ACTA VITAMINOL. ENZYMOL. 1985, 7 (3-4), 189-198

Repletion and Depletion of Serum Alpha and Beta Carotene in Humans with Carrots and an Algae-Derived Supplement.[1]

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Key words: Carotene - Carotenoids - Vitamin A - Antineoplastic agents.

SUMMARY

A 10-day low-carotene diet phase (LCD), followed by a 7-day treatment phase (TP) and then another LCD, was adhered to by 32 adult human subjects. Serum alpha carotene (SAC) and serum beta carotene (SBC) levels were examined using HPLC and found to have decreased 44.7% and 35.8% respectively in the first LCD. Alpha and beta carotene, from algae-derived capsules and raw carrots, significantly increased SAC and SBC levels during the TP. Half-lives of SAC and SBC averaged 7.8 days and 12.4 days respectively during the two LCD. Serum vitamin A remained unchanged throughout the study.

RIASSUNTO

Replezione e deplezione dell'alfa- e beta-carotene nell'uomo, mediante somministrazione di supplementi di carote e di derivati di alghe.

Trentadue soggetti adulti sani sono stati sottoposti ad una dieta a basso contenuto di carotene (LCD) per 10 giorni, poi ad un periodo di trattamento (TP) per 7 giorni, e quindi ancora a dieta carente. I livelli serici di alfa- e beta-carotene, misurati con HPLC, diminuivano del 44,7% e del 35,8% rispettivamente, durante il periodo LCD. La somministrazione di capsule contenenti beta-carotene estratto da carote o alghe provocava un aumento significativo di beta-carotene e di alfa-carotene nel periodo TP. L'emivita dei livelli serici era in media di 7,8 e 12,4 giorni rispettivamente, durante i due periodi LCD. I livelli serici di vitamina A restavano invece costanti durante l'intero studio.

INTRODUCTION

Recent attention has focused on the possible anticancer effects of consuming dark green and deep yellow vegetables.[1] While any number of dietary factors in these foods could be protecting against cancer, epidemiological observations indicate there is a below average risk of cancer among people consuming an above average intake of beta carotene from these foods.[2]

Peto *et al.*,[2] proposed a number of mechanisms to explain the possible effects carotenes may have on target tissues, thereby affecting cancer risks. Adequate circulating levels of carotenes might serve as an intermediate step to target tissue exposure.

Using a highly specific high-pressure liquid chromatography (HPLC) method, this study examined the effects of short-term consumption of alpha and beta carotene, in the form of an algae-derived carotene supplement and carrots, on serum alpha and beta carotene levels in humans. In addition, the effect of a short-term low-carotene diet on serum alpha and beta carotene levels was also determined.

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[1] Presented in part at the April 1985 FASEB meeting in Anaheim, CA. Fed. Proc. 44: 771, March, 1985.

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MATERIALS AND METHODS

Subjects

Twelve healthy male and 20 female adults between the ages of 18 and 60 yr participated. All subjects were employees of the research center or spouses of employees.

The design and procedures followed in the study were in accord with the Helsinki Declaration as updated in Tokyo, Japan in 1975. 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.

Baseline, non-fasting venous blood specimens were collected and analyzed for serum alpha and beta carotene. Subjects were then placed on a low-carotene diet (free of all yellow and green fruits and vegetables, margarine, and colored cheeses) and supplemented with multivitamin-mineral tablets 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 (Shaklee Corporation, Vita-Lea, San Francisco, CA). Due to the absence of fruits and vegetables, subjects were provided high fiber cereals, crackers, and breads to insure 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. Subjects were then randomized into 5 groups with similar mean beta carotene levels by the method of random permuted blocks.[3]

Treatments

The five groups were randomly assigned to one of five treatments (Table 1). Treatment groups 3 and 4 consumed an additional 2 placebo capsules/day so that all subjects appeared to be taking the same number of treatment units (i.e. either 3 capsules, 3 servings of carrots, or 2 capsules plus one serving of carrots). The carotene capsules contained carotenes, extracted from the algae *Dunaliella salina* into a vegetable oil base. Treatment were administered daily by investigators.

