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Enhanced bioavailability of lycopene when consumed as *cis*-isomers from *tangerine* compared to red tomato juice, a randomized, cross-over clinical trial

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Abstract

Scope—*Tangerine* tomatoes (*Solanum lycopersicum*) are rich in tetra-*cis*-lycopene resulting from natural variation in carotenoid isomerase. Our objective was to compare the bioavailability of lycopene from *tangerine* to red tomato juice, and elucidate physical deposition forms of these isomers in tomatoes by light and electron microscopy.

Methods and results—Following a randomized crossover design, subjects ($n=11$, 6M/5F) consumed two meals delivering 10 mg lycopene from *tangerine* (94% *cis*) or red tomato juice (10% *cis*). Blood was sampled over 12 hours and triglyceride-rich lipoprotein fractions of plasma (TRLs) were isolated and analyzed using HPLC-DAD-MS/MS. Lycopene was crystalline in red tomato chromoplasts and globular in *tangerine* tomatoes. With *tangerine* tomato juice we observed a marked 8.5-fold increase in lycopene bioavailability compared to red tomato juice ($P<0.001$). Fractional absorption was $47.70 \pm 8.81\%$ from *tangerine* and $4.98 \pm 1.92\%$ from red tomato juices. Large heterogeneity was observed among subjects.

Conclusions—Lycopene is markedly more bioavailable from *tangerine* than from red tomato juice, consistent with a predominance of *cis*-lycopene isomers and presence in chromoplasts in a lipid dissolved globular state. These results justify using *tangerine* tomatoes as a lycopene source in studies examining the potential health benefits of lycopene-rich foods.

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Keywords

bioavailability; carotenoids; chromoplast; *tangerine* tomato; tetra-*cis*-lycopene

1 Introduction

Epidemiological evidence suggests that diets rich in tomatoes and tomato products may be protective against risk for certain cancers, especially prostate cancer [1]. The carotenoid lycopene has received the most attention as the compound in tomatoes responsible for this noted decrease in cancer risk. In raw, red tomatoes, approximately 95% of the total lycopene is present in the all-*trans* form [2]. Despite the predominance of dietary all-*trans*-lycopene, *cis*-isomers account for 58-73% of total lycopene in human serum, and a surprisingly high 79-88% in benign or malignant prostate tissue [3]. This observation is related to isomerization *in vivo*, as demonstrated by conventional [4] and tracer studies [5,6], but also to the preferential absorption of *cis*-lycopene [7], with the exact mechanisms yet to be elucidated. Compared to the all-*trans* forms, *cis*-lycopene isomers are less likely to crystallize, more oil/hydrocarbon soluble, [8] preferentially micellized [9], and are more readily taken up by intestinal cells [10].

In contrast to red tomatoes, *tangerine* tomatoes are unique *cis*-lycopene rich tomatoes developed through conventional breeding techniques (Figure 1). These tomatoes have the recessive mutation *tangerine* [11] and lack a functional form of the enzyme carotenoid isomerase (CRTISO), which converts poly-*cis*- into all-*trans*-lycopene, and is necessary for the biosynthesis of downstream cyclized carotenoids [12]. As a result, *tangerine* tomatoes accumulate several *cis*-lycopene isomers, with tetra-*cis*-lycopene ((7*Z*, 9*Z*, 7'*Z*, 9'*Z*)-lycopene, also called prolycopene) predominating at the expense of the all-*trans* form. Tetra-*cis*-lycopene absorbs light maximally approximately 35 nm below all-*trans*-lycopene, resulting in tomatoes with an orange color (Figure 1, A2) [13]. Most processed foods containing tomato have concentrations of *cis* isomers less than 10% [14] although severe food processing conditions can increase the percentage of *cis*-lycopenes and thus increase bioavailability [7]. Stemming from this result, others have suggested tetra-*cis*-lycopene may be more bioavailable than all-*trans* [15].

