Carotenoid Absorption in Humans Consuming Tomato Sauces Obtained from Tangerine or High-*β*-Carotene Varieties of Tomatoes

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Tomato sauces were produced from unique tomato varieties to study carotenoid absorption in humans. Tangerine tomatoes, high in *cis*-lycopene, especially prolycopene (7*Z*,9*Z*,7′*Z*,9′*Z*), and high-*β*-carotene tomatoes as an alternative dietary source of *β*-carotene were grown and processed. Sauces were served after 2 week washout periods and overnight fasting for breakfast to healthy subjects ($n = 12$, 6M/6F) in a randomized crossover design. The serving size was 150 g (containing 15 g of corn oil), tangerine sauce containing 13 mg of lycopene (97.0% as *cis*-isomers) and high-*β*-carotene sauce containing 17 mg of total *β*-carotene (1.6% as the 9-*cis*-isomer) and 4 mg of lycopene. Blood samples were collected 0, 2, 3, 4, 5, 6, 8, and 9.5 h following test meal consumption and carotenoids determined in the plasma triacylglycerol-rich lipoprotein fraction by HPLC−electrochemical detection. Baseline-corrected areas under the concentration vs time curves (AUC) were used as a measure of absorption. $AUC_{0-9.5h}$ values for total lycopene in the tangerine sauce group were 870 ± 187 (nmol· h)/L (mean ± SEM) with >99% as *cis*-isomers (59% as the *tetra-cis*-isomer). The $AUC_{0-9.5h}$ values for total *β*-carotene and lycopene after consumption of the high-*β*-carotene sauce were 304 ± 54 (4% as 9-*cis*-carotene) and 118 ± 24 (nmol·h)/L, respectively. Lycopene dose-adjusted triacylglycerol-rich lipoprotein AUC responses in the tangerine sauce group were relatively high when compared to those in the literature and the high-*β*-carotene group. The results support the hypothesis that lycopene *cis*-isomers are highly bioavailable and suggest that special tomato varieties can be utilized to increase both the intake and bioavailability of health-beneficial carotenoids.

KEYWORDS: Lycopene; *β*-carotene; tangerine and high-*β*-carotene tomatoes; postprandial absorption; humans; electrochemical detection

INTRODUCTION

A diet high in carotenoid-rich fruits and vegetables is associated with a reduced risk of developing several diseases including cancer, cardiovascular diseases, macular degeneration, and cataract formation (*1−3*). Lycopene, the compound giving red color to tomatoes, is one of the major carotenoids in the diet of North Americans and Europeans, mostly consumed in the form of tomato and tomato-based products (*2*). Red tomatoes typically contain 94−96% *all-trans*-lycopene, which is the thermodynamically most stable form (*4*). In contrast, human plasma and tissues contain at least 50% *cis*-isomers (*5*), the most common isomeric lycopene forms being *all-trans*-, 5-*cis*-, 9-*cis*-, 13-*cis*-, and 15-*cis*-lycopene. Contrarily, in *tangerine* variety

tomatoes, the predominant lycopene isomer present is prolycopene (*tetra-cis*-lycopene, **Figure 1**), a geometric isomer of *all-trans*-lycopene, giving this fruit a characteristic orange color. Carotenoid isomerase is the enzyme in tomatoes responsible for the conversion of *poly-cis*-lycopene to *all-trans*-lycopene. Tangerine tomatoes lack this enzyme and therefore accumulate *tetra-cis*-lycopene with 4 (7*Z*,9*Z*,7′*Z*,9′*Z*-tetra-cis) of its 11 double bonds in the *cis*-configuration (*6*).

Another commonly studied carotenoid in the human diet is *β*-carotene because of its antioxidative and provitamin A activities. Provitamin A carotenoids, in particular *β*-carotene in fruits and vegetables, are the major source of vitamin A for a large proportion of the world population, and its deficiency is a serious health problem in many developing countries (*7*). Even though colored vegetables and fruits as well as dark green leafy vegetables are good sources of *β*-carotene, the food matrix affects its bioavailability, ranging from low in raw green leafy vegetables to intermediate in cooked vegetables and fruits (*8*).