Design

The study was a parallel comparison of the 5 treatments (Table 1), and was double blind with respect to capsule treatments. The experimental period consisted of three phases: 1) an initial depletion phase lasting 10 days; 2) a treatment phase lasting 7 days; and 3) a final depletion phase lasting 7 days. The low-carotene diet was maintained throughout the study.

On the first day of the treatment phase, baseline fasting venous blood specimens were collected and analyzed for serum carotenes. Treatments were then consumed and non-fasting venous

Table 1 - Treatments

Treatment	Carotene/day		Form	Amount/day
	alpha	beta		
1	3.2 mg	24.0 mg	Capsules	3 capsules
2	18.9 mg	24.0 mg	Carrots	207.3 g carrots
3	1.1 mg	8.0 mg	Capsules	1 capsule
4	6.3 mg	8.0 mg	Carrots	69.1 g carrots
5 (placebo)	0 mg	0 mg	Capsules	3 capsules

blood specimens taken on hr 24, 48, 72, 96, and 168 after consuming the initial treatment. In addition, treatments were taken daily following the collecting of blood or at approximately 9 a.m.
During the final depletion phase, nonfasting venous blood specimens were collected 24, 48, 72, 120 and 192 hr after carotene supplementation was discontinued. During this period, all subjects consumed 3 placebo capsules daily following blood collections or at approximately 9 a.m.
Serum vitamin A levels were assessed weekly during the study.

Laboratory Analyses
All venous blood specimens were collected in silicone coated vacuum tubes, allowed to clot for 15-20 minutes, and centrifuged at 2500·g for 10-15 minutes. The serum was removed from the clot, protected from light, and frozen at —20° C. Serum alpha and beta carotene levels of venous blood specimens were determined by HPLC procedure as follows: 1 ml of alcoholic KOH was added to a 2 ml aliquot of thawed serum and incubated for 30 minutes at 60° C. After cooling to room temperature, the solution was extracted with 4 ml of hexane. The phases were separated and the hexane removed. A total of 3 hexane extractions were completed, then combined, dried down, and resuspended in 2 ml of hexane. A 20 ul aliquot of this solution was injected into a 5 um C18 Chromasil column (4.6 * 250 mm) (Waters Associates, Milford, MA) and eluted isocratically with 40% acetonitrile in methanol pumped at 4.0 ml/min (Laboratory Data Control, Constametric 3, Riviera Beach, FL). A detector (Beckman, 104 LC, Berkeley, CA) with a 436 nm filter was used to monitor the column effluent (0.01 AUFS). Retention times of 12 min. and 14 min. were observed for alpha and beta carotene respectively. The peak areas of chromatographed stock solutions of alpha and beta carotene were used to calculate serum carotene levels.

As little as 3-5 ng of carotene per 20 ul were detectable. The SEM of a pooled serum sample was 0.56 ug/dl for alpha carotene at a level of 3.12 ug/dl, and 1.62 ug/dl for beta carotene at a level of 11.7 ug/dl. Recoveries of alpha and beta carotene from a spiked sample containing 2.5 ug/dl of each carotene were 94.4% and 110.0% respectively.
Serum vitamin A was measured with a dichloropropanol technique.[4]

Statistical Analysis
Serum alpha and beta carotene difference values were calculated by subtracting the values of each blood draw day from day 10 values. One-way analysis of variance was performed on the differences using the Systat Version 1.3 Statistical Package.[5] When the F-statistic indicated significance ($p < 0.05$), a two-tailed Dunnett's test[6] was used to determine critical differences on the adjusted serum carotene and vitamin A means. For the depletion phases, first-order rate constants were fitted by the method of least squares on the log transformed data. A two-tailed t-test was used to determine significant difference between half-lives in the depletion phases. For the treatment phase, zero-order rate constants were fitted by the method of least squares on the serum carotene difference values which were calculated by subtracting day 10 values. A two-tailed t-test was used to determine significant difference in rates of increase in serum carotenes between: high and low dose treatments; carrots and capsules; and dietary alpha and beta carotene.