In order to manifest their potential beneficial health effects, carotenoids must be liberated from the food matrix and subsequently solubilized into mixed micelles before they can be absorbed. In common red tomatoes, lycopene is found in large crystalline aggregates of up to 15 µm length within chromoplasts, the cellular organelle where carotenoids are biosynthesized and deposited [16]. Carotenoid liberation and solubilization from such crystals was hypothesized to be significantly lower when compared to lycopene from smaller aggregates [23]. This suggests that chromoplast morphology may play a role in post-prandial bioavailability of carotenoids. Non-crystalline deposition of lycopene is rarely found in natural plant foods since all-*trans*-lycopene easily crystallizes. Therefore, most common lycopene containing fruits (red tomatoes, watermelon, and red-fleshed papaya) have crystalline lycopene aggregates [16,17]. Often, in order to have lipid-dissolved carotenoids a high concentration of fat is required, as previously reported for carotenoids in peach palm (*Bactris gasipaes* Kunth) fruits [18]. Although *tangerine* tomatoes contain

negligible amounts of fat, tetra-*cis*- and other *cis*-isomers are expected to be deposited in a non-crystalline form. A lipid-dissolved deposition state within small lipid globules (plastoglobules) of *tangerine* chromoplasts has been suggested [17], although simultaneous investigations of the carotenoid profile and the chromoplast ultrastructure has not been conducted. Due to the importance for bioavailability, the first goal was to compare the carotenoid profiles and chromoplast ultrastructures of our red and *tangerine* tomatoes.

The main objective of this study was to evaluate, on an equal dose basis, the bioavailability of lycopene from *tangerine* tomato juice compared to red tomato juice in humans. This approach allows a direct comparison of the bioavailability of *cis*- vs *trans*-lycopene from a natural source. In addition, it allows inferences to be made about the relationship between lycopene *cis* isomer composition in conjunction with carotenoid chromoplast morphology and its resulting effect on bioavailability in humans. *Tangerine* tomatoes also contain considerable levels of phytoene, phytofluene, ζ -carotene and neurosporene, in addition to other mono-, di- and tri-*cis*-lycopene isomers in comparison to all-*trans*-lycopene containing red tomatoes. Our study also provides an opportunity to examine the absorption of these carotenoids from tomato products although the juices are not equal in concentrations, as the juice products were matched only in total lycopene. It is hypothesized that these carotenoids upstream in the biosynthesis of tomato products, especially phytoene and phytofluene, may also play a role in disease prevention [15, 16].

2 Materials and Methods

2.1 Microscopy

Light microscopy was performed as previously described by Schweiggert et al. [16]. Free hand sections of raw *tangerine* and red tomato mesocarp close to the skin were viewed without staining on a Leica DM IRB microscope (Buffalo Grove, IL, USA) equipped with a Q Imaging Retiga 2000 (Surrey, British Columbia, Canada). Transmission electron microscopy (TEM) sample preparation was performed according to Schweiggert et al. [16]. Raw tomato samples were viewed using a Hitachi H-7500 TEM (Hitachi High-Tech, Tokyo, Japan) at 80 kV. Photoshop CS4 (Adobe Systems, San Jose, CA, USA) was used to adjust contrast and brightness of the micrographs.

2.2 *Tangerine* and red tomato juices

Tangerine tomatoes (*Solanum lycopersicum* L. hybrid FG10-314) and red tomatoes (*Solanum lycopersicum* L., hybrid derived from OH8245xOH8243) were grown at the OSU's North Central Agriculture Research Station, Fremont, OH. These tomatoes were harvested, processed into juice, salted (7.4 g NaCl/L juice) and hot break processed at 93 °C, held for 3 min and hot filled into cans in the OSU Food Industries Center Pilot Plant (Columbus, OH, USA).

2.3 Subjects

A total of 11 subjects completed both intervention arms of this clinical trial in the fall of 2012 (Columbus, OH, USA). One subject dropped out after completing one of the two day-long visits due to an adverse effect determined to be unrelated to the study agent. Subject

number was calculated using previously published data [21,22] to provide >80% power with $\alpha = 0.05$. Volunteers (6 male, 5 female) were healthy, non-pregnant, non-smoking, normocholesterolemic (<200 mg/dL), normolipidemic (<200 mg/dL), hemoglobin and hematocrit levels at or above 10 g/dL and 30% respectively, and had BMIs between 18.5-30 kg/m². Additionally, subjects were free of any metabolic disease (including diabetes mellitus or thyroid dysfunction), malabsorption disorders (including ileus, Crohn's disease, ulcerative colitis and pancreatic insufficiency), history of cancer, esophageal, gastric or intestinal ulcers, history of liver or kidney insufficiency/failure, autoimmune disorders, chronic inflammatory syndromes (including rheumatoid arthritis) and allergies to tomatoes or tomato products. Baseline subject characteristics can be found in Table 1. At the initial visit, subjects were screened to ensure they met inclusion criteria using a Dimension Xpand Plus Automated Clinical Chemistry Analyzer (Siemens, New York, NY, USA) and LH 780 hematology analyzer (Beckman Coulter, Brea, CA, USA).

