Consumption of canned citrus fruit meals increases human plasma beta-cryptoxanthin concentration whereas lycopene and beta-carotene concentrations did not change in healthy adults

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- 19 Abbreviations
- 20
- 21 AUC; Area under the curve
- 22 BMI; body mass index
- 23 BCO1; 15,15'-beta-carotene oxygenase
- 24 CD36; cluster determinant 36
- 25 HDL; high density lipoprotein
- 26 LDL; low density lipoprotein
- 27 ND; not determined
- 28 SRB1; Scavenger Receptor class B-type 1
- 29 WHNRC; Western Human Nutrition Research Center
- 30

Abstract

Several studies suggest that beta-cryptoxanthin has a greater plasma response from its common food sources than other carotenoids such as beta-carotene and lycopene. The hypothesis of this study is that changes in plasma beta-cryptoxanthin concentrations will be greater than changes in plasma beta-carotene or lycopene concentrations even if these carotenoids are fed in a similar food matrix, such as citrus fruit. We tested this hypothesis by measuring changes in plasma concentrations of beta-cryptoxanthin, lycopene, and beta-carotene after feeding measured amounts of canned tangerines and pink grapefruit to healthy non-smoking adult humans. Volunteers served as their own controls and received both citrus fruit treatments randomly. In the first study, eight subjects ate single meals of 234-304 g tangerines or 60-540 g pink grapefruit. The second study compared changes in plasma carotenoid concentration caused by feeding 234 g tangerines or 540 g pink grapefruit to 11 subjects. Blood was collected 5 times within 24 hr after each citrus meal. Carotenoid concentrations were analyzed by reversed-phase HPLC. Plasma beta-cryptoxanthin concentrations increased within 5 hrs, and then stabilized, remaining high throughout the 24 hrs measured. Plasma concentrations of lycopene and beta-carotene did not change. These results show that beta-cryptoxanthin concentrations increased after a citrus fruit meal, but lycopene and beta-carotene concentrations did not change after a similar citrus fruit meal. These results support our hypothesis that changes in plasma beta-cryptoxanthin are greater than changes in plasma lycopene or beta-carotene, even when these carotenoids are fed in a similar food matrix.

Key words: carotenoid, plasma response, citrus, human, beta-cryptoxanthin, lycopene

1 Introduction

Beta-cryptoxanthin, alpha-carotene, and especially beta-carotene are vitamin A-forming carotenoids found in fruits and vegetables [1,2]. Vitamin A activity of pro-vitamin A carotenoid rich foods depends partially on the absorption of the carotenoid from foods [3 - 5] and on the carotenoid's suitability as a substrate for 15,15'-beta-carotene oxygenase (BCO1) [6]. Thus, differences in carotenoid absorption from a food may be important in determining the amount of vitamin A formed from that food.

Beta-carotene is found in a wide variety of vegetables, fruits, oils, and supplements [7]. The absorption, and thus the bioavailability of beta-carotene in food varies greatly, being highest in oils and lowest in green leafy vegetables. These differences in bioavailability are well known, and are represented by the estimates of retinol activity equivalents, which vary from 1 µg retinol activity:2 µg beta-carotene in oils; to 1 µg:12 µg for beta-carotene in fruits; up to 1 µg:28 µg beta-carotene in vegetables [4,5]. Once absorbed, beta-carotene is a highly effective substrate for BCO1 [6]. Therefore, the decrease of retinyl activity equivalent estimates (from 1 µg:2 µg in oil to 1 µg::28 µg from vegetables) suggest that the absorption and bioavailability of beta-carotene from most fruits and vegetables is relatively poor.

Lycopene is a non-vitamin A forming carotenoid that has a limited distribution in foods, with tomatoes, watermelon, pink grapefruit, pink guava, and papaya being good sources [7]. Lycopene consumption is high in many populations, including those from the United States and much of Europe [8]. The impact of food matrix on lycopene bioavailability is well established.

Specifically, including fat in the food matrix increases bioavailability dramatically. Food processing is also important, with more processed foods tending to have greater bioavailability [9-13]. Even with processing and the addition of fat, the absorption and bioavailability of lycopene is also relatively poor from most foods [9-13].

