field evolves. Thus, my conclusions about the overall importance of beta-carotene to human health may--and in fact in some cases probably will--be wrong. However, I believe that they may be useful because they suggest areas for further research.

First, I believe that beta-carotene--in small amounts--is useful for maintaining normal redox, immunological, and probably cell-to-cell communication activity in the general population. However, the amounts of beta-carotene that are necessary appear to be low, and easily attained by the consumption of a wide variety of diets. Therefore, I believe that it is likely that beta-carotene supplements will not be of significant value to most healthy well-fed populations. On the other hand, studies of the effectiveness of beta-carotene supplements for under-nourished populations should be continued or increased.

Second, more investigations on the factors controlling and limiting the effectiveness of beta-carotene supplements and supplementation programs to increase vitamin A status should be done. Unfortunately, these studies may not be very rewarding scientifically. There are too many variables in feeding, many of which cannot be identified beyond a reasonable doubt. Thus, these studies may have to be initiated as part of broad-based government programs. My hypothesis is that beta-carotene supplementation (of no more than 10 mg per day, through diet or otherwise) would be useful in populations with serum concentrations of <0.15 μmol/L, or intakes of <700 μg per day of beta-carotene. Any such supplementation trials should include intermediate endpoints and biomarkers, so that we may be able to understand the mechanisms that lead to the results. Such midpoint markers could include plasma and tissue malondialdehyde, fatty acid degradation products and conjugated dienes, premalignant lesions such as oral leukoplasia; DNA damage adducts (oxidation products and strand breaks); and biochemical markers (autocrine growth factors, prostaglandin concentrations, and ornithine decarboxylase).
Third, research should be directed into understanding the dose-response association of beta-carotene and the factors affecting its variability. These dose-response curves should be derived for vitamin A formation, antioxidant or redox reactivity, connexin 43 activity, and suitable indices of immunological response. However, more basic research is also needed to define optimal ranges of antioxidant, immunological, and connexin 43 activity. In the past most researchers, including myself, have tacitly assumed that the greater the immunological and antioxidant activity, the better. Results from the Phase III trial results suggest that this may be a dangerous assumption.

Fourth, researchers and physicians should continue to investigate the influence of beta-carotene on disease states that cause high levels of oxidative damage, suppress immunological response, or impair cell to cell communication. Beta-carotene is obviously beneficial to people with EPP. It might prove useful in cystic fibrosis, AIDS, and other diseases. However, we should no longer assume that beta-carotene supplementation—either through foods or nutritional supplements—carries no risk. Populations can, and should, be selected where the possibility of benefit most probably outweighs the potential risk.

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