NUTRITION REPORTS INTERNATIONAL

OBSERVATIONS ON THE EFFECTS OF INGESTING CIS- AND TRANS-BETA-CAROTENE ISOMERS ON HUMAN SERUM CONCENTRATIONS

Christopher D. Jensen, Timothy W. Howes, Gene A.Spiller, Thomas S. Pattison, James H. Whittam, and James Scala

Shaklee Research Center, 1992 Alpine Way, Hayward, CA 94545

An abstract of a portion of this research was published in the Am J Clin Nutr 43, 689 (1986)

ABSTRACT

It has been previously reported that cis rotation reduced by 60% the vitamin A activity of beta-carotene (BC) fed to rats. A similar biopotency reduction is presumed to hold true for humans. Other workers have shown in humans that up to 30% of absorbed BC is found intact in the lymphatic circulation. Consequently, serum BC concentrations can serve as an indicator of BC absorption. In this present study, 16 healthy adults, who had been on a low carotene diet for ten days, were fed either BC extracted from Dunaliella salina alga, containing approximately equal amounts of all-trans-BC (TBC) and 9-mono-cis-BC (CBC), or BC in the form of fresh carrots containing predominately TBC, or avocado oil-placebo capsules. Subjects were randomly divided into three groups: they consumed daily in a single dose either 3 BC capsules (24 mg BC); 207.3 g carrots (24 mg BC); or 3 BC-free placebo capsules for seven days. HPLC determinations of serum trans-cis BC ratios showed TBC to be the predominate serum isomer before and after all treatments. Serum TBC concentrations were significantly increased in the BC capsule ($p<0.02$) and carrot groups ($p<0.01$). CBC concentrations were increased in the carrot and placebo groups ($p<0.05$). However, the serum isomer increments for those taking BC capsules and carrots strongly favored TBC over CBC ($p<0.01$ and $p<0.01$ respectively). These data demonstrate a predominant absorption of intact TBC over intact CBC into human serum even when approximately equivalent amounts of these isomers were ingested. This selective absorption of intact BC isomers may be a factor in their biopotency in humans.

INTRODUCTION

All-trans-beta-carotene (TBC) has been shown to have a greater vitamin A biopotency in rats than the 9-monocis-beta-carotene (CBC) isomer (1). It is presumed that a similar enhanced biopotency of TBC over CBC holds true for humans (2), although supportive data are lacking.

Conversion of beta-carotene (BC) to vitamin A occurs largely in the intestinal mucosa (3). However, Goodman (4) and Blomstrand (5) have shown that in humans up to 30% of absorbed BC is found intact in the lymphatic circulation. Carotenoids are then transported in the blood to their tissue storage sites. Consequently, serum BC concentrations can serve as an indicator of BC absorption.

It is not known whether cis BC stereoisomers are in themselves active or must first be transformed in the digestive tract to the all-trans form. One explanation for the biological activity of the cis-carotene stereoisomer is that during digestion and/or absorption they are indeed converted to the all-trans form (3).

The finding that TBC and CBC isomers from the algae Dunaliella salina are available in approximately equivalent amounts (6), and that TBC predominates in carrots (7) provided an opportunity to observe the effects of ingesting these isomers on their serum concentrations in humans. The data presented here offer insight into the absorption of intact BC isomers into human serum. This may be a factor of importance in their vitamin A biopotency.

MATERIALS AND METHODS

Subjects. Participants were six healthy male and ten female adults, all employees of the Research Center, with ages ranging from 18 to 60 yr (34.3 ± 12.5 yr) and averaging 112.8 ± 16.6% of their ideal body weight (Table I). These data were derived from one of a group of studies in which carotene metabolism was examined and in which these individuals participated as subjects. Other data from these studies are published elsewhere (8,9). The procedures followed were in accord with the Helsinki Declaration, as updated in Tokyo, Japan in 1975.

Only subjects having serum vitamin A levels within normal limits were admitted to the study. Informed consent was obtained.