RESULTS

Compliance

One subject was removed from the study prior to randomization because of serum beta carotene levels which were four-times higher (202 ug/dl) than the next highest subject.

A second subject was removed on day 10 due to blood drawing difficulties.

Daily food records indicated that the dietary regimen was adhered to by subjects.

Baseline

At baseline, alpha carotene comprised approximately one-fourth (Table 2), and beta carotene three-fourths (Table 3), of the serum carotenes measured. For all subjects, mean serum alpha and beta carotene levels were 5.72 ± 3.05 ug/dl and 18.68 ± 2.09 ug/dl respectively.

Initial Depletion Phase

Within 10 days of converting to the low-carotene diet, mean serum alpha

Table 2 - Serum Alpha Carotene Values[1]

TXMT	Count	Baseline Day 0	Depletion Day 6	Depletion Day 10
One	6	4.73(1.55)	2.47(1.20)	2.40(0.76)
Two	6	6.1 (1.81)	3.77(1.42)	3.30(0.82)
Three	7	6.13(1.44)	3.56(0.85)	3.74(0.78)
Four	6	4.90(1.71)	3.00(1.42)	3.13(1.13)
Five	5	6.50(1.77)	4.08(1.71)	3.15(0.92)

TXMT	Count	Treatment Day 11	Treatment Day 12	Treatment Day 13
One	6	4.53(0.19)	5.30(1.07)^{a,b}	6.28(1.02)^{b}
Two	6	6.90(2.51)	7.95(1.38)^{a}	9.65(1.66)^{a}
Three	7	3.56(0.90)	5.39(1.10)^{b,c}	5.73(1.04)^{b,c}
Four	6	3.80(1.27)	5.77(1.32)^{a,b}	5.88(1.00)^{b}
Five	5	3.66(1.28)	3.66(1.05)^{c}	3.22(1.13)^{c}

TXMT	Count	Treatment Day 14	Treatment Day 17	Treatment Day 18
One	6	7.20(1.00)^{b}	15.35(3.13)^{b}	9.73(1.72)^{b,c}
Two	7	13.38(2.27)^{a}	25.93(2.96)^{a}	19.68(2.37)^{a}
Three	7	6.19(1.11)^{b,c}	10.2 (1.65)^{b,c}	8.64(1.50)^{c,d}
Four	6	8.03(1.25)^{b}	10.68(1.87)^{b,c}	11.88(1.80)^{b}
Five	5	3.56(1.16)^{c}	3.82(1.10)^{c}	3.46(0.83)^{d}

TXMT	Count	Depletion Day 19	Depletion Day 21	Depletion Day 24
One	6	9.64(2.05)^{b}	10.30(2.09)^{b}	6.72(1.15)^{b}
Two	6	18.86(2.65)^{a}	14.32(1.55)^{a}	13.15(2.08)^{a}
Three	7	8.41(1.41)^{b,c}	6.83(1.31)^{b,c}	5.96(1.10)^{b,c}
Four	6	10.7 (1.79)^{b}	9.18(1.99)^{b}	6.88(1.39)^{b}
Five	5	4.02(1.31)^{c}	3.80(0.93)^{c}	2.42(0.79)^{c}

1) Data are means and (SEM). Serum alpha carotene values are expressed in micrograms per deciliter. Superscripts refer to difference values of each day substracted from day 10 values (differences are not shown). Only days with values significantly different have superscripts. The differences are significant ($P<0.05$) if they do not share a common superscript letter.

carotene levels, for all subjects, decreased 44.7% ($P<0.001$) (Table 2). Mean serum beta carotene levels decreased 35.8% ($p<0.001$) (Table 3). The mean serum half-lives of alpha and beta carotene during this phase were 7.5 days and 14.3 days respectively (Table 4).