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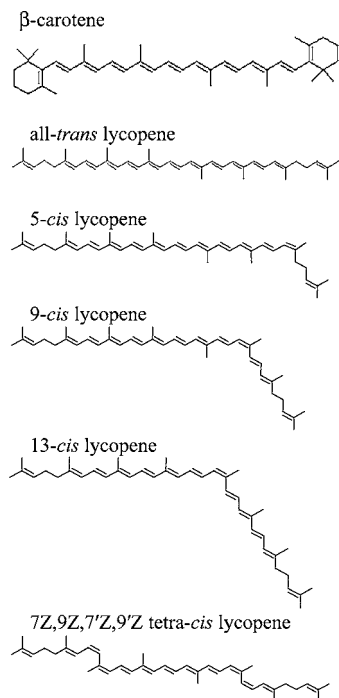‡ Department of Horticulture and Crop Science.
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Figure 1. Chemical structures of β-carotene and selected lycopene isomers.

In comparison to tomato-based foods, only carrots are a richer dietary source of vitamin A (*1*); however, biotechnological strategies have recently been attempted to obtain tomato fruits with elevated β-carotene content (*9*). In addition to lycopene and β-carotene, tomatoes can also be a good source of other potential health-beneficial carotenoids, such as the lycopene precursors phytoene, phytofluene, and ζ-carotene (*10*, *11*).

The aim of the present study was to assess lycopene bioavailability from tomato sauce produced from tangerine tomatoes and lycopene and β-carotene from tomato sauce obtained from a high-β-carotene tomato variety. For this purpose, tomato sauces containing added corn oil for superior palatability and to improve carotenoid absorption (*12*, *13*) were served in a postprandial trial to human subjects. Intestinal absorption of lycopene and β-carotene including its *cis*-isomers was assessed in the triacylglycerol-rich lipoprotein (TRL) fraction of plasma over 9.5 h following the consumption of a tomato-sauce-rich test meal. Carotenoids were determined using a highly sensitive electrochemical detector (ECD) (*14*) coupled with reversed-phase HPLC.

MATERIALS AND METHODS

Subjects. Healthy, nonpregnant, nonsmoking adults ($n = 12$, 6M/6F), age 19−45 (median 29.5 y) were enrolled in the study. Eligibility was based on a health and lifestyle questionnaire along with a screening test for blood lipids (cholesterol, triacylglycerol). Exclusion criteria were hyperlipidemia, any history of chronic disease influencing gastrointestinal function, use of medication affecting lipid absorption, transport, or metabolism, regular use of vitamin supplements containing carotenoids, and frequent alcohol consumption (>40 g/d). The study was approved by the Biomedical Sciences Institutional Review Board (IRB) of The Ohio State University (OSU), and written informed consent was obtained from the subjects. All clinical procedures were conducted at the General Clinical Research Center (GCRC) of OSU.

Tomato Sauce Processing. Two unique tomato (*Solanum lycopersicum* L.) varieties, Ohio FG99-77 and 97L97, were used for these studies due to their high prolycopene and β-carotene contents, respectively. Ohio FG99-77 was developed by crossing the tangerine allele *t*^{v} from LA3002 (Tomato Genetics Resource Center, Davis, CA)

into the Ohio 9242 genetic background (Department of Horticulture and Crop Sciences, OSU, Wooster, OH). The variety 97L97 contains the *B* allele of the fruit-specific β-cyclase from LA0317 crossed into the Ohio 8245 genetic background. Plants were grown with 30 cm within row spacing and 1.5 m between row spacing at the OSU North Central Agricultural Research Station near Fremont, OH, following standard practices (*15*). Following harvest, both tomato varieties were immediately processed into canned tomato juice (104 °C, 30 min). The tomato juice was then concentrated in a pilot plant scale rotary evaporator under vacuum at mild temperatures (60 °C). Following the addition of small amounts of Italian seasoning and 7% high fructose corn syrup to the concentrate, the mixture was heated to 75 °C (ca. 15−20 min) in an agitated steam-jacketed vessel, hot filled into glass jars (300 mL), sealed, and kept in the refrigerator after cooling. Approximately 1000 g of fresh tomatoes yielded 250 g of tomato sauce. Tangerine sauce high in prolycopene was developed from FG99-77 and sauce high in β-carotene from 97L97. Both tomato sauces had a brix value of 16.4°, indicating a similar amount of dissolved matter, especially carbohydrates.