The subjects were placed on a ten-day low-carotene diet (free of all yellow and green fruits and vegetables, margarine, and colored cheeses) to ensure that there was no

contribution of carotene from previous dietary intake. The diets were freely selected by subjects from lists of appropriate foods. Food records were completed by subjects and examined daily by a dietitian to ensure compliance. The participants' diets were supplemented with multivitamin-mineral tablets (Vita-Lea, Shaklee Corporation, San Francisco, CA) providing approximately U.S.RDA levels of essential vitamins and minerals, but no carotene. Due to the absence of fruits and vegetables in the diet, subjects were encouraged to include commercially available high fiber cereals and crackers in their diets as well as whole-grain breads to facilitate normal bowel function. The fat content of diets was not restricted.

On day six of the low-carotene diet, venous blood specimens were collected and analyzed for serum total BC concentrations. These concentrations were then used to randomize subjects into three groups having similar mean serum total BC concentrations.

Treatments. The three groups were randomly assigned to one of three treatments (Table I). BC and placebo were provided in gelatin capsule form. Capsules contained either BC in a solution of corn oil (MicroBio Resources, Inc., San Diego, CA) or BC-free avocado oil which was sufficiently dark in color to make the capsules indistinguishable. Carrots from the same lot were purchased from a local market, washed, diced, mixed thoroughly, protected from light and refrigerated until used. At nine am on each of the seven treatment days, subjects ingested either three capsules (BC or placebo) or 207.3 g carrot with six oz water.

Table I. Subjects and Treatments.

Treatment	Form	Sex	Age	Carotene Intake Per Day TBC mg	CBC mg	Total BC mg
1	3 capsules per day	3 F 2 M	40±16	9.6	14.4	24.0
2	207.3 g carrots per day	3 F 3 M	33±14	23.5	0.5	24.0
3 (placebo)	3 capsules per day	4 F 1 M	30±6	0	0	0

Ages are expressed as $\bar{x} \pm$ STD. F and M refer to female and male respectively.

Design. The study was double-blind (except of course for the carrots). The experimental period consisted of the initial low-carotene diet phase lasting ten days to ensure no contribution of BC from previous intake, followed by the treatment phase lasting seven days. On the first day of the treatment phase, baseline fasting venous blood specimens were collected. Post-treatment fasting venous blood was collected 24 hours after the ingestion of the final treatment dose.

Laboratory Analyses. Venous blood specimens were collected in silicone coated tubes, allowed to clot for 15-20 minutes, and centrifuged at 2500*g for 10-15 minutes. The serum was protected from light and stored frozen at -20°C for one week prior to analysis. Serum specimens were extracted with hexane using a procedure similar to that of Mathews-Roth (10) with the following modifications: three extractions with hexane were performed instead of two. They were then combined and evaporated using low heat (30-40°C). The residue was reconstituted with 2 ml hexane. Extracts were analyzed immediately by HPLC to determine serum total BC concentrations. HPLC conditions for total beta-carotene determinations are described elsewhere (8). The remaining portions of the extracts were protected from light and stored at -20°C for two weeks prior to HPLC analysis for TBC and CBC isomers present. TBC and CBC were found to be the predominant BC isomers in the serum. Only trace quantities of other cis isomers were detected. Consequently, the ratios of TBC to CBC found in the remaining portions of the extracts were then multiplied by the serum total BC concentrations to yield TBC and CBC concentrations (Table II).

Capsules were cut open and their contents dissolved in methylene chloride (containing 0.1% BHT to prevent isomerization) and diluted with hexane. Carrots were minced and extracted with acetone using a blender. The residue was then extracted with hexane.

The serum, capsule and carrot hexane extracts were analyzed by HPLC to determine the amounts of CBC and TBC isomers present. For these analyses a Vydac 201 TP54 5u C18 column (4.6*250 mm) (Separations Group, Hesperia, CA) was used. The solvent system was 10% acetonitrile in methanol and the flow rate was 1.5 ml/min. A variable wavelength Perkin-Elmer LC-75 detector (Norwalk, CT) was used to monitor the column effluent at 436 nm. An external standard of all-trans-beta-carotene (Sigma Company, St. Louis, MI) was used for quantitation. Monitoring for quantitation at 436 nm compensates for the lower extinction coefficients of cis isomers. Samples of each HPLC peak were collected and their spectra were measured using a CARY

Model 14 recording spectrophotometer (Varian Instruments, Palo Alto, CA). Spectra obtained were then compared to those previously reported (11-13).