Beta-cryptoxanthin is found in high concentrations in only a few foods such as tangerines, oranges, and pumpkin [7]. Unlike beta-carotene and lycopene, beta-cryptoxanthin is present in modest amounts (about 1 mg/100 g) even in its richest food sources. Therefore, typical dietary intakes of beta-cryptoxanthin in the United States are lower than intakes of beta-carotene and lycopene. However, despite its scarcity in foods, beta-cryptoxanthin is one of the most abundant carotenoids in blood [14]. The abundance of beta-cryptoxanthin in blood compared to its scarcity in foods suggest that beta-cryptoxanthin may be more bioavailable than beta-carotene and lycopene from common food sources. Currently there is little information on beta-cryptoxanthin absorption and bioavailability from food [15], and indeed only a few studies of any aspect of beta-cryptoxanthin metabolism in humans [3,16-18]. Even so, several studies suggest that beta-cryptoxanthin is better absorbed from foods and more bioavailable than beta-carotene or lycopene [3,14,18].

There are a few reasons why beta-cryptoxanthin may be more bioavailable than other common carotenoids from food. First, beta-cryptoxanthin is a xanthophyll, and therefore more polar and potentially more easily absorbed from food than more hydrophobic carotenes such as beta-carotene and lycopene [1,2]. Second, the most common food sources for beta-cryptoxanthin, beta-carotene, and lycopene differ. A common food source of beta-cryptoxanthin is citrus fruit,

while lycopene is found primarily in tomatoes and beta-carotene in a wide variety of fruits and vegetables [1,2,7,14]. Carotenoids in citrus fruit may be more bioavailable than carotenoids in tomatoes and vegetables. Third, carotenoid cleavage activity in intestinal mucosal cells differs between carotenoids [19], so it is possible that the rate or extent of beta-cryptoxanthin cleavage to its metabolites might be much lower than then lycopene or beta-carotene cleavage.

The hypothesis of these studies is that feeding equivalent amounts of beta-cryptoxanthin, beta-carotene, and lycopene from a similar food matrix will result in higher plasma concentrations of beta-cryptoxanthin compared to the other carotenoids. We tested this hypothesis by comparing the changes in concentrations of plasma beta-cryptoxanthin, beta-carotene and lycopene after feeding healthy well-nourished humans single meals of canned citrus fruit. These studies will generate useful information for scientists investigating carotenoid absorption and metabolism.

2 Methods and materials

2.1 Chemicals

Lycopene, beta-carotene, beta-apo-8-carotenal, and butylated hydroxytoluene were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Beta-cryptoxanthin, alpha-carotene and zeaxanthin were from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Lutein was from ChromaDex, Inc (Irvine, CA, USA). Hexanes, dichloromethane and methanol (HPLC grade) were purchased from Fisher Scientific Inc. (Waltham MA, USA). Ethanol (100%) was

purchased from Decon Labs, Inc. (King of Prussia, PA, USA).; Inter-day variance was measure with human plasma purchased from UTAK Laboratories, Inc (Valencia, CA, USA). Geisha JFE SHOJI Trade America, Inc. canned mandarin oranges segments and Dole Food Company grapefruit segments were purchased from local markets in Davis, California, USA.

2.2 Study Design

Two studies are described. The first study determined the amount of tangerine and pink grapefruit that needed to be consumed to cause carotenoid concentration changes in plasma. It compared carotenoid plasma responses after feeding single meals of 60, 105, or 540 g pink grapefruit versus 234 or 304 g tangerines to healthy adult humans (Table 1). Eight volunteers participated in this crossover study (with one male and one female testing each treatment).

After analyzing results from this study, the second study was conducted as a randomized cross-over single blind study (with researchers analyzing carotenoid concentrations blinded to the intervention) by feeding 540 g pink grapefruit or 234 g tangerines as single meals to 11 volunteers (6 male, 5 female). Aside from the amount of citrus fruit fed during the studies, the studies followed similar procedures for recruiting and feeding subjects, and for blood collection and analysis.

The time lines for both studies are shown in Figure 1. On treatment days (day 14 and 28), subjects came to Western Human Nutrition Research Center (WHNRC) in the morning after an overnight fast. A baseline blood draw (25 mL) was collected by catheter from a forearm vein

by a registered nurse. After the baseline blood draw, subjects were fed a carotenoid-containing fruit (either tangerines or pink grapefruit) as part of a controlled brunch that contained 35% fat and was low in carotenoids. Test meals were fed to the volunteers under the supervision of the WHNRC dietary staff. Subjects received their canned tangerine segments or canned pink grapefruit segments in a randomized fashion, as part of a meal including a bagel, cream cheese, and fruit. Approximately 7 ½ hours after receiving their citrus fruit intervention, subjects were fed a low carotenoid, moderate fat dinner consisting of a chicken burrito, rice, and beans. The next day, subjects ate a breakfast snack of bread and cream cheese (Figure 1). Each subject was fed both citrus treatments, one on day 14 and the other on day 28, separated by a two-week washout period during which subjects consumed their usual diet that excluded foods on the list of high lycopene or high beta-cryptoxanthin containing foods.

