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# Carotenodermia in men with elevated carotenoid intake from foods and $\beta$ -carotene supplements<sup>1,2</sup>

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**ABSTRACT** We evaluated the relation between plasma levels of carotenoids and carotenodermia in 30 men receiving carotenoid supplementation for 42 d. Five subjects each were randomly assigned to one of six treatment groups: 30 mg purified  $\beta$ -carotene supplement, 12 mg  $\beta$ -carotene supplement, 272 g cooked carrots, 300 g cooked broccoli, 180 g tomato juice, and placebo. Definite carotenodermia was observed only in the five subjects who took 30 mg of purified  $\beta$ -carotene daily. Carotenodermia was first noted between 25 and 42 d after supplementation and persisted from 14 to > 42 d posttreatment and was observed only after plasma total carotenoid levels exceeded 4.0 mg/L. These observations may be useful to investigators planning clinical trials with  $\beta$ -carotene and to clinicians assessing the significance of carotenodermia in men taking  $\beta$ -carotene supplements or following diets high in carotenoid-containing foods. *Am J Clin Nutr* 1988;48:1061-4.

**KEY WORDS** Carotenodermia, foods,  $\beta$ -carotene, supplements, carotenoids.

## Introduction

It has been suggested that dietary intakes of carotenoids, particularly  $\beta$ -carotene, may reduce the risk of cancer (1). Numerous clinical trials are currently in progress to test the potential cancer-modifying activity of  $\beta$ -carotene (2). Carotenodermia (yellowing of the skin) with carotenoid administration has been observed in the United States (3) and in Finland (D Albanes, personal communication, 1987) but plasma levels and dietary intake levels of carotenoids were not comprehensively assessed in these studies. This phenomenon is important to patient compliance in clinical trials and may also provide insight into the bioavailability of administered carotenoids. We conducted a study to observe and compare plasma response to carotenoid supplementation from various sources including pharmaceutical preparations and foods high in carotenoids. Carotenodermia was observed and is reported in detail here.

## Subjects and methods

Thirty healthy Caucasian men participated in a carotenoid-supplementation study conducted during October-December, 1985. Volunteers were screened by medical and dietary questionnaires, physical examination, hematologic and biochemical profiles, plasma carotenoid levels, and selected anthropometric measurements. Eligible subjects were between the ages of 20 and 45 y, had no history of chronic diseases and had normal physical examinations and blood profiles, were not smokers, were within 10% of ideal body weight for size, and had no

unusual dietary patterns (eg, vegetarianism). Individuals not conforming to these eligibility criteria were excluded. Vitamin self-supplementation was stopped in all subjects taking vitamins  $\geq$  4 wk before the start of the study. Procedures used in the study were approved by committees for human subjects' protection at the US Department of Agriculture, the National Cancer Institute, and Georgetown University, Washington, DC.

Subjects were randomly assigned to one of six treatment groups that were defined by the amount and type of total carotenoid supplementation received (Table 1). All vegetables used as treatments were purchased in single lots before the study began in amounts sufficient to last the entire study. Multiple analyses of samples of carrots, broccoli, and tomato juice were performed using the methods of Khachik and Beecher (4) and Khachik et al (5) and quantities given to subjects were adjusted to provide the desired daily total carotenoid amount (Table 1). The  $\beta$ -carotene capsules contained dry gelatin beadlets of 10%  $\beta$ -carotene by weight compounded with butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and sodium benzoate as preservatives (Hoffmann-La Roche Inc, Nutley, NJ). The placebo contained beadlets with BHT, BHA, and so-

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TABLE 1  
Treatment groups by amount and type of carotenoid supplementation

Group	Weight	Carotenoid content				
		Lutein	Lycopene	$\alpha$ -Carotene	$\beta$ -Carotene	Total
	<i>g</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>	<i>mg</i>
30 mg carotenoid						
Purified $\beta$ -carotene ( <i>n</i> = 5)	Capsule	ND*	ND	ND	30	30
Carrots ( <i>n</i> = 5)	272	Trace†	ND	9	29	38
12 mg carotenoid						
Purified $\beta$ -carotene ( <i>n</i> = 5)	Capsule	ND	ND	ND	12	12
Tomato juice ( <i>n</i> = 5)	180	ND	12	ND	Trace	12
6 mg carotenoid						
Broccoli ( <i>n</i> = 5)	300	3	ND	ND	3	6
Placebo ( <i>n</i> = 5)	Capsule	ND	ND	ND	ND	ND

\* Not detectable.