Informed consent was obtained from all subjects prior to any study activity and all study procedures were performed at The Ohio State University's (OSU) Clinical Research Center (CRC). This study was approved by the OSU Institutional Review Board (IRB, protocol #2012H0189), the CRC (Clinical Center for Translational Science ID #1995) and registered with ClinicalTrials.gov (NCT01696773).

2.4 Study design

This study was conducted using a randomized, two-way cross-over design. All subjects were provided with a list of lycopene-containing foods to avoid and were instructed to consume a low-lycopene diet for 14 days prior to each day-long clinic visit to sufficiently washout and avoid carryover of carotenoids from previous meals [23]. Subjects refrained from consuming any foods with more than 1 mg/serving of lycopene, according to the USDA Nutrient Database and Standard Reference Release 25. Subjects were instructed to fast overnight (12 hr) prior to their first day-long visit. After drawing a sample of baseline blood (0 hr) via lower arm catheter into glass, EDTA vacutainer[®] tubes (BD and Co., Franklin Lakes, NJ, USA) subjects were instructed to consume their tomato juice containing breakfast within 20 min. Blood was then taken at 2, 3, 4, 5, 6, 8, 10 and 12 hrs with lunch at 4.5 hrs. No snacks were permitted during the 12-hr collection period and subjects were allowed to consume water *ad libitum*. Subjects then left the clinic, continued their washout diet for the next 14 days and returned to the clinic for their second day-long visit to consume the test meal with the tomato juice they did not consume on their first day-long visit.

2.5 Test meal composition

Tomato juices were provided in conjunction with a breakfast at the CRC. Breakfast contained either red or *tangerine* tomato juice providing 10 mg of total lycopene (94 g from red tomato juice or 505 g from tangerine tomato juice), 10 g of canola oil, 1 English muffin (57 g), 2 large egg whites (66 g) scrambled without any fat, 1 banana (118 g), fat-free vanilla Greek yogurt (170 g), honey (14 g) and coffee (356 g) with non-fat creamer and sugar. Breakfast provided 521 kcal, 30 g protein, 11.7 g fat, 77 g carbohydrates and 5.7 g fiber. A very low-fat lunch was provided at 4.5 hours which included fat-free multigrain bread (52 g), fat-free turkey breast (90 g), fat-free Swiss-style cheese (42 g), 1 banana (118

g), fat-free pretzels (56 g), canned white peaches (166 g), cauliflower (62 g) cooked without fat and fat-free mayonnaise-like spread (5.3 g). Lunch provided 692 kcal, 42 g protein, 1.6 g fat, 127 g of carbohydrates and 7.8 g fiber. Values were calculated using the Nutrient Data System for Research software (University of Minnesota, Minneapolis, MN, USA). Neither breakfast nor lunch contained appreciable carotenoids, except for the test tomato juice.

2.6 Triglyceride-rich lipoprotein fraction of plasma (TRL) isolation and carotenoid extraction

TRLs were isolated using a protocol from trial 1 of Kopec et al. [24] using a SW 55 Ti swinging bucket rotor and an Optima L-100XP ultracentrifuge (Beckman Coulter, Brea, CA, USA). Carotenoids were extracted from tomato juices and TRLs as previously described by Kopec et al. [24].

2.7 Carotenoid analysis by HPLC-DAD and HPLC-DAD-MS/MS

All solvents and NaCl were obtained from Fisher Scientific (Pittsburgh, PA, USA). Acetone and methyl *tert*-butyl ether (MTBE) were HPLC grade and methanol and water were Optima grade. Ammonium acetate was purchased from J.T. Baker (Phillipsburg, NJ, USA). Lycopene was isolated and crystallized from tomato paste as previously described [25]. Phytoene, phytofluene, ζ -carotene, neurosporene and tetra-*cis*-lycopene were isolated from *tangerine* tomato extracts using preparative HPLC. Identity and purity (>95%) was confirmed with HPLC/accurate mass before using as an external calibrant.