2.3 Subjects

Volunteers for both studies were recruited from the Yolo and Sacramento counties, CA, USA through advertisements, postings, and word-of-mouth. Subjects underwent a screening procedure that included a medical history and diet questionnaire, physical examination, and clinical laboratory tests. Subjects were excluded if they had total cholesterol concentrations >6.48 mmol/L (250 mg/dL), total triacylglycerol concentrations >1.98 mmol/L (175 mg/dL), or a red blood cell count, white blood cell count, creatinine concentration or glucose concentration outside of the clinically normal range. Subjects were also excluded if their blood pressure was >150/90 mm Hg, or their body mass index was <18 or >35.

Both studies were conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Institutional Review Committee of the University of California at Davis. Written informed consents were obtained from all subjects. The trial registry name is Human Lycopene and Beta-Cryptoxanthin Absorption from Citrus Fruit, registration identification number NCT02348164, and URL for the registry is <https://clinicaltrials.gov>. Subject demographics and carotenoid concentrations (measured on study day 14) are shown in Table 2.

2.4 Study diet

Subjects were given dietary counseling and a list of foods that are good sources of lycopene and beta-cryptoxanthin, and asked to avoid eating these foods throughout the 29 d study. Both carotenoids are found in a small number of foods, and can be avoided by limiting intakes of tomatoes, red and orange peppers, pumpkin, watermelon, pink guava, pink grapefruit, tangerines, oranges, papayas, and mangos. No attempt was made to limit the amount of beta-carotene consumed from foods because it is found in a great variety of foods, but beta-carotene supplements were prohibited.

Carotenoid concentrations in the experimental diets were analyzed by reversed-phase HPLC, as described below. Otherwise, the macro- and micronutrient composition of the experimental diets were estimated using the Nutrition Data System for Research database (The Nutrition Coordinating Center, University of Minnesota), and are listed in Table 3.

2.5 Plasma collection and analysis

Twenty-five ml aliquots of blood were collected into EDTA tubes from the subjects on - 0.2, 3, 5, 6, 9, 21, and 24 h after the citrus fruit meal (for a total blood collection of 175 mL). Immediately after collection, each blood sample was centrifuged at 1500 g on an RP5 Sorvall Centrifuge (Newton, CT, USA) for 10 minutes (1300x g, 4°C), and the plasma collected and stored at -70°C until analyzed.

Health status at baseline was assessed by blood screening chemistries (complete blood counts and comprehensive metabolic and lipid panels) performed by the University of California Davis Medical Center Pathology Labs during screening. Total, HDL- and LDL cholesterol and triacylglycerol concentrations were also measured with an automated Hitachi analyzer, on days 14 and 28 of the study.

2.6 Carotenoid analysis

Carotenoids (lycopene, lutein, beta-carotene, and beta-cryptoxanthin) were extracted and measured with reverse-phase liquid chromatography using two in-house methods. Carotenoid extraction and analysis were performed under yellow light. Samples were thawed at room temperature.

2.6.1 HPLC analysis for first study

Plasma (500 μ l) was denatured by adding 500 μ l methanol, then extracted with an equal volume of hexane. Then 100 μ L $\sim$ 1 μ g/g beta-apo-8-carotenal was added as internal standard in 50:50 v:v hexane/methylene chloride. The sample mixture was centrifuged for 10 min (1500x g, 4°C). Then the hexane layer was transferred to a new glass tube. The extraction was repeated two times, the hexane layers combined and evaporated to dryness; then the sample was re-suspended in 100 μ L pure methanol. Carotenoid concentrations in citrus fruits were analyzed by the method of Rajendran *et al.* [20], using 1 g samples of drained minced pink grapefruit or tangerine fruit sections, analyzed in triplicate.

HPLC analysis was performed on an Agilent 1100 gradient chromatograph with a binary pump, degasser, refrigerated autosampler, column heater, and diode array detector (Hewlett-Packard series 1100, Waldbronn, Germany). Data analyses were done with an HP Chemstation for LC 3D revision A.08.03 (847) for Agilent Technologies. Separation was run on an YMC 5 μ m C30 ODS 3 250 x 4.6mm reverse-phase column (YMC America, Allentown, PA, USA. It consisted of a binary gradient of 100% methanol (A) and 100% methylene chloride (B) at the flow rate of 1 ml/min. The gradient elution program was initialized at 90% A and 10% B for 5 minutes. After 5 minutes, the gradient was increased linearly to 22%B at 15 min, 38%B at 30 min, 48%B at 40 min, 59%B at 50 min, 62%B from 55 to 58 min, and returned abruptly to 10%B at 58 min, maintained for 7 minutes Rajendran *et al.* [20]. The column temperature was set at 20 °C with detection at 452 nm. Injection volume was 20 μ l.