Test Meals. Test meals were served at 08:00 h and consisted of tomato sauce (150 g, containing 15 g of corn oil) served with spaghetti (200 g) along with a slice (25 g) fat-free white wheat bread to soak up the tomato sauce leftover on the plate and bottled water (240 mL). Tomato sauces were served at room temperature, added over warm, not hot spaghetti, to not cause any change in the carotenoid composition. The test meals provided 2.19 MJ of energy, with 29% of the energy coming from fat, with the remainder coming almost entirely from carbohydrates (Food Processor version 8.1, ESHA Reach, Salem, OR).

Experimental Design. All 12 subjects completed both absorption studies. At enrollment, subjects consumed a diet devoid of tomato products and β-carotene for 13 d as a washout period. Subjects then completed a 12 h overnight fast and presented to the OSU-GCRC for the first test meal, which was consumed in randomized order, 6 subjects starting with tangerine and 6 with the high-β-carotene variety. After the baseline blood draw (0 h, 12 mL), test meal ingredients were served and consumed completely under supervision within 20 min. Consecutive blood samples were collected 2, 3, 4, 5, 6, 8, and 9.5 h after completion of the test meal. At 4.5 h, subjects consumed a standardized lunch low in carotenoids and fat (mushroom soup, saltines, tuna salad sandwich, two vanilla sandwich cookies, diet soda, water). This meal provided 1.92 MJ of energy from 77 g of carbohydrates, 22 g of protein, and 7 g of fat. No other foods and beverages except water (ad libitum) were allowed during the 10 h stays. Following the 10 h clinical stay, subjects then completed another 13 d washout and presented for the second test meal in a crossover design.

Blood Sampling and TRL Fraction Isolation. HPLC-grade solvents and water were used throughout the experiments. Blood samples were drawn into 10 mL EDTA tubes via a catheter placed in a forearm vein and centrifuged immediately for plasma separation at 1250*g* for 10 min at 4 °C with a Sorvall Legend TM T/RT centrifuge (Kendro, Newtown, CT). TRL fractions were isolated from the plasma by ultracentrifugation (*16*) at 155.000*g* for 30 min at 20 °C with an L8-M ultracentrifuge, SW 50.1 swinging bucket rotor (Beckman, Fullerton, CA). The top layer (0.5 mL) containing the TRL fraction was removed and stored at −80 °C until carotenoid analysis. All procedures were conducted under dim or red light. Measurements of plasma cholesterol and triacylglycerol levels were based on a spectrophotometric method (*17*) by a Synchron LX 20 system (Beckman Coulter).

Carotenoid Extractions from the Food Matrix. Carotenoid extractions from tomato sauces followed a method developed by Ferruzzi et al. (*14*). In brief, tomato sauce (5 g) was combined with Celite, $CaCO_3$, and methanol and homogenized. Carotenoids were extracted three times with 50 mL of acetone/hexane (1:1, v/v). The combined hexane layer was collected quantitatively after filtering through anhydrous sodium sulfate. The aliquots (1 mL) were dried under nitrogen, reconstituted in methyl *tert*-butyl ether (MTBE)/methanol (1:1, v/v), filtered through a 0.2 μm 13 mm nylon syringe filter (type no. 2166, Alltech, Deerfield, IL), and injected (25 μL) for HPLC analysis.