Carotenoids from tomato juices were analyzed using HPLC-DAD (Alliance 2695, 996 DAD, Waters Corporation, Milford, MA, USA) and TRL extracts were analyzed using HPLC-DAD-MS/MS (Agilent 1260, Santa Clara, CA, interfaced with an AB Sciex QTrap 5500 mass spectrometer, Foster City, CA, USA). Analytes were separated on a C30 column (4.6×250 mm, 3 μ m, YMC Inc., Wilmington, NC, USA) at 35 °C using a gradient of A: 60% methanol, 35% MTBE, 3% water, 2% aqueous ammonium acetate (2% w/v), and B: 78% MTBE, 20% methanol, 2% aqueous ammonium acetate (2% w/v) flowing at 1.3 mL/min. A linear gradient was applied as follows: 0% B to 35.6% B over 9 min, to 100% B over the next 6.5 min, hold for 3.5 min at 100% B, and equilibrate for 3.5 min at initial conditions. Tomato juice extracts were re-dissolved in 2 mL of 1:1 MTBE:methanol, filtered using a 13 mm, 0.2 μ m pore nylon filter, and 10 μ L was injected. TRL extracts were re-dissolved in 200 μ L 1:1 MTBE:methanol, centrifuged (model 5424, Eppendorf, Hamburg, Germany) at 21,130 $\times$ g for 2 min, and 20 μ L of the supernatant was injected. Phytoene, phytofluene and ζ -carotene were quantified using DAD while neurosporene and all lycopene isomers were quantified using MS/MS. HPLC-DAD-MS/MS parameters are shown in Table 2.

2.8 Statistical and data analyses

Statistical analysis was performed using SPSS version 21 (IBM, Armonk, NY, USA). Baseline corrected AUC over 12 hours was derived using trapezoidal approximation. AUC for total lycopene from *tangerine* and red tomato juices were compared using a paired Student's *t*-test, and significance reported at $P < 0.05$. Fractional absorption was calculated using the following equation:

Fractional absorption = $(\ln 2/t_{1/2}) * [(AUC_{\text{lycopene}} * MW_{\text{lycopene}} * \text{plasma volume}) / \text{dose}_{\text{lycopene}}]$ This calculation assumes that the half-life ($t_{1/2}$) of lycopene is equal to that of chylomicrons (0.192 h) and plasma volume (mL) is equal to $927 + (31.47 * \text{body weight in kg})$ [26,27]. This calculation of fractional absorption also assumes that the entirety of this fraction is composed of intestinal derived chylomicrons. Some groups have reported some very low density lipoproteins (VLDLs) in TRL fractions, indicating some presence of liver derived lipoproteins [26].

3 Results and Discussion

3.1 Carotenoid analysis of tomato juices

Carotenoid content in the *tangerine* and red tomato juices as dosed are listed in Table 3. The *tangerine* and red tomato juices each contained an identical 10 mg of total lycopene, a dose achievable through diet alone. The red tomato juice provided approximately 90% of the lycopene in the all-*trans*- configuration while the *tangerine* tomato juice provided 94% of the lycopene in *cis*- configurations. Tetra-*cis*-lycopene made up 58% of the lycopene from the *tangerine* tomato juice with an additional 36% as mono-, di- and tri-*cis*-lycopene. This distribution allows us to compare the absorption of *cis* vs. *trans* lycopene, both within a tomato matrix. In addition to providing lycopene, both juices contain phytoene and phytofluene (although present at about 20 fold higher concentration in the *tangerine* tomato juice), and the *tangerine* tomato juice had ζ -carotene and neurosporene.

3.2 Carotenoid physical deposition forms in red and *tangerine* tomatoes

Light microscopy and TEM revealed stark differences between the two types of tomato fruits. In Figure 1, the typical needle-shaped elongated chromoplasts of red tomato fruit are depicted (B1), containing large crystalline aggregates of the predominant red pigment, all-*trans*-lycopene. These elongated chromoplasts are absent in *tangerine* tomato mesocarp and much smaller, round chromoplasts were observed by light microscopy (Figure 1, B2). The crystalline deposition form of all-*trans*-lycopene in red tomatoes has been reported frequently in literature [16,17,28–30] and, in agreement, our transmission electron micrographs show typical all-*trans*-lycopene crystal remnants with characteristic electron-dense, undulated internal structures (Figure 2A). *Tangerine* tomato chromoplasts were devoid of these characteristics elements, instead containing numerous plastoglobules as the only carotenoid-bearing element according to the classification of Sitte et al. [17]. Previous work has demonstrated that lycopene from red tomatoes remains present as crystalline bodies after processing into juice [31].