Treatment Phase

At the end of the 7 day treatment phase, serum alpha carotene was significantly increased for treatments 1 ($P<0.05$) and 2 ($P<0.01$) as compared to placebo (Table 2). Mean serum levels of beta carotene were also significantly increased for treatments 1 ($P<0.01$) and 2 ($P<0.05$) as compared to placebo (Table 3). The lower dose treatments tended to show higher levels of serum alpha and beta carotene as compared to placebo, but did not reach significance ($P>0.05$) by 7 days.

Alpha carotene from treatment 1 and 2 produced 540% and 686% increases in mean serum alpha carotene levels after

Table 3 - Serum Beta Carotene Values[1]

TXMT	Count	Baseline Day 0	Depletion Day 6	Depletion Day 10
One	6	17.77(3.72)	9.33(2.26)	11.02(2.41)
Two	6	17.28(3.33)	12.08(2.48)	11.67(2.05)
Three	7	18.89(5.11)	12.53(3.50)	13.07(3.19)
Four	6	20.63(5.88)	15.22(4.36)	13.08(4.25)
Five	5	18.82(4.28)	11.20(3.33)	10.74(2.51)

TXMT	Count	Treatment Day 11	Treatment Day 12	Treatment Day 13
One	6	16.05(2.60)	27.02(2.9) ^{a}	29.88(3.17)^{a}
Two	6	17.24(4.49)	20.42(3.4) ^{a,b}	23.03(3.60)^{b}
Three	7	10.56(2.90)	21.93(5.44)^{a,b}	21.77(5.16)^{b}
Four	6	11.58(3.52)	20.15(5.49)^{b}	19.75(4.11)^{b,c}
Five	5	9.14(2.42)	13.04(3.1) ^{b}	11.6 (3.09)^{c}

TXMT	Count	Treatment Day 14	Treatment Day 17	Treatment Day 18
One	6	32.87(5.86)^{a}	51.5 (9.43)^{a}	38.1(6.78)^{a}
Two	6	31.3 (5.21)^{a}	38.15(4.96)^{a,b}	27.73(2.45)^{a,b}
Three	7	25.77(5.41)^{a,b}	26.39(6.47)^{b,c}	25.59(5.26)^{b,c}
Four	6	23.33(4.71)^{a,b}	18.47(4.07)^{b,c}	21.95(4.86)^{b,c}
Five	5	11.7 (3.19)^{b}	9.88(2.53)^{c}	9.82(1.86)^{c}

TXMT	Count	Depletion Day 19	Depletion Day 21	Depletion Day 24
One	6	31.1 (5.60)^{a}	27.9 (5.72)^{a}	21.57(3.52)^{a}
Two	6	26.42(4.34)^{a,b}	20.7 (2.95)^{a,b}	19.73(3.15)^{a,b}
Three	7	23.77(5.26)^{a,b,c}	19.04(4.49)^{a,b}	16.01(3.86)^{b,c}
Four	6	19.28(4.57)^{b,c}	17.73(4.74)^{a,b}	13.17(3.2) ^{c}
Five	5	10.16(2.32)^{c}	9.6 (1.93)^{b}	7.02(1.86)^{c}

1) Data are means and (SEM). Serum beta carotene values are expressed in micrograms per deciliter. Superscripts refer to difference values of each day substracted from day 10 values (differences are not shown). Only days with values significantly different have superscripts. The differences are significant ($P<0.05$) if they do not share a common superscript letter.

7 days respectively (Table 2). Beta carotene from treatment 1 and 2 yielded 367% and 227% increases in mean serum beta carotene levels after 7 days respectively (Table 3).

Final Depletion Phase

For all treatments, after 7 days of the final depletion phase, mean serum alpha and beta carotene levels decreased 46.7% ($P<0.001$) and 46.3% ($P<0.001$)

Table 4 - Rates of Depletion and Half-Lives of Serum Alpha and Beta Carotene[1]

Serum Carotene	Initial Depletion[2] Rate	Initial Depletion[2] Half-life	Final Depletion[2] Rate	Final Depletion[2] Half-life
Alpha	—0.040(.010)	7.5	—0.037(.011)	8.1
Beta	—0.021(.007)	14.3	—0.029(.009)	10.4

1) Rates of depletion data are means and (SEM) for all subjects, and are expressed in days minus one. Half-lives are means for all subjects and are expressed as days.
2) Rates of depletion and half-lives for serum alpha and beta carotene were not significantly different ($P>0.05$).