Carotenoid Extractions from the Plasma TRL Fraction. Extraction followed the method of Ferruzzi et al. (*18*) with minor modifications. Briefly, 200 μL aliquots of each TRL fraction were transferred

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into microcentrifuge tubes and deproteinated by the addition of equal volumes of ethanol containing 0.1% 2,6-di-*tert*-butyl-4-methylphenol (BHT) by mass. Carotenoids were extracted two times with 1 mL of hexane/acetone (2:1, v/v) containing 0.02% BHT by mass. For each extraction, the samples were vortexed and centrifuged at 5600*g* for 1 min at room temperature with a 10 MVSS microcentrifuge (Corning-Costar, New York). The combined hexane layers were dried under nitrogen, reconstituted in 400 μL of MTBE/methanol (30:70, v/v), filtered, and 100 μL aliquots were analyzed by HPLC.

Quantification of Carotenoids by HPLC. *all-trans* standards of lycopene and β-carotene were purchased from Sigma (St. Louis, MO). Stock solutions of carotenoids were prepared in hexane, and their concentrations were determined spectrophotometrically, using their specific absorption coefficients (*19*). *all-trans* standards were used for the quantification of both *trans*- and *cis*-isomers of carotenoids except for prolycopene. As the prolycopene absorption maximum was lower (438 nm compared to 470 nm) than that of the *all-trans* configuration and had a lower extinction coefficient (102900 m²/mol as compared to 184000 m²/mol (*20*)), it was purified from tangerine tomatoes on a preparatory HPLC system employing a C_{18} column and 85% MeOH/ 15% MTBE with a flow rate of 5 mL/min. Identification of *cis*-isomers was based on the comparison to previously reported UV−VIS and electrochemical data (*18*, *21*).

The HPLC system consisted of a Hewlett-Packard model 1050 (Santa Clara, CA) solvent delivery system. An eight-channel 5600 Coularray electrochemical detector (ESA, Chelmsford, MA) with cell potentials set from 200 to 620 mV in 60 mV increments was used. Separations were achieved using a 150 mm × 4.6 mm i.d., 5 μm YMC C_{30} column (Waters, Wilmington, NC). The solvent was methanol/MTBE/water/ ammonium acetate buffer (88:5:5:2, mobile phase A; 28:70:0:2, mobile phase B). The linear gradient used was 100% A at 0 min; changed to 15% A from 0 to 40 min; changed to 100% B from 40 to 50 min, at a flow rate of 1 mL/min (*18*).

Statistical Analysis. Statistical analyses were performed using SPSS 14.0 (SPSS Inc., Chicago, IL). Postprandial absorption for each carotenoid in the plasma TRL fraction expressed as the baseline-corrected area under the concentration vs time curve (AUC) over 9.5 h after test meal consumption was calculated by trapezoidal approximation using Igor Pro 4.06 software (Wave Metrics, Lake Oswego, OR). Normal distribution of the data was tested by Q−Q plots and Kolmogorov−Smirnov tests, equality of variance by Levene's test and box plots. A general linear multivariate model was developed with lycopene and β-carotene baseline-corrected and dose-adjusted (by dividing the AUC values through the amount of lycopene consumed) AUC values and fractional absorption as the dependent variables (all log-transformed data) and subject and meal (tangerine or high-β-carotene sauce) as fixed factors. AUC ((nmol·h)/L), peak concentrations (C_{max}, nmol/L, baseline-corrected), and carotenoid content (mg/100 g serving size) of the test meals were expressed as means ± SEM. *P* values of <0.05 were considered significant. Fractional lycopene absorption from the test meals was estimated according to O'Neil and Thurnham (*22*): fractional absorption = (ln 2)/$t_{1/2}$ × (calculated oral AUC × mass × plasma volume)/oral dose, assuming $t_{1/2}$ of chylomicrons to be 0.192 h and the plasma volume (mL) to be 927 + (31.47 × body mass (kg)), with mass being the molecular mass (g/mol) of lycopene.

RESULTS

All subjects recruited (**Table 1**) completed the study and consumed the test meals without any complaint and compliance problem. By mistake, however, the same sauce (high-β-carotene) was served twice to one subject. For this reason, only 11 observations are present for the tangerine sauce consumption. There was no significant difference among BMIs and plasma cholesterol and plasma triacylglycerol levels of the females and males, except the females were older than the males (*P* < 0.01). Fasting plasma triacylglycerol and cholesterol levels were all within the normal reported range (<2.3 mmol/L for tri-acylglycerol and <5.2 mmol/L for cholesterol (*23*)).