As shown in Figure 2B and C, these plastoglobules were different in size and frequently contained an electron-dense surface area, potentially representing a lipoprotein layer [32]. Based upon these observations, the *cis*-lycopene of our *tangerine* tomato variety is deposited in a lipid-dissolved physical state. This finding is consistent with previous work on Golden Jubilee tomatoes, a variety reported to contain the *tangerine* gene and similar globular chromoplasts [17]. Globular chromoplasts containing carotenoids were previously observed in comparatively lipid-rich fruits such as peach palm (*Bactris gasipaes* Kunth) fruits, which contain enough lipids to dissolve all present carotenoids [18]. Since *tangerine* tomato does

not contain appreciable lipid, the *cis*-lycopene itself might be responsible for the oily aggregate form. A similar carotenoid deposition was reported in globular structures of the microalgae *Dunaliella* sp., which contains extremely high concentrations (up to 10% of the dry weight) of all-*trans*- β -carotene and its 9-*cis*-isomer [33]. It was proposed that the presence the 9-*cis*-isomer at 40% of total β -carotene might help maintaining the oily rather than a crystalline deposition form of all *Dunaliella* carotenoids. Supporting this hypothesis, the visual appearance of the plastoglobules in *tangerine* tomato was different as compared to those of red tomato chromoplasts (Figure 2), potentially indicating a compositional difference. As described below, naturally different chromoplastidal deposition forms have previously been hypothesized to substantially influence carotenoid bioavailability.

In vitro studies have compared the bioaccessibility of carotenoids from a variety of fruits containing chromoplasts with differing morphology. One study found significantly higher bioaccessibility of β -carotene from mangoes (present as globular-tubular structures) compared to carrots (crystalloid structures) [30]. A human clinical study conducted by Schweiggert et al. [34] compared the bioavailability of lycopene (very small crystalloid structures) and β -carotene (liquid-crystalline structures) from papaya with dose-matched tomatoes (large crystals) and carrots (large crystals) respectively. These authors found lycopene to be 2.6 times and β -carotene 3 times more bioavailable from papaya compared to carrot, suggesting that physical deposition form may be a determinant factor in post-prandial absorption differences. Similarly, the highly different deposition forms of lycopene in *tangerine* and red tomatoes might help explaining the extreme differences in bioavailability noted in this study.

3.3 Lycopene bioavailability from tomato juices

The 10 mg lycopene dose resulted in a mean AUC value ($\pm$ SEM, $n=11$) for total lycopene of 690.9 ± 117.8 nmol·hr/L TRL from *tangerine* tomato juice and 81.6 ± 32.1 nmol·hr/L TRL from red tomato juice, with significantly higher bioavailability from *tangerine* tomato juice ($P<0.001$) (Figure 3). Lycopene from *tangerine* tomato juice was, on average, approximately 8.5 times more bioavailable compared to red tomato juice. A HPLC-MS/MS chromatogram showing carotenoids present in a 6 hr TRL after consumption of *tangerine* tomato juice is shown in Figure 4.

One previous report from our group found a lycopene AUC of 870.2 ± 186.9 nmol·h/L TRL and $C_{\max}$ of 189.8 ± 44.2 nmol/L TRL from *tangerine* tomatoes when given 13 mg with 15 g of lipid [22]. These data are in good agreement with the results found in this work, although Unlu et al. did not directly compare lycopene bioavailability from *tangerine* tomatoes to an equal dose from red tomatoes. Richelle and others have found that 5-*cis*-lycopene is better absorbed and/or less isomerized to other *cis* forms compared to 9- and 13-*cis*-lycopene suggesting differential isomerization of lycopene isomers in TRLs [35]. The profile of lycopene isomers in TRLs after consumption of *tangerine* tomato juice was consistent with the profile in the juice.

The average ($\pm$ SEM) fractional absorption of this 10 mg dose was $47.70 \pm 8.81\%$ from *tangerine* tomato and $4.98 \pm 1.92\%$ from red tomato. Fractional absorption ranged from

4.59-88.30% from *tangerine* tomato juice and 0.24-19.0% from red tomato juice, demonstrating heterogeneity in carotenoid absorption among individuals.

Since this study was conducted using a cross-over design, each subject can be compared to him or herself in terms of lycopene bioavailability from the two tomato sources. Thus, the ratio of $AUC_{tangerine}/AUC_{red}$ reflects how each individual absorbed lycopene from *tangerine* tomatoes with reference to red. The mean $AUC_{tangerine}/AUC_{red}$ ($\pm$ SEM) was 52.5 ± 22.0 . Only one subject absorbed less lycopene from *tangerine* tomato juice ($AUC_{tangerine}/AUC_{red} = 0.51$). The remaining ten of the eleven subjects absorbed between 2.3 and 247 times more lycopene from *tangerine* compared to red tomato juice. There was also greater variation in absorption from the red tomato juice (80 fold difference between the two most extreme subjects) than from the *tangerine* tomato juice (20 fold difference between the two most extreme subjects). The data appears heterogeneous, with some subjects gaining considerably larger fold increases in blood lycopene from *tangerine* tomato relative to red tomato. Specifically, subjects who have the lowest lycopene AUC_{red} on red tomato juice have the largest fold increase (lycopene $AUC_{tangerine}/AUC_{red}$) when consuming *tangerine* tomato juice and vice versa. This suggests that those subjects who are comparably worse at absorbing lycopene from red tomato juice gain the most benefit (in terms of increase in lycopene AUC) from *tangerine* tomato juice.