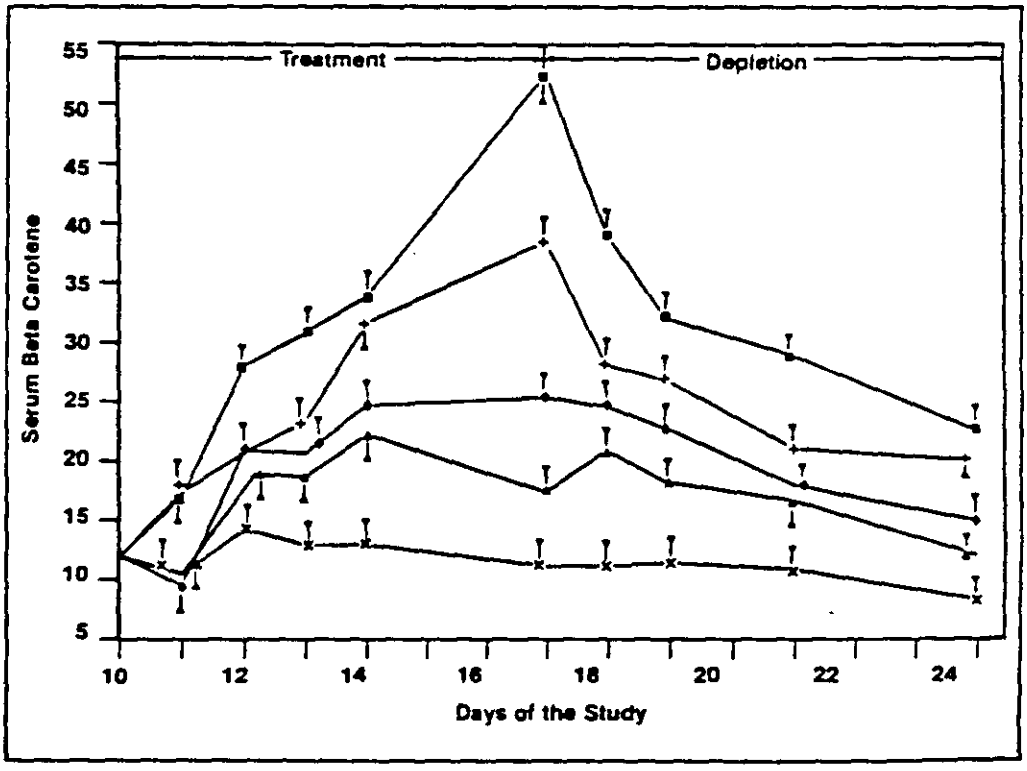

Fig. - Mean serum alpha carotene levels during the treatment and final depletion phases.
Values are normalized to the same starting point. Symbols represent the means for each treatment group expressed as ug/dl. Symbols are: ■, treatment 1 (3.2 mg alpha carotene from capsules); +, treatment 2 (18.9 mg alpha carotene from carrots); •, treatment 3 (1.1 mg alpha carotene from capsules); ▪, treatement 4 (6.3 mg alpha carotene from carrots); X, treatment 5 (placebo); respectively. Flags indicate standard errors of the mean.

respectively (Fig. 1, 2). The mean serum half-lives of alpha and beta carotene during depletion were 8.1 days and 10.4 days (Table 4) respectively. During the final depletion period, the rates of decline were similar ($P>0.05$) for individual treatments (data not shown).

Rates of Increase: High versus Low Dose Treatments

The increases in serum carotenes per milligram of carotenes fed were greater for lower dose treatments ($P<0.001$) as compared to the same source higher dose treatments (Table 5).

Rates of Increase: Capsule versus Carrot Treatments

The increases in serum beta carotene per milligram of beta carotene fed were greater in capsule treatments ($P<0.001$) as compared to carrot treatments (Table 5).

