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# ACUTE EFFECTS OF DIETARY CAROTENES ON SERUM ALPHA AND BETA CAROTENE IN HUMANS

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## ABSTRACT

The acute effects of consuming alpha and beta carotene from carrots on serum alpha carotene (AC) and serum beta carotene (BC) levels were investigated in 17 adult subjects (18-58 yr). After a 10-day low carotene (LC) diet, subjects were randomized into three groups based on day 6 BC levels. On day 11, fasting baseline blood was drawn. Treatments of either 3 carrots (3C), 1 carrot (1C), or 3 placebo capsules (3P) were then consumed following a LC breakfast. Blood was drawn 1,2,3,4,5,7 and 24 hr post-treatment (PT) and AC and BC determined by HPLC. Treatment 3C yielded significantly greater ($p<0.05$) peak AC and BC levels at 5 hr PT than did treatments 1C or 3P. These results suggest the best condition for drawing blood samples to assess the serum carotene status of adults is at the fasting state and that significant alterations in serum can occur within 5 hr of a carotene rich meal.

## INTRODUCTION

The determination of plasma carotenoid levels has been used as one means of assessing vitamin A nutritional status (1). More recently, with the interest in dietary carotenes as possible cancer-preventive agents (2), scientists have attempted to establish a correlation between cancer rates and total serum carotenoid levels (3). However, the question on the best time and under what conditions blood should be sampled (such that blood carotene levels remain unaffected by the carotene content of a previous meal) has received little attention. A number of studies has demonstrated the sensitivity of blood carotene levels to dietary intake of carotene over a period of months (4-6) and even days (7). However, very little is known about the acute effects of dietary carotenes on blood carotene levels. Results from one study, presumably using synthetic beta carotene, demonstrated a peak in serum beta carotene 5 hours after ingestion (8). Another study revealed no significant alterations in plasma carotenoid levels up to 4 hours after consuming a vitamin A and carotene-rich meal (9).

It was desirable therefore to clarify the effects of ingesting a carotene-rich meal on postprandial serum carotene concentrations. For this a modified HPLC technique was employed to assess the 24-hour response to a single administration of 2 doses of carotenes from carrots on serum alpha and beta carotene levels in fasting adult humans.

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## MATERIALS AND METHODS

Subjects. Participants were 8 healthy male and 9 female adults, all employees of the Shaklee Research Center or their spouses, with ages ranging from 18 to 58 years, and averaging 112% of ideal body weight (Table 1). This study was one of a group of studies examining carotene metabolism in which these individuals also participated as subjects. Procedures followed were in accord with the Helsinki Declaration as updated in Tokyo, Japan in 1975.

Table 1. Subjects

| INITIALS | TREATMENT | SEX | AGE (YEARS) | HEIGHT (CM) | WEIGHT (KG) | PERCENT OF IBW |
|---|---|---|---|---|---|---|
| G.K. | 1 | Male | 18 | 179.07 | 89.55 | 1.17 |
| G.S. | 1 | M | 26 | 184.15 | 81.14 | 0.99 |
| J.T. | 1 | Female | 33 | 172.72 | 83.86 | 1.32 |
| L.W. | 1 | M | 29 | 173.99 | 69.09 | 0.97 |
| P.P. | 1 | F | 58 | 148.59 | 50.91 | 1.21 |
| P.T. | 1 | F | 32 | 167.64 | 64.55 | 1.09 |
| A.J. | 2 | F | 27 | 152.40 | 62.50 | 1.38 |
| H.B. | 2 | M | 28 | 184.15 | 59.55 | 0.72 |
| M.R. | 2 | M | 28 | 179.07 | 79.55 | 1.04 |
| M.T. | 2 | F | 29 | 161.29 | 64.32 | 1.20 |
| W.B. | 2 | M | 30 | 154.31 | 73.41 | 1.46 |
| Y.C. | 2 | M | 57 | 154.94 | 57.27 | 1.12 |
| D.L. | 3 | F | 33 | 156.21 | 55.45 | 1.13 |
| E.H. | 3 | F | 26 | 194.31 | 53.64 | 0.65 |
| E.M. | 3 | F | 36 | 154.94 | 69.77 | 1.46 |
| J.W. | 3 | F | 35 | 165.10 | 62.73 | 1.10 |
| K.B. | 3 | M | 22 | 172.72 | 70.45 | 1.01 |

Details of the study were explained extensively in group meetings and privately prior to enlistment. Only subjects found to be healthy by a physician, and having serum vitamin A levels within normal limits were admitted to the study. Informed consent was obtained in private.