We hypothesize that this relationship between fold change and red tomato lycopene absorption is largely explained by inter-individual differences in ability to dissolve crystalline lycopene from red tomato, as subjects were more similar in their absorption of tetra-*cis*-lycopene from *tangerine* tomatoes. This explains the decrease in variation after consumption, and increase in absorption of lycopene from *tangerine* tomato juice, as these carotenoids are already present in lipid dissolved droplets.

Often, when discussing absorption of carotenoids, the process is considered in two phases we can loosely categorize as bioaccessibility and absorption. The bioaccessibility portion measures the amount of carotenoid that can be liberated from a food matrix and subsequently incorporated into mixed micelles, providing the theoretical maximum available for absorption. In reality, less carotenoid is absorbed than this theoretical amount, and the true amount absorbed by enterocytes and passed into circulation is termed bioavailability. Since *tangerine* tomatoes have *cis*-lycopene present in lipid dissolved droplets and *cis*-carotenoids (including tetra-*cis*-lycopene) do not crystallize, this form of lycopene should be more bioaccessible since it does not first need to be dissolved before it can be absorbed. We can thus hypothesize that the increase in lycopene AUC from *tangerine* tomatoes is a function of more dissolved carotenoid available for absorption. It is also important to note that different amounts of tomato juices were given in this study in order to provide the same dose of lycopene. The effects of meal volume on the bioavailability of carotenoids requires further investigation.

Many factors impacting heterogeneity into carotenoid absorption were controlled for in this study. Our subjects were of similar age, were non-smokers, were not affected by malabsorption disorders, received the same amount of lycopene in each test meal, received carotenoids in the same food matrix and consumed meals with the same composition.

However, we still observed heterogeneity in carotenoid absorption among individuals. Other host factors, including the genetics of each individuals, which have been shown to influence provitamin A carotenoid absorption and metabolism, may be partially responsible for this observation. Leung et al. found that 2 nonsynonymous SNPs within the coding region of β -carotene oxygenase 1 (BCO1) caused a reduced catalytic activity of this cleavage enzyme *in vitro* as well as a reduced ability to convert β -carotene to retinyl palmitate in humans, causing higher baseline levels of β -carotene in plasma [36]. The same group found that there were large variation in these affected alleles by ethnicity, suggesting this may also be a factor in carotenoid absorption and conversion [37]. *In vitro* data has found that, contrary to conventional thought, BCO1 can act on lycopene despite the absence of a unsubstituted β -ionone ring [38]. BCO1 SNPs were also recently related to a strong or weak plasma response after the consumption of watermelon or tomato juice [39], further suggesting that BCO1 plays some role in lycopene metabolism. Currently, there is little published on the role SNPs in β -carotene oxygenase 2 (BCO2), which cleave carotenoids (including lycopene) eccentrically, play in carotenoid absorption or metabolism in humans. Recent work has found that 73% of the variability of postprandial lutein in chylomicrons can be explained by SNPs in 15 genes involved in carotenoid and chylomicron metabolism [40].

Additionally, we noticed differences in total lycopene AUC values between males and females. In this study, all males had higher AUC values for lycopene on *tangerine* tomatoes than all females, a statistically significant increase ($P = 0.004$). Most studies in the literature report higher free-living serum carotenoid levels in women [43, 44], likely a function of higher consumption of carotenoids in the diet. Additionally, it has been shown that chronic supplementation of β -carotene over one year leads to a relatively higher increase in plasma β -carotene in women [43]. It has been suggested that for retinol, women have a higher transfer coefficient from the plasma to the extravascular pool, and this faster clearance is responsible for an apparent lower appearance of ^{13}C labeled retinyl palmitate derived from ^{13}C labeled β -carotene or retinyl acetate [44]. Despite lycopene being a non-provitamin A carotenoid, we hypothesize that this data on turnover to the extravascular pool may explain an apparent lower AUC for lycopene in females in this study despite trends in the literature towards higher plasma carotenoids, but are cautious in making claims on sex differences with an $n = 5$ or 6 for each group.