The increases in serum alpha carotene per milligram of alpha carotene fed in treatment 1 (3.2 mg alpha fed from capsules) was higher ($P<0.001$) than treatment 4 (6.3 mg alpha fed from carrots) (Table 5).

Effectiveness of Alpha versus Beta Carotene at Raising Serum Carotene Levels

The increases in serum alpha carotene per milligram alpha carotene fed for carrot treatments 2 and 4 were similar ($P>0.05$) to the increases in serum beta carotene per milligram beta carotene fed for those treatments (Table 5). The increase in serum alpha carotene per

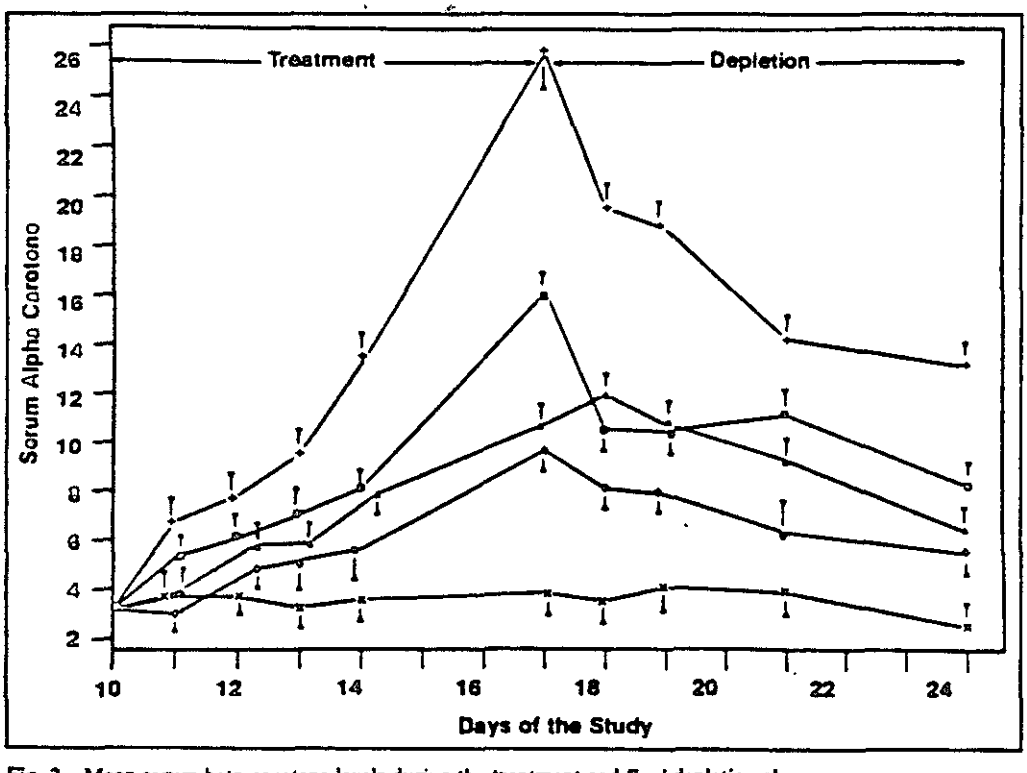

Fig. 2 - Mean serum beta carotene levels during the treatment and final depletion phases.
Values are normalized to the same starting point. Symbols represent the means for each treatment group expressed as ug/dl. Symbols are: ■, treatment 1 (24 mg beta carotene from capsules); +, treatment 2 (24 mg beta carotene from carrots); ◆, treatment 3 (8 mg beta carotene from capsules); ▲, treatment 4 (8 mg beta carotene from carrots); ×, treatment 5 (placebo); respectively. Flags indicate standard errors of the mean.