2.6.2 HPLC analysis for second study

Because we saw no change in lycopene and beta-carotene concentrations after pink grapefruit feeding (even after feeding 540 g grapefruit), we extracted and analyzed samples from 11 subjects from the second study using a second, more sensitive method. In this method, 600 μ L ethanol (with 0.25% butylated hydroxytoluene) was added to 500 μ L of sample in a 15 mL centrifuge tube. Then 100 μ L $\sim$ 1 μ g/g beta-apo-8-carotenal was added as internal standard in hexane/methylene chloride. Sample preparations were vortexed for 30 s, then 300 μ L DI water and 2 mL hexane were added and vortexed 1 min. Preparations were centrifuged at 2280 rpm for 5 min. The top layer was collected and placed into a second 15 mL centrifuge tube, and the extraction repeated. The extracts were combined, evaporated under N, and reconstituted with 50 μ L methylene chloride with 20 s vortexing followed by 50 μ L methanol, also vortexed 20 s; Rajendran *et al.* [20].

HPLC conditions were similar to those given above for the first method [20], using a similar with the following modifications. Chromatography was run on a YMC Carotenoid S3 4.6 x 250 mm column with a YMC Carotenoid S5 4.0 x 20mm guard column on an Agilent 1260 (Agilent, San Jose, CA, USA), with column temperature at 25 $^{\circ}$ C, and 1 mL/min flow rate. The binary gradient and run times were identical to those of the first method. Injection volume was 70 μ L. Analysis was with diode array detection set at 452 nm for beta-carotene, beta-cryptoxanthin and their isomers, and 476 for lycopene and its isomers. Chromatograms of plasma from the 5th hour blood draw for typical subjects are provided as supplementary material in Appendix A.

2.7 Statistical analyses

The experimental group size was estimated using a two-tailed power calculation (SAS, Cary NC) with effect $1.3 \times \sigma$, $\alpha = 0.05$, $\beta = 0.20$) based on a study comparing the apparent absorption of beta-cryptoxanthin to other carotenoids [14]. The power calculation suggested a minimum group size of 8. However, we enrolled 11 subjects.

Carotenoid concentrations were measured over 24 hrs. Areas under the concentration-time curve (AUCs) were calculated for each individual using a trapezoidal approximation after subtracting the appropriate baseline carotenoid concentration. The individual AUCs were combined and the means $\pm$ SEM of AUCs arising from each citrus fruit meal calculated.

Time points were compared by 2-way ANOVA using GraphPad Prism Statistical Software (GraphPad Software, San Diego, CA, USA). Significant differences between time points were determined with Tukey's multiple comparison test. Statistics comparing citrus food intervention maximum peak concentrations and mean AUCs were calculated with SAS version 9 (Cary, NC, USA), with significance set at $p=0.05$.

3 Results

3.1 Subject characteristics

The demographic characteristics and plasma carotenoid concentrations of the volunteers on study day 14, before the first citrus fruit intervention, are shown in Table 2. These results

show that the subjects in both studies had baseline cholesterol, triacylglycerol, and glucose concentrations in the normal range, and body mass index (BMI) suggesting that they were had body weights in the normal or somewhat over weight range. Baseline blood pressure, complete blood counts, creatinine, protein, calcium, albumin, sodium, potassium, alkaline phosphatase, and tests of liver function such as total bilirubin, alanine transaminase, and aspartate aminotransferase were all in the normal range (data not shown). Thus, all the volunteers from both studies were healthy and appeared to have normal liver functions, and cholesterol and triacylglycerol concentrations.

3.2 Results of first study

In the first study, an attempt was made to match the citrus fruit interventions fed the subjects, so that the concentration of beta-cryptoxanthin in the tangerines was similar to the concentration of lycopene in the pink grapefruit (see column for 60 g pink grapefruit, Table 3). The meals were also very similar in proximate composition (kcal, fat, carbohydrate, protein, P/S ratio). The concentration of naringenin, a polyphenol that is believed to influence the absorption of some medicines and supplements [21,22], was also similar.

Both tangerine interventions (234 or 304 g) increased plasma concentrations of beta-cryptoxanthin, in all individuals. The increase of plasma concentrations did not differ between the 234 and 304 g treatments, presumably because of the small number of subjects tested.