Many post-prandial studies investigating lycopene absorption use relatively high doses, above 20 mg [45]. Two studies feeding 10, 30, 60, 90 and 120 mg of lycopene were given in a tomato beverage, and found no significant increases in fractional or net absorption between the 10 mg dose and the higher doses [48, 49]. This observation suggests lycopene absorption is saturable, or at minimum, reduced, at doses above 10 mg. We hypothesize that the barrier to lycopene absorption occurs more at dissolution compared to uptake. This observed saturation rationalizes the need for dose matching, as to appropriately compare bioavailability of lycopene from different food sources.

3.4 Phytoene, phytofluene, ζ -carotene and neurosporene bioavailability from tomato juices

The unique carotenoid profile of *tangerine* tomatoes allowed us to investigate the post-prandial absorption of phytoene, phytofluene, ζ -carotene and neurosporene into

chylomicrons in humans. Mean ($\pm$ SEM) AUC and $C_{\max}$ of these carotenoids from *tangerine* tomato juice can be found in Table 4. These lycopene precursors appear to follow approximately the same absorption curve and time-course as lycopene and other carotenoids (Figure 6). Since concentrations of phytoene, phytofluene, ζ -carotene and neurosporene were not dose matched between the two juices, we are unable to make comparisons on absorption. Some hypothesize that phytoene and phytofluene contribute to the benefits observed in those consuming tomatoes over purified lycopene [48]. Additionally, phytoene and phytofluene are thought to exist primarily in *cis* configurations in plants [12], and have been shown to be more bioavailable than lycopene from red tomatoes in animals [49,50]. In this study, the net amount of phytoene and phytofluene absorbed from red tomato juice was quite consistent among subjects, despite great variability in lycopene absorption. This suggests that for phytoene and phytofluene as well, physical structure plays a role in bioavailability. Tomatoes are likely the best source of phytoene and phytofluene in a Western diet [16, 51, 52] and further work is needed to elucidate the role they may play in chronic disease prevention.

4 Concluding Remarks

We investigated the post-prandial absorption of lycopene from *tangerine* and red tomato juices in humans. In this study, lycopene from *tangerine* tomato juice was, on average, 8.5 times more bioavailable as compared lycopene from red tomato juice ($P<0.001$). We attribute this increase in bioavailability to *tangerine* tomatoes being rich in *cis*-lycopene and this lycopene present in lipid-dissolved globular structures in chromoplasts. In contrast, lycopene in red tomatoes, present as all-*trans*-lycopene, exists in large crystalline aggregates contributing to poor solubilization and comparatively lower bioavailability.

Tangerine tomatoes are a unique hybrid with the ability to greatly increase plasma lycopene and could represent a unique source of lycopene for studies of chronic disease prevention. If increased plasma lycopene is responsible for a decreased risk for certain chronic diseases, the *tangerine* tomato is a novel, highly bioavailable source of lycopene allowing individuals to consume reasonable amounts of tomatoes while still conferring health benefits.

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Abbreviations

AUC	area under the curve
BCO1	β-carotene oxygenase 1
BCO2	β-carotene oxygenase 2
C_{max}	concentration maximum
CRTISO	carotenoid isomerase
CRC	The Ohio State University's Clinical Research Center
IRB	Institutional Review Board
MTBE	methyl <i>tert</i> -butyl ether
OSU	The Ohio State University
TEM	transmission electron microscopy

TRL triglyceride-rich lipoprotein fraction of plasma

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Figure 1. Photographs of red (A1) and *tangerine* (*Solanum lycopersicon* L. hybrid FG10-314) tomatoes (B1) with corresponding light micrographs at 400× magnification of fresh red tomato (A2) and *tangerine* tomato (B2) mesocarp. Arrows and arrowheads denote crystalline and non-crystalline carotenoid containing structures, respectively.

Figure 2. Transmission electron micrographs of fresh red tomato (A) and *tangerine* tomato (B, C) mesocarp. Arrows: crystal (remnants), m: mitochondrion, pg: plastoglobules, w: cell wall, #: internal membranes

Figure 3.

Post-prandial absorption of total lycopene (*cis* + *trans*) from *tangerine* (▲) and red (■) tomato juices. The 10 mg dose of lycopene resulted in TRL concentrations (average ± SEM) of 690.9 ± 117.8 nmol·hr/L from *tangerine* tomato juice and 81.6 ± 32.1 nmol·hr/L from red tomato juice, making lycopene from *tangerine* tomatoes, on average, 8.5 times more bioavailable than lycopene from red tomatoes.

Figure 4. HPLC-MS/MS chromatogram of a 6 hr TRL after the consumption of *tangerine* tomato juice. HPLC-DAD-MS/MS parameters and peak labeled are given in Table 2.