Table 5 - Increases in Serum Carotenes per Day and per Milligram of Carotenes Fed[1]

Treatment	Dose (mg)	Alpha Carotene Increases Ug/(dl* day)	Increases Ug/(dl* mg fed)
1	3.2	1.945 (.277)	0.608 (.087)
2	18.9	3.592 (.366)	0.190 (.019)
3	1.1	1.057 (.135)	0.961 (.123)
4	6.3	1.275 (.207)	0.202 (.033)
5	0	0.070 (.088)	

Treatment	Dose (mg)	Beta Carotene Increases Ug/(dl* day)	Increases Ug/(dl* mg fed)
1	24	6.427 (.849)	0.268 (.035)
1	24	4.904 (.555)	0.204 (.023)
1	8	2.617 (.629)	0.327 (.079)
1	8	1.327 (.512)	0.166 (.064)
5	0	—0.050 (.218)	

1) Data are means and (SEM).

milligram alpha carotene fed in capsule treatment 1 (3.2 mg alpha) was higher ($P<0.025$) than the increase in serum beta carotene per milligram fed (8 mg beta) in capsule treatment 3 (Table 5).

Serum Vitamin A

No significant changes ($P<0.05$) in serum vitamin A levels were seen during the study (data not shown).

DISCUSSION

This study employed a highly specific HPLC technique to differentiate between serum alpha and beta carotene levels. Previous studies which examined blood levels of carotenes[7-11] employed a spectrophotometric method to measure total plasma carotenoid levels. This makes quantitative comparisons of serum carotene levels between this and other published studies difficult. However, trends in serum carotene levels are comparable.

Upon entry into the study, subjects were consuming self-selected diets and carotene was found to be the predominate serum carotene isomer (Tables 2, 3). This predominance in the serum appears to mimic carotene ratios in fruits and vegetables, where beta carotene is the most abundant carotene isomer.[12] This study demonstrated that, in the short-term, serum alpha and beta carotene levels were sensitive to dietary intakes of carotenes in the form of carrots and an algae-derived beta carotene supplement (Fig. 1, 2). It had been previously shown in longer-term studies that total plasma carotene levels were sensitive to dietary intakes of beta carotene in the form of capsule supplements.[7-9] No plateauing in serum alpha carotene was observed for any carotene treatment during the 7 day treatment phase (Fig. 1). Similar observations were made for serum beta carotene levels except that treatment 4 showed a decline on the last day of treatment (Fig. 2). However, this decline was due to a sharp drop in the serum beta carotene levels of one subject, which then rebounded the following day. Willett[7] showed that with a 30 mg beta carotene supplement, total

plasma carotenoid levels reached a plateau within a period of 8 weeks.

In the final depletion phase of the study, subjects demonstrated rapid declines in both serum carotenes within a few days of returning to the low-carotene diet (Tables 2, 3). This supports the findings of Hartzler[13] who showed in a single subject that total plasma carotenoid levels dropped immediately and rapidly when a low vitamin A diet was fed.

The fact that the half-lives of serum alpha and beta carotene during the depletion periods were 1 to 2 weeks (Table 4) suggests a greater serum sensitivity to carotene intakes than indicated by a previous study which described fluctuations in total plasma carotenoid levels as seasonal.[8]

The finding that higher dose treatments were less effective at raising serum carotene levels per milligram of carotenes fed, as compared to lower dose treatments (Table 5), is in line with an animal study where a 4-fold increase in dietary beta carotene produced a 2.5-fold increase in the carotene content of cow's milk and a 16-fold increase in dose produced only a 4-fold increase.[14] The greater efficiency of encapsulated carotenes extracted from algae at raising serum carotene levels per milligram fed, as compared to carotenes from carrots (Table 5), is in accord with other studies which have shown that carotene is better absorbed from oily solution than food sources such as vegetables.[10-15]

The result that alpha and beta carotene from carrots showed similar rates of increase in their respective serum carotenes, and that alpha carotene from capsules showed a higher rate of increase than beta carotene from capsules (Table 5), contrast other studies which showed beta carotene preferentially absorbed over other carotenoids.[16-18]

The lack of significant alteration in serum vitamin A levels is consistent with the findings of other studies.[7-9].