Subjects were placed on a 10-day low-carotene diet (free of all yellow and green fruits and vegetables, margarine, and colored cheeses), to ensure no contribution of carotene from previous dietary intake, and supplemented with multivitamin-mineral tablets (Shaklee Corporation, Vita-Lea, San Francisco, CA) containing 12 essential vitamins at 100-150 % of the U.S. RDA, 7 essential minerals at 45-100 % of the U.S. RDA, and no carotene. Due to the absence of fruits and vegetables, subjects were provided high fiber cereals, crackers, and breads to ensure a dietary fiber intake of at least 15-20 g per day.

On day 6, non-fasting venous blood specimens were collected and analyzed for serum alpha and beta carotene (levels ranged from 0.5-35.8 mcg/dl). Subjects were then randomized into 3 groups with similar mean beta carotene levels by the method of random permuted blocks (10).

Treatments. The 3 groups were randomly assigned to one of three treatments (Table 2).

Table 2. Treatments

| TREATMENT | CAROTENE/DAY ALPHA | BETA | FORM | AMOUNT/DAY |
|---|---|---|---|---|
| 1 | 18.9 mg | 24.0 mg | Carrots | 207.3 g |
| 2 | 6.3 mg | 8.0 mg | Carrots | 69.1 g |
| 3 (placebo) | 0 mg | 0 mg | Capsules | 3 capsules |

Treatment group 2 consumed an additional 2 placebo capsules so it appeared to the subjects that they were all taking the same number of treatment units (i.e. either 3 capsules, 3 servings of carrots, or 2 capsules plus 1 serving of carrots).

Design. The study was a parallel comparison of the 3 treatments and was blind with respect to capsule treatments. The experimental period consisted of an initial low-carotene diet depletion phase lasting 10 days, followed by the single administration of treatments on day 11.

On treatment day, baseline fasting venous blood specimens were collected. Subjects then consumed identical breakfasts consisting of 1 bran muffin, 1 pat of butter, 1/2 cup lowfat milk, and 1 cup black coffee or tea. Treatments were ingested immediately after breakfast with 4 oz of cranberry juice cocktail and 8 oz tap water. Venous blood specimens were collected 1,2,3,4,5,7 and 24 hr after consuming treatments. All subjects consumed equal quantities of low-carotene foods at similar times during this acute blood drawing period, and ate freely from low-carotene foods at home thereafter. During the acute blood draw period fat intake was strictly controlled.

Laboratory Analyses. Venous blood specimens were collected and the serum was frozen at -20°C. Serum alpha and beta carotene levels were determined by the HPLC procedure as described by Driskel (11) except for the following modifications: 1 ml of alcoholic KOH was added to a 2 ml aliqout of just previously thawed serum and incubated for 30 minutes at 60°C. After cooling to room temperature, the solution was extracted 3 times with 4 ml of hexane. A 20 ml injection was eluted isocratically with 40 % acetonitrile in methanol pumped at 4.0 ml/min.

Statistical Analyses. Differences in serum alpha and beta carotene values were calculated by subtracting the values of the blood draws 1,2,3,4,5,7 and 24 hr after treatment from the baseline values. One-way analysis of variance was performed on the differences using the Systat Version 1.3 Statistical Package (12). When the F-statistic indicated significance ($p<0.05$), a two-tailed Dunnett's test (13) was used to determine critical differences on the adjusted mean serum carotene values.