Figure 5. Post-prandial absorption of 44.9 mg phytoene (A), 13.9 mg phytofluene (B), 29.3 mg ζ-carotene (C) and 6.26 mg neurosporene (D) which are found in substantial concentrations in *tangerine* tomato juice.

Table 1

Mean ($\pm$ standard deviation) baseline subject characteristics.

Sex	Age (years)	BMI (kg/m ²)	Plasma total cholesterol (mg/dL)	Plasma triglycerides (mg/dL)	Hematocrit (%)	Hemoglobin (g/dL)
All subjects (n = 11)	26.5 $\pm$ 10.1	23.3 $\pm$ 1.49	174 $\pm$ 11.1	77.9 $\pm$ 46.6	42.4 $\pm$ 3.31	14.2 $\pm$ 1.09
Female (n = 5)	29.2 $\pm$ 14.9	22.7 $\pm$ 1.23	182 $\pm$ 9.53	70.4 $\pm$ 53.9	40.3 $\pm$ 3.17	13.5 $\pm$ 1.08
Male (n = 6)	24.2 $\pm$ 3.54	23.8 $\pm$ 1.62	167 $\pm$ 7.20	84.2 $\pm$ 43.8	43.4 $\pm$ 2.93	14.5 $\pm$ 0.94

Table 2

HPLC-PDA-MS/MS parameters for carotenoid analysis of triglyceride rich lipoprotein fractions of plasma (TRLs).

Peak label	Compound identity	HPLC-PDA λ_{max} (nm)	HPLC-APCI(+)-MS/MS experiments	CE ^a (eV)	Dwell ^b (msec)
1	phytoene	286	545.51>463.6, 421.6, 395.6, 327.4	22, 22, 29, 25	165
2	phytofluene isomer	348	543.49>461.6, 393.6, 325.4	29, 29, 22	165
3	phytofluene isomer	348	543.49>461.6, 393.6, 325.4	29, 29, 22	165
4	α -carotene	445	537.45>455.3, 269.2	22, 20	135
5	tetra- <i>cis</i> -lycopene	439	537.45>455.3, 269.2	22, 20	135
6	neurosporene	410	539.45>457.6, 415.4, 389.6	22, 29, 29	135
7	ζ -carotene isomer	400, 425	541.47>391.4, 349.7, 271.27	22, 22, 20	135
8	β -carotene	451	537.45>455.3, 269.2	22, 20	135
9	ζ -carotene isomers	400, 425	541.47>391.4, 349.7, 271.27	22, 22, 20	135
10, 11	neurosporene isomers	440	539.45>457.6, 415.4, 389.6	22, 29, 29	180
12	other- <i>cis</i> -lycopenes	440-471	537.45>455.3, 269.2	22, 20	180
13	all- <i>trans</i> -lycopene	471	537.45>455.3, 269.2	22, 20	180
14	5- <i>cis</i> -lycopene	471	537.45>455.3, 269.2	22, 20	180

^aCE: collision energy,

^b multiple reaction monitoring experiments were sorted into periods to maximize dwell times for each analyte. All analytes were run with the following parameters: declustering potential: 185 V, entrance potential: 10 V, collision cell exit potential: 11 V, curtain gas: 30 psi and source temperature: 450 °C. Phytoene, phytofluene, ζ -carotene and tetra-*cis*-lycopene were quantified using PDA. Neurosporene and all lycopene isomers were quantified using the sum of the MS/MS transitions listed. A chromatogram is provided in Figure 4.

Table 3

Carotenoid mean values in test meals as determined using HPLC-PDA.

Carotenoid	Tangerine tomato juice ^a (mg)	Red tomato juice ^b (mg)
Phytoene	44.9 ± 0.42	2.20 ± 0.06
Phytofluene	13.9 ± 0.16	0.69 ± 0.04
ζ-carotene	29.3 ± 1.81	ND
Neurosporene	6.26 ± 0.20	ND
Tetra- <i>cis</i> -lycopene	5.80 ± 0.09	ND
Other- <i>cis</i> -lycopene	3.64 ± 0.05	1.00 ± 0.25
All- <i>trans</i> -lycopene	0.57 ± 0.05	9.0 ± 0.31
Total lycopene	10.0 ± 0.16	10.0 ± 0.33
Total carotenoid	104.3 ± 2.96	13.1 ± 0.32

^a Values are reported as means ± SD in 505 g of tangerine tomato juice, *n* = 4, to provide 10 mg of total lycopene^b Values are reported as means ± SD in 94 g of red tomato juice, *n* = 4 to provide 10 mg of total lycopene

nND: not detected