In light of the current attention paid to the possible role dietary carotenes may play in reducing the incidence of cancer, this study indicates that the discontinuation of dietary carotene intakes can lead to rapid serum decreases in these nutrients within a few days, and that short-term, regular consumption of dietary carotenes can restore serum levels.

Received: June 26, 1985

Acknowledgements: Placebo and beta carotene capsules were provided by Shaklee Corporation, San Francisco, CA. The technical help of Caron Nelson, Tim Howes, Dr. Sriram Vemuri, Robert Skluzacek and Barbara Gifford is gratefully acknowledged. A special thanks to Harriet Nicolay for preparation of the typescript.

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REFERENCES

(1) Committee on Diet, Nutrition, and Cancer. Diet, nutrition and cancer. Washington, D.C.; National Academy Press, 1980.

(2) Peto, R., Doll, R., Buckley, J.D. & Sporn, M.B. - Can dietary beta-carotene materially reduce human cancer rates? Nature. (1981) *290*, 201-208.

(3) Pocock, S.J., «Clinical trials: a practical approach», New York; John Wiley & Sons, (1983) 76-84.

(4) Henry, R.J., «Clinical chemistry, principles and techniques», New York; Harper and Row, (1963) 709.

(5) Systat Manual - Version 1.3. Evanstan, I.L: Systat Inc.

(6) Dunnett, C.W. - New tables for multiple comparisons with a control. Biometrics (1964) *20*, 482-491.

(7) Willett, W.C., Stampfer, M.J., Underwood, B.A., Taylor, J.O. & Hennekens, C.H. - Vitamins A.E. and carotene: effects of supplementation on their plasma levels. Am. J. Clin. Nutr. (1983) *38*, 559-566.

(8) Urbach, C., Hickman, K. & Harris, P.L. - Effect of individual vitamins A, C, E and carotene administered at high levels on their concentration in the blood. Exp. Med. Surg. (1952) *10*, 7-20.

(9) Mathews-Roth, M., Pathak, M.A., Fitzpatrick, T.B., Harbor, L.C. & Kass, E.H. - B-carotene as an oral photoprotective agent in erythrocytic protoporphyria. JAMA (1974) *228*, 1004-1008.

(10) Roels, O.A., Trout, M. & Dujacquier, R. - Carotene balances on boys in Ruanda where vitamin A deficiency is prevalent. J. Nutr. (1958) *65*, 115-127.

(11) Merrow, S.B., Krause, R.F., Browe, J.H., Newhall, C.A. & Pierce, H.H. - Relationships between intake and serum levels of ascorbic acid, vitamin A, and carotene of selected groups of children with physical signs of vitamin deficiencies. J. Nutr. (1952) *46*, 445-458.

(12) Committee on Dietary Allowances, Food and Nutrition Board - Fat-soluble vitamins. «Recommended dietary allowances», Ninth Ed., Washington, D.C.: National Academy of Sciences, (1980): 55-60.

(13) Hartzler, E. - The vitamin A content of human blood plasma as an index of carotene utilization. J. Nutr. (1946) *6*, 381-390.

(14) Thompson, S.Y. - Wissenschaftliche Veröffentlichungen der Deutschen Gesellschaft für Ernahrung. Lang, K. ed. Carotine und Carotenoide. Darmstadt: Dr. Dietrich Steinkopff Verlag, (1963) Vol. *9*, 263.

(15) Fujita, A., Kawaguchi, T. & Kimura, K. - Absorption of carotene in man. J. Vitaminol. (1957) Japan. *3*, 223-234.

(16) Kubler, W. - Studies on the infant absorption of carotene from carrots. Intern. Rev. Vitamin Res. (1958) *29*, 339-357.

(17) Wilson, H.E.C., Gupta, S.M.D & Ahmad, B. - Studies on the absorption of carotene and vitamin A in the human subject. Ind. J. Med. Res. (1937) *24*, 807-811.

(18) Rao, N.C. & Rao, B.S.N - Absorption of dietary carotenes in human subjects. Am. J. Clin. Nutr. (1970) *23*, 105-109.