However, the 60 g pink grapefruit intervention resulted in no detectable increase in either plasma lycopene or beta-carotene. The 105 g pink grapefruit intervention resulted in a very small increase of lycopene (maximum change 0.01 and 0.03 μmol , respectively for the two subjects tested) and again no change in beta-carotene concentrations. After the pink grapefruit intervention was increased to 540 gm, the maximum change in plasma lycopene concentrations increased to 0.02 and 0.06 μmol , respectively for the two subjects tested while beta-carotene concentrations increased 0.01 and 0.02 μmol . Thus, the results showed that lycopene concentrations might have increased slightly after the pink grapefruit intervention, although the concentration changes were very small. We considered feeding an even larger grapefruit meal, but decided that we could not increase the size of the pink grapefruit meal, since it was already > than 500 g (2 ½ cups) of drained pink grapefruit, which is a very large serving of this food. These results meant that we used the largest grapefruit serving size tested for the second study, which increased the lycopene, beta-carotene, naringenin and kcals in the pink grapefruit intervention diet (Table 3). We also developed a more sensitive HPLC method that we hoped would be able to measure very small changes in carotenoid concentrations.

3.3 Results from second study

3.3.1 Plasma carotenoid concentrations

Figure 2A shows carotenoid concentrations after feeding the tangerine meal. Plasma beta-cryptoxanthin concentrations increased after the tangerine meal. Lycopene and beta-carotene concentrations did not change after the tangerine meal, as expected.

Figure 2B shows carotenoid concentrations after feeding the pink grapefruit meal. Beta-cryptoxanthin, lycopene, and beta-carotene concentrations did not change after the subjects were fed pink grapefruit (Figure 2B).

A comparison of Figures 2A and B shows that even though the lycopene concentration in the grapefruit intervention was much greater (about 7X >) than the beta-cryptoxanthin concentration in the tangerine intervention, the tangerine intervention resulted in increased beta-cryptoxanthin concentrations, while the pink grapefruit intervention did not increase lycopene or beta-carotene concentrations. Similarly, beta-carotene concentrations did not change after the pink grapefruit intervention, despite the fact that beta-carotene concentrations in the pink grapefruit intervention were about 5X > than beta-cryptoxanthin concentrations in the tangerine intervention.

3.3.2 Changes in beta-cryptoxanthin concentration during tangerine and pink grapefruit interventions

Plasma beta-cryptoxanthin concentration changes after each dietary treatment are shown in Figure 3. Plasma beta-cryptoxanthin concentrations increased within 3 hours, then plateaued at 5 hr after subjects were fed tangerines. Beta-cryptoxanthin concentrations remained high from 5 to 24 hr. This pattern occurred in all subjects, although the amount of the beta-cryptoxanthin concentration increase varied between subjects, as is typical for carotenoids [4,6,14,18].

4 Discussion

4.1 Pink grapefruit as a source of beta-carotene and lycopene

The hypothesis of these studies was that feeding equivalent amounts of beta-cryptoxanthin, beta-carotene, and lycopene from citrus fruit would result in higher plasma concentrations of beta-cryptoxanthin compared to other carotenoids. Therefore, the studies compared human plasma carotenoid response to two similar citrus fruit meals: canned pink grapefruit (a good source of beta-carotene and lycopene) and canned tangerines (a good source of beta-cryptoxanthin). In the first study, we attempted to match the concentrations of beta-carotene and lycopene in the citrus fruit meals. However, the pink grapefruit meal did not change carotenoid concentrations. The size of the pink grapefruit meal was then increased up to 540 g. Even after feeding this large amount of grapefruit, results from both studies showed that canned pink grapefruit did not increase plasma carotenoid concentrations over baseline.

This result suggests that canned pink grapefruit is a poor dietary source of lycopene and beta-carotene. Although we had predicted a somewhat greater plasma response to the grapefruit meal, this result was not unexpected, because both beta-carotene and especially lycopene are known to be poorly absorbed from many foods unless the carotenoid is emulsified or well-mixed with fat [2,11,12,23–29]. In this study, the pink grapefruit was minimally processed and was not emulsified with fat.

4.2 *Tangerines as a source of beta-cryptoxanthin*

Plasma beta-cryptoxanthin concentrations increased approximately 45% after the tangerine meal. People varied in the change of plasma beta-cryptoxanthin concentration after treatment, as is typical for this and other carotenoids [2,6,8,17,18,29-33]. However, every volunteer showed measurable increases in plasma beta-cryptoxanthin. These results suggest that canned tangerines are a good dietary source of beta-cryptoxanthin. The results from these studies support the hypothesis that feeding equivalent amounts of beta-cryptoxanthin, beta-carotene, and lycopene from citrus fruit results in higher plasma concentrations of beta-cryptoxanthin compared to these other carotenoids.