Table 1. Subject Characteristics at the Onset of the Study[a]

	age (y)	BMI (kg/m²)	plasma triacylglycerol level (mmol/L)	plasma cholesterol level (mmol/L)
female (*n* = 6)	35.5 ± 2.4[b]	26.3 ± 3.0	0.891 ± 0.080[c]	4.35 ± 0.19[d]
male (*n* = 6)	25.5 ± 2.2	24.6 ± 1.5	1.298 ± 0.304	4.26 ± 0.53

[a] Values are means ± SEM. [b] *P* < 0.05, unpaired Student's *t* test (two-tailed). [c] The conversion factor to mg/dL is ×88.57. [d] The conversion factor to mg/dL is ×38.67.

The serving size of 150 g of tomato sauce processed from tangerine tomatoes contained 13 mg of lycopene, and the high-β-carotene tomato sauce contained 17 mg of total β-carotene and 4 mg of lycopene (**Tables 2** and **3**). Food processing caused a decrease in the abundance of *tetra-cis*-lycopene while increasing the amount of other *cis*-isomers in the tangerine sauce compared to raw tomatoes. Contrarily, processing did not cause significant isomerization in high-β-carotene sauce as the percentage of 9-*cis*-β-carotene in the total β-carotene increased only slightly (**Tables 2** and **3**).

The predominant lycopene isomer detected both in tangerine sauce and in the corresponding TRL fraction was *tetra-cis*-lycopene (**Tables 2** and **3**). In addition to the *tetra-cis*-isomer, several other *cis*-isomers of lycopene (**Figure 1**) were detected as described by Hadley et al. (*21*) along with relatively low amounts of the *all-trans* form. The sum of all *cis*-isomers (*tetra-cis*, 9-*cis*, 13-*cis*, and 5-*cis* in addition to further but minor quantities of unidentified ones) is reported as total *cis*-isomers as compared to *all-trans*-isomers. After *tetra-cis*-lycopene, 9-*cis*-lycopene was found to be the second major *cis*-isomer followed by 13-*cis*-lycopene in the TRL fraction. In the high-β-carotene sauce and the corresponding TRL fractions, *all-trans*-β-carotene was the major component; the sum of *all-trans*-β-carotene and its 9-*cis*-isomer was expressed as total β-carotene.

As seen in **Figure 2**, the postprandial absorption curves for the carotenoids examined followed a similar time course after the consumption of tangerine and high-β-carotene sauces in the plasma TRL fraction. All carotenoids studied visually indicated a shoulder for carotenoid incorporation into the TRL fraction, with the first moderate increase at 3 h after test meal consumption, followed by the major response observed at 5 h, the time after the lunch meal (**Tables 2** and **3**). Both total β-carotene and total lycopene TRL concentrations in the subjects consuming the tomato sauce based on the high-β-carotene and the tangerine tomato varieties, respectively, were significantly higher at 5 h compared to 4 h and 3 h (*P* < 0.044, Fisher protected LSD test), the latter two being not significantly different.

When adjusted for the amount of lycopene intake by dividing the TRL-AUC values through the amount of lycopene consumed, AUC was found to be 68.1 ± 14.6 and 28.0 ± 5.6 (nmol·h)/L (*P* = 0.02) following consumption of the tangerine and high-β-carotene varieties, respectively. Fractional absorption of total lycopene was found to be 38 ± 31% from the tangerine and 16 ± 11% from the high-β-carotene variety (*P* = 0.03). Correlation between lycopene absorption from the tangerine vs the high-β-carotene variety was low and not significant, indicating high intraindividual absorption differences within the subjects.