† Trace = <1 mg.

dium benzoate but without  $\beta$ -carotene. Multiple analyses confirmed the dosage levels. Each subject daily consumed one of the six treatments at meals together with a strictly controlled diet for 6 wk (42 d). The controlled diet itself was formulated to have a constant and low total carotenoid content (0.5–1.6 mg/d for the 3000 kcal intake level) and to exclude carotenoid-containing foods used as treatments (eg, carrots, broccoli, and tomatoes). All meals were prepared under the supervision of a dietitian at the Human Studies Facility of the Beltsville Human Nutrition Research Center (BHNRC) at the US Department of Agriculture in Beltsville, MD. On weekdays breakfast, lunch, and supper were consumed in the dining facility. All meals on weekends and holidays were prepared for carryout. Seven days of menus were formulated from commonly available foods and provided at 200-kcal increments from 2600 to 4000 kcal/d. Subjects started at their estimated caloric intake level based on stature and weight and adjustments were made as needed to maintain constant weight throughout the study. All daily diets regardless of caloric levels had fixed percentages of calories from fat (40%), carbohydrate (43%), and proteins (17%). Alcohol was not permitted during the controlled-diet period but instant coffee and diet lemon-lime carbonated soft drinks were allowed ad libitum throughout.

Fasting blood samples were collected 1 wk before the start of the study, at base line, and twice weekly during the study for determination of plasma carotenoid values. Blood collection continued at 7, 14, and 28 d posttreatment for all study participants and at 42 and 75 d posttreatment for those who developed carotenoderma, in order to monitor carotenoid status.

As in other studies and clinical practice, the assessment of carotenoderma was avowedly subjective. Carotenoderma was assessed by physical examination of the skin, especially that of the face (zygomatic prominence), hands, elbows, knees, and feet. Yellowing of the skin was graded based on clinical assessment as "yellowing definitely not present," "yellowing may be present," or "yellowing definitely present." Examinations were performed on all subjects on treatment days 25, 32, and 42 and at 7, 14, 28, and 42 d posttreatment. All clinical assessments were performed by the same observer (MSM) in the same manner. The observer did not know the treatment groups of the subjects and the subjects did not know the nature of the clinical assessment.

At the termination of the study all participants completed

an exit questionnaire that contained several questions about skin yellowing: whether yellowing was noted, when it was first noted, who first noted it, where on the body it was first noted, intensity of skin yellowing over time course, and subjective concerns about yellowing.

Blood for carotenoid analysis was collected in all-plastic syringes, with  $\sim 9 \mu\text{g}$  (4.5 units) of heparin/mL whole blood. All samples were protected from light and centrifuged within 1 h after blood drawing. Aliquots of plasma were stored at  $-70^\circ\text{C}$  and were not thawed until analysis. Individual carotenoids and total carotenoids were analyzed by the high-pressure liquid chromatography (HPLC) methods of Bieri et al (6) with a Beckman model 114M solvent delivery system (Beckman Instruments, Inc, San Ramon, CA), a Beckman model 160 ultraviolet-visible-range (UV/VIS) detector with a 436-nm filter, a Beckman 450 data system and/controller, and an Altex model 270A injector (Altex, Deerfield, MA). Crystalline  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene from Sigma (St Louis, MO) and zeaxanthin, cryptoxanthin, and echinenone from Hoffmann-La Roche Inc (Nutley, NJ) were used as standards.

A pooled plasma reference material was analyzed daily in conjunction with all samples analyzed. Coefficients of variation for the seven carotenoid peaks that were summed and reported as total carotenoids varied from 2.8% (zeaxanthin and lutein) to 10.0% (pre-cryptoxanthin).

Because of the small number of subjects in each treatment group, statistical analysis of these data was limited to a description of the percentage of subjects who developed carotenoderma and to a graphical presentation of clinical assessment of yellowing by treatment group in relation to plasma levels over time.

## Results

The 30 subjects weighed between 64.1 and 92.7 kg and had base-line levels of plasma total carotenoids that ranged from 0.9 to 2.6 mg/L (median, 1.5 mg/L).