4.3 *Possible mechanisms controlling differences in carotenoid plasma response*

These results suggest that beta-cryptoxanthin itself may be more easily absorbed from food than lycopene or beta-carotene. They are consistent with other studies that also suggest that beta-cryptoxanthin specifically, or xanthopylls in general, are better absorbed from foods, or more bioavailable than carotenes such as lycopene and beta-carotene [3,14,18,34-38].

There are a couple of alternative hypotheses that could explain the greater plasma response of beta-cryptoxanthin, compared to beta-carotene and lycopene. First, beta-cryptoxanthin clearance from plasma might be much slower than the plasma clearance of beta-carotene or lycopene. This explanation is unlikely, because the clearance rates of beta-carotene and beta-cryptoxanthin from healthy adults appear to be similar, with $t_{1/2}$ 37 versus 39 d in

399 carotenoid depleted women for beta-carotene and beta-cryptoxanthin, respectively [2,39].
400 Second, beta-carotene, lycopene, and beta-cryptoxanthin can all be cleaved by carotenoid
401 cleavage enzymes, most importantly by BCO1 [1,4,6,19,33,40,41]. Theoretically, carotenoid
402 cleavage could be fast and effective for both beta-carotene and lycopene, but not beta-
403 cryptoxanthin. However, subjects were fed high doses of lycopene and beta-carotene in our
404 second study, so this hypothesis would require that almost all of the lycopene and beta-carotene
405 fed was cleaved before it appeared in plasma. This is unlikely. Stable isotope and radioisotope
406 studies of beta-carotene metabolism show that some beta-carotene is cleaved rapidly, but most is
407 not cleaved into metabolites even when fed in very low doses [30,31,42,43]. Thus, there is little
408 evidence to suggest that either beta-carotene or lycopene are efficiently and completely cleaved
409 to metabolites in most people. Reports of poor carotenoid absorption from food, on the other
410 hand, are common [1,2,4-6,10,14,18,23]. Thus, differences in carotenoid cleavage efficiency
411 might contribute to the differences in plasma response seen in this study, but differences in
412 carotenoid absorption from food appear to be larger and more important.

413
414 The most likely reason for the greater plasma response of beta-cryptoxanthin from citrus
415 fruit (compared to lycopene and beta-carotene) is its chemical structure. Unlike beta-carotene
416 and lycopene, which are carotenes, beta-cryptoxanthin is an oxygenated carotenoid, a
417 xanthophyll [1,2]. The presence of this oxygen moiety is expected to increase the hydrophilicity
418 of beta-cryptoxanthin compared to carotenes [1,2]. To be absorbed a carotenoid must be freed
419 from its food matrix, emulsified into oil droplets, and then taken up by cells of the intestinal
420 lining. Carotenoids appear to be absorbed into the intestine by two mechanisms: facilitative

transport assisted by enzymes such as Scavenger Receptor class B-type 1 (SRB1) and cluster determinant 36 (CD36) [35,44], and by passive diffusion at higher concentrations [45,46].

SRB1 preferentially facilitates the absorption of xanthophylls (such as beta-cryptoxanthin) compared to carotenes (such as beta-carotene and lycopene) [35,44]. Moreover, polar, more hydrophilic carotenoids such as beta-cryptoxanthin are located nearer the exterior of the lipid micelle where they are more available for absorption. Indeed, the percentage of beta-cryptoxanthin formed into micelles during *in vitro* digestion is 3X> than for beta-carotene under similar conditions [47].

The chemical structure of beta-cryptoxanthin also appears to increase its stability during digestion, compared to carotenes such as lycopene and beta-carotene [37]. Finally, beta-cryptoxanthin appears to be less likely to appear in crystalline form in foods than lycopene or beta-carotene [48]. Thus, there are several potential mechanisms that could result in a greater plasma response of beta-cryptoxanthin, compared to lycopene or beta-carotene.

4.4 Effects of food matrix on carotenoid absorption

The results from these studies show that the plasma response of beta-cryptoxanthin to a citrus meal appears to be greater than plasma responses of beta-carotene and lycopene to a citrus meal. These results do not mean that food matrix is not important in beta-cryptoxanthin plasma response. Beta-cryptoxanthin plasma response, absorption, and bioavailability can be influenced by the consistency, fat content, and degree of processing in a food [3,34,38,49-52]. In this

respect, beta-cryptoxanthin is similar to other carotenoids such as beta-carotene and lycopene. The plasma response of beta-cryptoxanthin, like the plasma response of other carotenoids, appears to be determined by a complex interaction of food matrix, chemical structure, and genetics [1,2,10-13,23-28,53-55]. However, the greater plasma response for beta-cryptoxanthin was generated in individuals who served as their own controls, and were fed carotenoids from a similar food matrix. These results suggest that the structure of beta-cryptoxanthin is important in its plasma response.