DISCUSSION

The present study addressed the hypothesis of lycopene *cis*-isomers being highly bioavailable from tomato sauce obtained from tangerine tomatoes with elevated *cis*-isomer concentrations,

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Table 2. Distribution of Lycopene Isomers in the Test Meal Prepared from Tangerine Tomatoes and the Corresponding AUC and C_{max} Responses in the TRL Fractions[a]

	all-trans-lycopene	*tetra-cis*-lycopene	total *cis*-isomers	total lycopene
tomatoes (mg/ 100g), $n = 6$	0.10 ± 0.01 $(3.1)^b$	2.60 ± 0.04 (80.0)	3.15 ± 0.05 (96.9)	3.25 ± 0.07
tomato sauce (mg/ 100g), $n = 6$	0.28 ± 0.04 (3.0)	5.66 ± 0.13 (59.8)	9.19 ± 0.23 (97.0)	9.47 ± 0.23
AUC$_{0-9.5h}$ ((nmol·h)/L),^{c} $n = 11$	3.5 ± 2.8 (0.4)	515.1 ± 113.7 (59.2)	866.7 ± 185.4 (99.6)	870.2 ± 186.9
C_{max} (nmol/L),^{d} $n = 11$	1.30 ± 0.33 (0.7)	117.3 ± 28.2 (61.8)	188.5 ± 44.1 (99.3)	189.8 ± 44.2

^{a} Values are means $\pm$ SEM. ^{b} The percentage of lycopene isomers in total lycopene is given in parentheses. ^{c} Measured as baseline-corrected area under the curve of the TRL fraction. ^{d} Maximum concentration reached in the baseline-corrected TRL fraction during the 9.5 h.

Table 3. Distribution of β-Carotene Isomers in the Test Meal Prepared from High-β-Carotene Tomatoes and the Corresponding AUC and C_{max} Responses in the TRL Fractions[a]

	all-trans-β-carotene	9-*cis*-β-carotene	total β-carotene	total lycopene
tomatoes (mg/100g), $n = 6$	2.29 ± 0.21 $(98.7)^b$	0.03 ± 0.01 (1.3)	2.32 ± 0.24	0.55 ± 0.03
tomato sauce (mg/100g), $n = 6$	12.6 ± 0.50 (98.4)	0.20 ± 0.04 (1.6)	12.8 ± 0.5	3.13 ± 0.10
AUC$_{0-9.5h}$ ((nmol·h)/L),^{c} $n = 12$	291.2 ± 53.9 (95.9)	12.4 ± 3.6 (4.1)	303.6 ± 53.9	118.2 ± 23.8
C_{max} (nmol/L),^{d} $n = 12$	75.2 ± 11.5 (92.1)	6.5 ± 1.4 (7.9)	81.6 ± 12.1	35.9 ± 6.3

^{a} Values are means $\pm$ SEM. ^{b} The percentage of β-carotene isomers in total β-carotene is given in parentheses. ^{c} Measured as the baseline-corrected area under the curve of the TRL fraction. ^{d} Maximum concentration reached in the baseline-corrected TRL fraction during the 9.5 h.
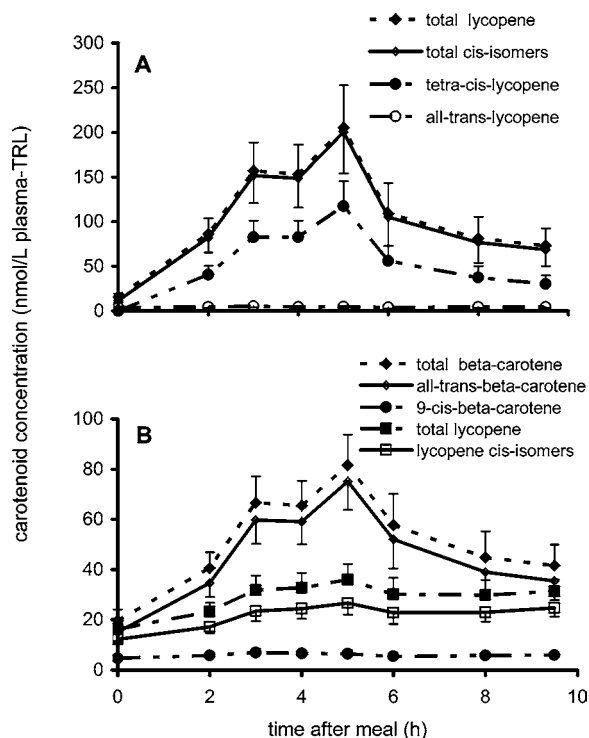