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RESULTS AND DISCUSSION

Conventional spectrophotometric methods for determining beta carotene do not distinguish between the different carotenoids that may be present (14). Consequently, the conventional measurement affords higher than actual values (11). This present study employed a highly specific HPLC method capable of separating serum alpha and beta

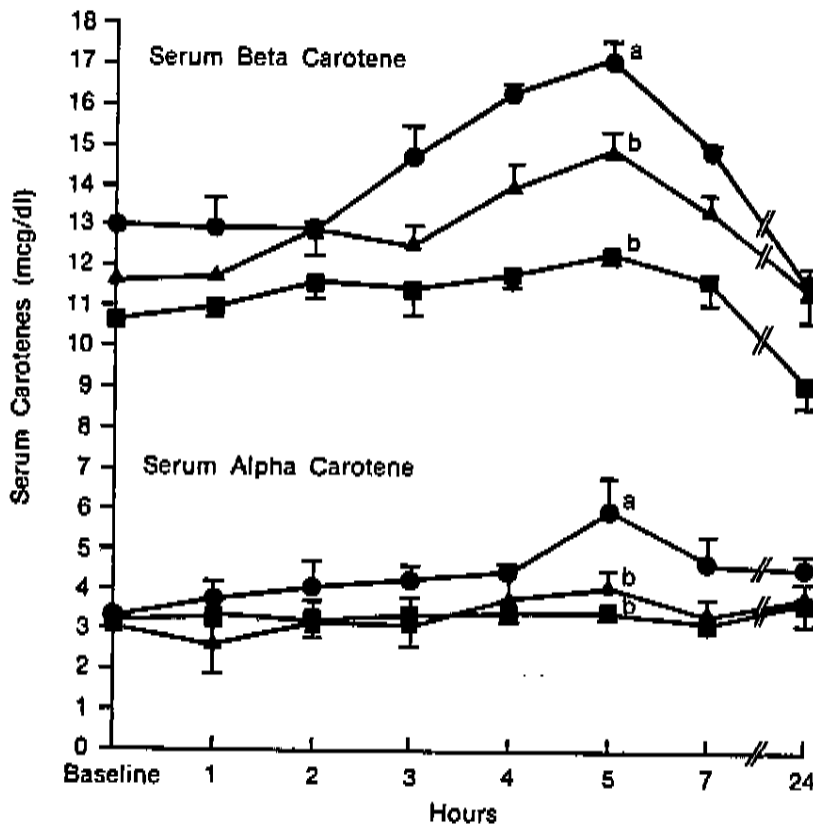


Figure 1. Serum alpha and beta carotene. Data are means. SEM is for the baseline corrected residual. Only blood draws with values significantly different have superscripts. The differences are significant ($p<0.05$) if they do not share a common superscript letter.

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carotene. Combining these individual values yields total serum carotenoid levels, and as expected they are significantly lower than conventional methods would provide. Therefore, quantitative comparisons with other studies is inappropriate. However, comparison of trends between studies is warranted.

Figure 1 shows serum alpha and beta carotene levels during fasting and 1,2,3,4,5,7 and 24 hours after ingesting treatments. All treatments showed some fluctuation in blood carotene levels. However, treatments 1 and 2 tended to result in higher serum carotene levels than placebo. The peak serum alpha and beta carotene responses to treatment 1 (high dose carrots) were significantly greater ($p<0.05$) than the peak serum carotene responses to treatments 2 and 3. Peak responses for both serum carotenes occurred approximately 5 hours after treatment consumption (figure 1).

These results are in accord with the previous observation of Fujita (8) who found that peak beta carotene levels in serum were achieved 5 hours after ingestion of a non-whole food source of beta carotene. These data reported here also support the findings of Goodman (15) who demonstrated in humans that labeled beta carotene, apparently absorbed intact, reached peak levels in the thoracic duct lymph 3-4 hours after intake.

In practice, the fact that serum carotene levels can be significantly altered within 5 hours of consuming a carotene-rich meal, suggests the importance of sampling blood in the fasted state. However, under field conditions, this is not always possible. These results provide some assurance, and confirm the suggestion of Mejia (9), that blood samples required for the purposes of determining carotene concentrations in adults can be obtained within approximately 4 hours of a previous meal without significantly altering the results.

In conclusion, the finding that serum carotenes can be significantly altered within hours of consuming a carotene-rich meal, coupled with the fact that serum carotenes have been shown to change dramatically in a period of days or weeks in response to an alteration in dietary carotene intake, suggests that caution be exercised when attempting to correlate a single blood carotene measurement to vitamin A nutriture or incidence of disease. On the contrary, repeated measurements at frequent intervals may be warranted.

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