4.3 Limitations

One important limitation of this study is that it assumes that canned tangerines and canned pink grapefruit share a similar food matrix. Although canned tangerines and pink grapefruit appear to be similar foods, they still might differ in ways that influence carotenoid plasma response. A second limitation is that we began these studies by matching lycopene and beta-cryptoxanthin concentrations in the citrus fruit meals, but then increased lycopene concentrations substantially because of the poor plasma response from the pink grapefruit meals given to the first four subjects of the first study. Increasing the pink grapefruit meal size meant that the citrus meals were less well matched than originally planned, potentially weakening the conclusions of the studies. A third limitation is that we were not able to measure carotenoid cleavage metabolites, such as vitamin A.

4.4 Conclusion

The results of these studies show that beta-cryptoxanthin plasma response is greater than the plasma response of beta-carotene or lycopene after feeding similar citrus fruit meals to the same individuals. These results suggests that the biochemical structure of beta-cryptoxanthin itself contributes to its greater plasma response. This information may be useful for scientists investigating the absorption and metabolism of this vitamin A-forming carotenoid.

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Table 1. Dietary interventions given in study 1.

Citrus fruit	Amount (g)	Beta-cryptoxanthin (mg)	Beta-carotene (mg)	Lycopene (mg)
Pink grapefruit	60	Trace	0.4	1.8
Pink grapefruit	105	Trace	0.7	3.2
Pink grapefruit	504	Trace	5.7	15.1
Tangerine	234	1.4	0.2	trace
Tangerine	304	1.8	0.3	trace

Eight volunteers participated in this crossover study, with one male and one female testing each treatment combination. Treatments consisted of one citrus fruit meal fed with brunch. Each subject received one treatment at random, then the alternate treatment after a 14 day washout period, as described in the text.

Treatment combinations were:

A: 60 g pink grapefruit versus 234 g tangerines

B: 60 g pink grapefruit versus 304 g tangerines

B: 105 g pink grapefruit versus 304 g tangerines

C: 504 g pink grapefruit versus 234 g tangerines

Table 2. Baseline characteristics of the subjects

	Study 1 ^a		Study 2 ^a	
Sex ratio (male : female)	4 : 4		6 : 5	
Age (years)	33.8	3.1	30.4	2.6
Body mass index (kg/m ²)	23.7	0.9	24.5	1.0
Total Cholesterol (mmol/L) ^b	4.78	0.26	4.45	0.23
(mg/dL)	185	10	172	9
HDL (mmol/L) ^b	1.50	0.18	1.32	0.05
(mg/dL)	58	7	51	2
LDL (mmol/L) ^b	2.87	0.10	2.66	0.16
(mg/dL)	111	4	103	6
Triacylglycerol (mmol/L) ^b	0.85	0.09	0.99	0.11
(mg/dL)	75	8	88	10
Glucose (mmol/L) ^b	4.77	0.06	4.16	0.17
(mg/dL)	86	1	75	3
β-Cryptoxanthin (μmol/L) ^c	0.08	0.01	0.10	0.01
Lycopene (μmol/L) ^c	0.24	0.06	0.21	0.03
β-Carotene (μmol/L) ^c	0.35	0.05	0.39	0.08
Retinyl palmitate (μmol/L) ^c	1.86	0.11	ND ^c	ND

^a Values are means $\pm$ SEM. Study 1, n = 8. Study 2, n = 11. Total triacylglycerol, glucose, and total, HDL- and LDL cholesterol concentrations were measured with an automated Hitachi analyzer and are shown for study day 14.

^b Analyzed by HPLC, methods described in text for study 1 and 2, respectively. Data is shown for study day 14.

^c ND = not determined

Table 3. Composition of the standardized meals

	Tangerine	Pink	Pink
	(234 g) ^a	Grapefruit ^b	Grapefruit ^c
		(60 g)	(540 g)
Energy (Kcal) ^d	2038	2070	2252
Energy (KJ) ^d	8525	8660	9424
Total Fat (g) ^d	80	90	91
Total Carbohydrate (g) ^d	252	234	280
Total Protein (g) ^d	86	87	90
P/S ratio ^d	0.78	0.99	0.99
Alcohol ^d	0	0	0
Retinol (mg) ^d	0.4	0.4	0.6
Beta-cryptoxanthin (mg) ^e	1.4	0.01	0.04
Lycopene (mg) ^e	0.0	1.8	15.1
Beta-carotene mg) ^e	2.2	2.6	5.7
Alpha-carotene (mg) ^e	1.7	1.4	1.4
Lutein/Zeaxanthin (mg) ^e	1.1	0.77	0.79
Hesperetin (mg) ^d	18	0.4	1.5
Naringenin (mg) ^d	23	32	172
Luteolin (mg) ^d	0	0.6	3.2
Quercetin (mg) ^d	0	0.19	1.0
Anthocyanins (mg) ^d	0	0	0

^aAmount of tangerines ingested in the second study.