Potential Effects Of Analytical Procedures On Isomerization. Base (e.g. alcoholic KOH) is not known to cause isomerization of BC (3). In our laboratory, mild heating for short periods of time (60°C for 20-30 min) has not been observed to produce significant isomerization. In fact, thermal isomerization of BC, albeit in dry form, is performed at 190-200°C for 15 min in an evacuated tube (11). Previous research has shown that BC is relatively stable in hexane solutions (10). In our laboratory, very little isomerization of TBC standard material in hexane has been observed when protected from light and stored refrigerated for two days, or at -20°C for two weeks. In addition, in our laboratory we have observed that the presence of 0.1% BHT in methylene chloride inhibits BC isomerization.

Statistical Analyses. For each treatment, a Student's paired t test was used to determine the significance of serum BC isomer concentration increments and changes in isomer ratios. A two-sample t test was used to compare the significance of the change in CBC concentration to the change in TBC concentration for a given treatment. A two-tailed test was used for each case.

RESULTS AND DISCUSSION

Daily meetings to review diet records with subjects indicated that the low-carotene diet regimen was followed. This was substantiated by the finding that mean serum BC isomer concentrations for the placebo group remained fairly constant during the one week treatment phase (Table II). Despite the dietary alterations including the ommission of fruits and vegetables and emphasis on dietary fiber from wholegrain sources, there were no complaints of altered bowel function. Diet records indicated that liberal amounts of fat were consumed throughout each day and that fat was obtained from a variety of food sources. No subjects adopted a low-fat diet during the experimental period.

After the initial ten-day low-carotene diet phase, the serum TBC/CBC ratios and isomer concentrations strongly favored TBC for all groups (Table II).

Table II. Serum Beta-Carotene Isomer Concentrations and Trans-Cis Ratios.

Treatment	Treatment Period	Serum TBC	Serum CBC	Trans-cis Ratio
1 (capsule)	Pre-	8.35±2.34	0.81±0.21	0.91
	Post-	43.53±10.34**	4.19±2.18	0.91
2 (carrots)	Pre-	10.67±2.26	1.03±0.22	0.91
	Post-	35.38±5.65***	2.77±0.55*	0.93
3 (placebo)	Pre-	9.76±2.82	0.98±0.39	0.91
	Post-	8.07±2.61	1.81±0.24*	0.82

TBC and CBC concentrations are $\bar{x}$ ± SEM expressed in mcg/dl. Asterisks indicate differences between post- and pre-treatment concentrations are significant (*=$p<0.05$) **=$p<0.02$, and ***=$p<0.01$).

Following the ingestion of TBC-rich carrots for seven days, the serum TBC/CBC ratio remained constant (Table II), and both mean serum TBC ($p<0.01$) and CBC ($p<0.05$) concentrations were increased. However, the CBC increment (1.75 mcg/dl) was only 7.1% ($p<0.01$) that of the TBC increment (24.71 mcg/dl).

When BC capsules were ingested over seven days, the TBC/CBC ratio again remained constant (Table II). The mean serum TBC concentration was significantly increased ($p<0.02$), but the increase in mean CBC concentration did not achieve significance. In spite of ingestion of approximately equivalents amounts of each isomer, the CBC increment (3.39 mcg/dl) was only 9.6% ($p<0.01$) that of the TBC increment (35.17 mcg/dl).

The placebo group showed slight fluctuations in mean serum isomer concentrations and the TBC/CBC ratio was decreased somewhat although not significantly (Table II). A slight decrease (1.69 mcg/dl) in the mean serum TBC concentration was observed during the treatment phase but did not achieve significance. The mean serum CBC concentration showed a slight but significant ($p<0.05$) increase (0.83 mcg/dl).