All five men who took the 30-mg purified  $\beta$ -carotene supplement developed carotenoderma ("yellowing definitely present") during the course of the study. No men in any of the other treatment groups developed un-

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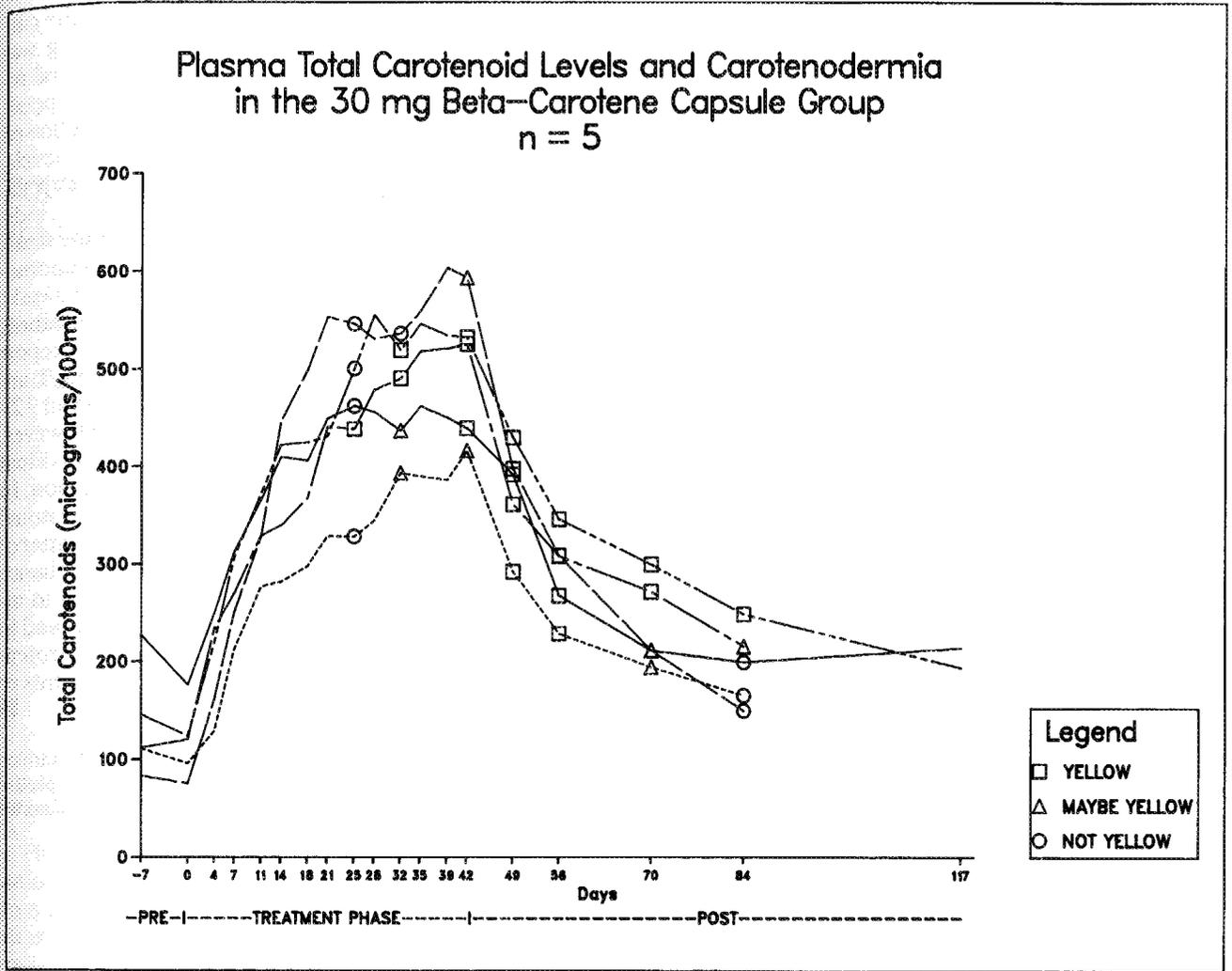


FIG 1. Plasma carotenoid responses of individuals who received a 30-mg purified  $\beta$ -carotene supplement daily ( $n = 5$ ). Observation on three individuals terminated at 42 d posttreatment, when carotenoderma disappeared. (Two curves exactly intersect each other at day 56 and at day 70 of the study.)

equivocal carotenoderma. The five men who took 30 mg of purified  $\beta$ -carotene were first observed to have definite carotenoderma at intervals ranging from day 25 of treatment to 7 d posttreatment and they all remained yellow for  $\geq 14$  d after the treatment was stopped. One subject remained yellow at 42 d posttreatment, the end of our observation period. In two of the five men carotenoderma was not definite until after peak total carotenoid plasma levels were attained and had begun to fall (ie, after treatment had stopped). In the remaining three men, carotenoderma developed before or at peak plasma total carotenoid levels. In all five carotenoderma persisted after plasma carotenoid levels approached mean base-line levels.