^bAmount of pink grapefruit ingested by the first four subjects participating in the first study, chosen to match the lycopene concentration of the pink grapefruit meal with the beta-cryptoxanthin concentration in the tangerine meal.

^cAmount of pink grapefruit ingested in the second study.

^dEstimated with the Nutrition Data System for Research database (The Nutrition Coordinating Center, University of Minnesota).

^eAnalyzed by HPLC, Rajendran *et al* method [19] on Agilent 1100, on diet composites for the brunch fed on study days 14 and 28. Values listed are means (n=3).

Figure Legends

Figure 1. Time line for studies. Top time line shows days that citrus treatments were given and blood draws collected. Bottom time line shows times of blood collections and dietary interventions.

Figure 2. Effect of citrus meals on plasma carotenoid concentrations. Figure 2A: plasma carotenoid concentrations after ingesting the tangerine meal. Figure 2B: plasma carotenoid concentrations after ingesting the pink grapefruit meal. ● = Plasma beta-cryptoxanthin concentration. ○ = plasma lycopene concentration. ▽ = plasma beta-carotene concentration. Values are means $\pm$ SEM (n = 11). * = significant difference from baseline concentration, p = 0.05.

Figure 3. Change in beta-cryptoxanthin concentrations after consuming citrus meals. ● = Change in plasma beta-cryptoxanthin concentrations with time after consumption of tangerine meal. ○ = Change in plasma beta-cryptoxanthin concentrations after consumption of pink grapefruit meal. Values are means $\pm$ SEM of the change in beta-cryptoxanthin concentration after subtraction of its baseline concentration (n=11). * = significant difference from baseline, p = 0.05.

Appendix A. Chromatographs of plasma extracts from a typical participant after the pink grapefruit (top) and mandarin orange (bottom) treatments. Chromatographs are generated by the Rajendran *et al* method [19], using Agilent 1260 as described in text as the second HPLC method. Peaks were identified by co-migration with authentic standards, and by diode array scans. Peak identifications: (1) *cis*-lutein, (2) all-*trans*-lutein, (3) all-*trans*-zeaxanthin, (4), (5) unidentified isomers, (6) beta-apo-8-carotenal standard, (7) all-*trans*-alpha-cryptoxanthin, (8) all-*trans*-beta-cryptoxanthin, (9) *cis*-beta-carotene, (10) all-*trans*-alpha-carotene, (11) all-*trans*-beta-carotene, (12) 13-*cis*-lycopene, (13) unknown-*cis*-lycopene, (14) 15-*cis*-lycopene, (15) 9-*cis*-lycopene, (16) 5-*cis*-lycopene, (17) all-*trans*-lycopene.

Figure 1. Timeline of studies

Time line for interventions, for studies 1 and 2.

Time line for blood draws (± 30 min), for studies 1 and 2:

Day 14 and Day 28

Day 15 and Day 29

0hr--X-----3hr-----5hr-----7hr---Y-----9hr-----21hr---Z---24hr- Blood draw

10:30AM 1:30PM 3:30PM 5:30PM 7:30PM 7:30AM 10:30AM Time of day for blood draw

X = Citrus interventions (pink grapefruit or tangerines)

Y = Dinner, 6:30 PM

Z = Breakfast, 8:30 AM

Figure 2A

Figure 2B

Figure 3.

Appendix A

Chromatographs of plasma extracts of a typical participant after the pink grapefruit (top) and mandarin orange (bottom) treatments.

(1) *cis*-lutein, rt=9.4 min (2) *all-trans*-lutein, rt=9.9 min (3) *all-trans*-zeaxanthin, rt=11.7 min (4), (5) unidentified isomers (6) beta-apo-8-carotenal standard, rt=13.8 min (7) *all-trans*-alpha-cryptoxanthin, rt=15.4 min (8) *all-trans*-beta-cryptoxanthin, rt=18.3 min (9) *cis*-beta-carotene, rt=21.2 min (10) *all-trans*-alpha-carotene, rt=23.0 min (11) *all-trans*-beta-carotene, rt=25.8 min

(12) 13-*cis*-lycopene, rt=36.7 min (13) unknown-*cis*-lycopene, rt=40.7 min (14) 15-*cis*-lycopene, rt=41.1 min (15) 9-*cis*- lycopene, rt=41.9 min (16) 5-*cis*-lycopene, rt=42.6 min (17) all-*trans*-lycopene, rt=46.9 min