Figure 2. Change in plasma TRL concentrations of lycopene and β-carotene over 9.5 h following the consumption of (**A**) tangerine tomato sauce ($n = 11$) and (**B**) high-β-carotene tomato sauce ($n = 12$), both containing 15 g of corn oil/150 g serving size, in a postprandial crossover study. Plasma TRL concentrations (nmol/L of plasma) are expressed as the mean $\pm$ SEM.

especially *tetra-cis*-lycopene. In addition, absorption of β-carotene and lycopene from tomato sauce produced from a high-β-carotene tomato variety was investigated. The tomato sauce obtained from tangerine provided a good source to investigate lycopene absorption when consumed predominantly as *cis*-isomers (97%), in contrast to most commercial tomato products rich in the *all-trans* form. The results revealed efficient absorption of lycopene consumed as *cis*-isomers when compared to both results from previous studies and lycopene absorption

from the high-β-carotene variety with lycopene existing predominantly in the *all-trans* form. To our knowledge, postprandial absorption of lycopene and β-carotene from these varieties was tested in humans for the first time. So far, very limited information has been available regarding the bioavailability of lycopene isomers from tomatoes or tomato products, and most studies focused on the blood plasma response after a longer feeding period, not on TRL reflecting newly absorbed carotenoids.

Lycopene absorption from the tangerine variety versus the high-β-carotene variety was about 2.5 times higher, even when adjusted for lycopene doses. The lower dose given within the high-β-carotene variety could have been expected to result in a rather higher fractional absorption, as lower lycopene doses are assumed to be better absorbed compared to large doses (*24*). Thus, the results suggest high lycopene bioavailability when ingested predominantly in the form of *cis*-isomers. In a recent human study by Allen (*25*), lycopene plasma responses were studied in a human crossover study following the consumption of 140 g/d of *cis*-lycopene-rich tangerine or *all-trans*-lycopene-rich roma sauces for 4 d. Even though the total amount of lycopene consumed in the tangerine group was lower, a 20% vs 2% increase in plasma lycopene concentration after tangerine and roma sauce consumption was observed, respectively, suggesting that *cis*-lycopene was more efficiently absorbed than the *all-trans*-isomer. Similarly, preliminary results by Ishida et al. (*26*) reported higher plasma lycopene responses following tangerine vs red tomato sauce consumption.

Lycopene absorption measured by TRL-AUC in previous studies tended to be lower as measured in the present study. When 23 mg of lycopene within tomato paste was consumed, 12 h AUC responses of 110 (nmol·h)/L for total lycopene following meal consumption were reached (*27*). Tyssandier et al. (*28*) reported TRL-AUC$_{0-6h}$ responses of 273 $\pm$ 32 (nmol·h)/L following the consumption of 30 mg of lycopene from tomato puree. A high (38 mg) lycopene capsule (containing 43 g of fat) resulted in a mean TRL-AUC value of 147 (nmol·h)/L (*22*). TRL-AUC responses of up to 89 (nmol·h)/L were obtained from test meals including canned tomatoes containing 22 mg of lycopene and 23 g of fat (*29*), and C_{max} (21 nmol/L)

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was also lower compared to that in our study (190 nmol/L) following tangerine sauce consumption. Thus, relatively strong TRL-AUC$_{0-9.5h}$ responses of total lycopene were obtained in the present study following the consumption of *cis*-isomer-rich tangerine tomato sauce, while the lycopene TRL-AUC response from the high-β-carotene variety was in a more similar range when compared to literature values.