Equivocal carotenoderma ("yellowing may be present") was observed on at least one examination in four of the five men who took the 12-mg purified  $\beta$ -carotene supplement, in three of the five men in the carrot group, in two of the five men in the broccoli group, and in one

of the five men in the placebo group. None of the men consuming tomato juice (the major ingredient of which is lycopene) was ever observed to have any yellowing.

Results of plasma analyses for the five subjects who took 30 mg of purified  $\beta$ -carotene, with indication of carotenoderma status, are shown in Figure 1. Peak plasma total carotenoid levels in individuals who developed definite carotenoderma ranged from 4.2 to 6.0 mg/L (median, 5.3 mg/L). By comparison, peak levels in persons with possible yellowing were only 1.3-3.5 mg/L (median, 2.0 mg/L) whereas subjects with no yellowing had peak levels ranging from 1.2 to 3.1 mg/L (median, 1.3 mg/L). All subjects appeared normal physically and had normal serum biochemical indices at the end of the trial.

In the self-assessment at the conclusion of the study, six individuals noted yellowing of the skin at some point during the study: four of the five men from the 30-mg purified  $\beta$ -carotene group and one each from the groups

consuming carrots and broccoli. Comments ranged from "yellowing of the face and hands," to "yellowing of the entire body." Some of the subjective impressions were that yellowing was "not at all unpleasant," "looks a little bit tanned," and resulted in "better skin tone." Of those men who developed yellowing, three responded that they first noted it themselves whereas one each responded that yellowing was first noted by a coworker, a friend, or a doctor.

Yellowing was first noted by subjects at times ranging from the second treatment week to 2 wk posttreatment. All but one of the subjects responded that yellowing was first observed on the hands or palms, or the face and hands. Three respondents indicated that the intensity of yellowing increased with time whereas three indicated it stayed the same. One also reported fluctuations over time. Skin yellowing did not affect the feelings of the subjects about participating or complying with the treatments, either in this study or in future studies. All subjects who noted yellowing responded that the quantities of the treatment vegetables were palatable. Most felt they could have consumed larger quantities although there were a few subjective complaints of indigestion.

### Discussion

This study demonstrated that unequivocal carotenoderma occurred in all five men who consumed 30 mg of a purified  $\beta$ -carotene supplement daily whereas possible yellowing was observed in four of the five men who took 12 mg of the purified  $\beta$ -carotene daily. This observation suggests an intake threshold for yellowing in the range of 12–30 mg/d for 6 wk for purified  $\beta$ -carotene. Of five persons who received 6 mg/d total carotenoids (lutein and  $\beta$ -carotene) as broccoli, only two had possible yellowing (none were definite). Ingestion of 12 mg/d of lycopene from tomato juice did not result in skin yellowing. The 38 mg/d of total carotenoids from carrots produced possible yellowing in only three (of five) men and definite yellowing in none. These findings suggest that for a 6-wk period for the amounts ingested, carotenoids in food sources are less likely to cause yellowing of the skin than is purified  $\beta$ -carotene and that lycopene in tomato juice does not cause carotenoderma at these levels of intake. The conclusions are based on consumption of a relatively high-fat diet in men of average body fat. Also, broccoli and carrots were cooked but not pureed, so that the plant cell walls of these vegetables were probably intact. The carotenoids from food sources are likely to be less efficiently absorbed than those from purified supplements.

For those five persons who demonstrated definite carotenoderma on clinical assessment, the yellowing was first noted from 25 to 49 d after ingestion began and required  $\geq 42$  d to disappear once ingestion was stopped. The development of carotenoderma generally followed the attainment of peak plasma total carotenoid levels, indicating that skin-tissue levels lag behind those in the blood. Carotenoderma remained present in some individuals after treatment for periods longer than the treatment period itself. The applicability of this lag phenomenon to other tissues remains to be tested.

Carotenoderma may be seen under the circumstances and in the type of population described in this report once blood plasma total carotenoid levels  $> 4.0$  mg/L are attained or, alternatively, with the consumption of 12–30 mg of purified  $\beta$ -carotene daily for 25–42 d. However, our sample was small and there may be wide individual variations in response to  $\beta$ -carotene supplementation. In the subjective comments yellowing of the skin (as noted by participants themselves) was not viewed as a matter of concern and did not affect participation in or compliance with the study. These observations may be useful to investigators planning clinical trials with  $\beta$ -carotene and to physicians assessing the clinical significance of carotenoderma in persons who take  $\beta$ -carotene supplements or follow diets high in carotenoid-containing foods. 

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