Isomerization of BC stereoisomers can occur upon exposure to heat, acid, light and solvents (3). Therefore, the potential for isomerization during the digestion and/or absorption process (e.g. exposure to gastric acid) or chemical analysis (e.g. solvent extraction) has to be taken

into account. Cautionary steps (see Laboratory Analyses) were taken to minimize the potential for isomerization resulting from the procedures employed in the handling, extraction and analysis of samples (10-13). The pronounced and consistent shift in the serum cis-trans BC equilibrium in favor of TBC supports the contention that significant isomerization from the analytical procedures did not occur. Had substantial isomerization occurred, the shift in the equilibrium would have been in the direction of increasing the amount of cis present (11).

The finding at baseline of a predominance of TBC over CBC in the serum for all groups may be explained by the fact that blood carotenoid levels directly reflect the amounts of carotenoids ingested in the diet (8,9,14-16) and that TBC is the predominate BC isomer found in carotene-rich fruits and vegetables (7). The fact that ingestion of TBC-rich carrots yielded a large increase in mean serum TBC concentration also points to the ability of the body to absorb intact TBC.

However, the pronounced shift toward TBC in the serum cis-trans equilibrium, despite ingestion of approximately equivalent amounts of these BC isomers from capsules, suggests that the intact trans form is preferentially absorbed over intact CBC into human serum.

Deuel (1,17-18) has shown with the rat growth response assay, that with the exception of pro-y-carotene, cis isomers of the provitamin A structures have a lower biopotency than do the naturally occurring all-trans forms. Sweeney and Marsh measured liver vitamin A storage values in rats after feeding various BC stereoisomers and also found a higher biopotency with the all-trans from (19). That a similar enhanced biopotency of the trans forms holds true for humans remains unknown but is assumed based on these animal studies (2).

Goodman (4) and Blomstrand (5) have shown in humans that up to 30% of absorbed BC is absorbed intact. Also, the fact that deposits of BC in fatty tissues throughout the body leading to carotenemia results from excessive carotene ingestion points to the fact that substantial absorption of intact BC can occur in humans. The rat on the other hand differs substantially in the way it processes BC. Workers have previously shown that the rat absorbs little carotene beyond the intestinal mucosa (20). A more recent study showed that rat plasma BC levels increased rapidly during supplementation and decreased rapidly during depletion (21). In contrast, humans taking large doses of BC require 2-4 weeks for plasma

concentrations to level off (22). Furthermore, no evidence of BC accumulation in the subcutaneous fat tissue of supplemented Sprague-Dawley rats has been observed (21), while in humans, BC is known to accumulate in subcutaneous fat. Therefore, the possibility that the rat and man differ in their processing of BC must be considered when extrapolating biopotency results from rat studies to man.

Goodman (4) and Blomstrand (5) also demonstrated that BC absorbed intact was found in association with lymph chylomicrons and newly absorbed retinyl esters. This suggests that BC absorbed intact is derived from the same intestinal mucosal pool of BC from which retinyl esters are also formed. It is possible that only TBC comprises this pool (perhaps due to an inability of the nonlinear cis molecule to pass into the mucosal cell), with some portion eventually being converted to vitamin A and some portion absorbed intact. It seems likely that if both trans and cis forms make up this pool, as would be expected if both forms were ingested in equivalent amounts, both forms would be equally represented in the serum. That this did not occur suggests that either CBC passes unabsorbed through the gastrointestinal tract, CBC is preferentially converted to vitamin A in the intestinal mucosa leaving TBC to be absorbed intact, or that some isomerization of cis isomers to the all-trans form takes place prior to absorption. An inefficiency in this conversion may explain the greater vitamin A biopotency of the all-trans form over the cis form demonstrated in animal studies. These are put forth only as theories which would account for our observations. The data do not allow conclusions to be drawn, but do suggest the need to study the fate of ingested cis and trans BC isomers further.

ACKNOWLEDGEMENTS

The assistance of Barbara Gifford in searching the literature, and the editorial comments of Dr W. Glenn Howells are greatly appreciated. Special thanks is due to Mabel Rich for preparation of the typescript. Placebo capsules were supplied by Shaklee Corporation, San Francisco, CA.

REFERENCES

1. Deuel H.J., Johnston C., Sumner E., Polgar A. and Zechmeister L. Stereochemical configuration and provitamin A activity, I. All-trans-beta-carotene and neo-beta-carotene. U. Arch Biochem 5, 107 (1944).

2. Recommended Dietary Allowances, Committee on Dietary Allowances, Food and Nutrition Board, Commission on Life Sciences, National Research Council, 9th ed., National Academy Press, Washington D.C., 1980.

3. Bauernfeind J.C., Adams R.C. and Marusich W.L. Carotenes and vitamin A precursors in animal feed. In: Bauernfeind J.C., ed. Carotenoids as colorants and vitamin A precursors: technological and nutritional applications, Academic Press, New York, 1981, p. 563.

4. Goodman D.S., Blomstrand R., Werner B., Huang H.S. and Shiratori T. The intestinal absorption and metabolism of vitamin A and Beta-Carotene in Man. J Clin Invest 45, 1615 (1966).

5. Blomstrand R. and Werner B. Studies on the intestinal absorption of radioactive beta-carotene and vitamin A in Man: conversion of beta-carotene into vitamin A. Scand J Clin Lab Invest 19, 339 (1967).

6. Ben-Amotz A., Katz A. and Avron M. Accumulation of beta-carotene in halotolerant algae: purification and characterization of beta-carotene-rich globules from Dunaliella bardawil (Chlorophyceae). J Phycol 18, 529 (1982).

7. Sweeney J.P. and Marsh A.C. Effect of processing on provitamin A in vegetables. J Am Diet Assn, 59, 238 (1971).

8. Jensen C.D., Pattison T.S., Spiller G.A., Whittam J.H. and Scala J. Repletion and depletion of alpha and beta carotene in humans with carrots and an algae-derived supplement. Acta Vitamin Enzymol 7, 189 (1985).

9. Jensen C.D., Spiller G.A., Pattison T.S., Whittam J.H. and Scala J. Acute effects of dietary carotenes on serum alpha and beta carotene in humans. Nutr Rep Int 33, 117 (1986).

10. Mathews-Roth M.M. and Stampfer M.J. Some factors affecting the determination of carotenoids in serum. Clin Chem 30, 459 (1984).

11. Tsukida K., Saiki K., Takii T. and Koyama Y. Separation and determination of cis/trans-beta-carotene by high-performance liquid chromatography. J Chrom 245, 359 (1982).

12. Zechmeister L. and Polgar A. Cis-trans isomerization and spectral characteristics of carotenoids and some related compounds. J Am Chem Soc 65, 1522 (1942).

13. Braumann T. and Grimme, L.H. Reversed-phase high-performance liquid chromatography of chlorophylls and carotenoids. Biochim Biophys Acta 637, 8 (1981).

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

15. Urbach C., Hickman K. and 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 10, 7 (1952).

16. Hartzler E. The vitamin A content of human blood plasma as an index of carotene utilization. J Nutr 6, 381 (1948).

17. Deuel H.J., Johnston C., Meserve E.R., Polgar A. and Zechmeister L. Stereochemical configuration and provitamin activity. IV. Neo-a-carotene B and neo-beta-carotene B. Arch Biochem 7, 247 (1945).

18. Deuel H.J., Johnston C., Sumner E., Polgar A., Schroeder W.A. and Zechmeister L. Stereochemical configuration and provitamin A activity. II. All-trans-y-carotene and pro-y-carotene. Arch Biochem 5, 365 (1944).

19. Sweeney J.P. and Marsh A.C. Liver storage of vitamin A in rats fed carotene stereoisomers. J Nutr 103, 20 (1973).

20. Thompson S.Y., Ganguly J. and Kon S.K. The conversion of beta-carotene to vitamin A in the intestine. Br J Nutr 3, 50 (1949).

21. Shapiro S.S., Mott D.J. and Machlin L.J. Kinetic characteristics of beta-carotene uptake and depletion in rat tissue. J Nutr 114, 1924 (1984).

22. Mathews-Roth M.M., Pathak M.A., Parrish J., Fitzpatrick T.B., Kass E.H., Toda K. and Clemens W. A clinical trial of the effects of oral beta-carotene on the responses of human skin to solar radiation. J Invest Derm 59, 349 (1972).

Accepted for publication: November 25, 1986.