The percent distribution of lycopene *cis*-isomers in the tangerine tomato sauce was reflected in a very similar TRL response pattern, with a further shift of *all-trans*-lycopene to its *cis* form. Interestingly, only relatively low concentrations of 5-*cis*-lycopene were detected following tangerine sauce consumption, while it was frequently found in comparable high concentrations following tomato feeding studies (*21*, *30*). It could therefore be speculated that 5-*cis*-lycopene is isomerized predominantly from *all-trans*-lycopene. The literature suggests that, in addition to preferred absorption of lycopene *cis*-isomers (*31*), further isomerization of *all-trans*-lycopene into *cis*-lycopene occurs in vivo (*5*, *32*). In a recent study, the *cis*:*all-trans* ratio of lycopene isomers changed from 55:45 in blood plasma prior to intervention to 65:35 following a tangerine intervention, while no significant change occurred following red tomato consumption (*25*). Both further isomerization of *all-trans*-lycopene and effective absorption of *cis*-isomers in vivo are supported by one of our former studies investigating the effect of processing to increase the *cis*-isomer lycopene content. A baseline-corrected TRL-AUC$_{0-9.5h}$ response of 289.6 ± 57.6 (nmol·h)/L was reached after the consumption of 150 g of *cis*-isomer-rich (45%, 40 mg of lycopene) tomato sauce, while it was significantly lower (230.7 ± 52.5 (nmol·h)/L) following the consumption of a very similar, *all-trans*-isomer-rich (95%, 49 mg lycopene) sauce (unpublished results). These results however are still clearly below the AUC responses observed in the present study. Boileau et al. (*32*) investigated the *cis*-isomer composition of micelles in vitro and suggested that *cis*-lycopene is more bioavailable than *all-trans*-lycopene, most likely due to increased solubility in mixed micelles. The enrichment of *cis*-isomers in micelles may be attributed to the lower virtual chain length of *cis*-isomers due to structural bending compared to that of the *all-trans* form, improving solubility in micelles and making this structure less susceptible to crystallization. It can be speculated that the higher number of *cis*-bonds present in prolycopene further increases solubility during micellization.

In addition to *cis*:*trans* isomer distribution, carotenoid bioavailability is influenced by a number of factors. Among these are food processing by disrupting the cell matrix and carotenoid–protein complexes (*33*) mechanically and thermally for the release of lycopene from the food matrix (*4*, *27*). Heating is also known to influence the *cis*:*trans* ratio of lycopene and therefore potentially its bioavailability when tomatoes are processed into sauce (*34*, *35*). In our study, the percentage of *tetra-cis*-lycopene of total lycopene decreased after processing, while that of *all-trans*-lycopene stayed about the same, and the sum of other *cis*-lycopene isomers increased. These findings are consistent with earlier observations (*25*) and the fact that *all-trans*-lycopene is the more thermodynamically stable compound (*4*). Coconsumption of lipids also has been shown to be important (*12*, *36*). In addition to an increase in carotenoid solubility during digestion, it was postulated that carotenoids are kept in the enterocyte and are not released until long-chain fatty acids (12:0−18:0) from a present or subsequent meal enable carotenoid packaging into chylomicrons (*37*). This could explain the observed shoulder in the postprandial TRL curves (**Figure 2**), which might indicate a double peak absorption

pattern which has been observed in a number of previous studies (*22*, *27*, *29*). Monounsaturated fatty acids especially were shown to be incorporated preferably into lipoproteins as compared to oils higher in saturated fatty acids (*38*, *39*). Short- and medium-chain fatty acids seem to have a less strong effect on increasing carotenoid absorption (*37*). For this reason, we chose to add 10% corn oil, rich in long-chain, unsaturated (linoleic) acid, to the tomato sauce to enhance carotenoid bioavailability.

The second objective of the present study was to investigate β-carotene absorption in humans from tomato sauce produced from a high-β-carotene tomato variety as an alternative dietary source for β-carotene, as tomato and its products are commonly consumed. The present results indicate effective absorption of β-carotene from the tomato sauce obtained from this unique variety. TRL-AUC$_{0-9.5h}$ responses of 304 (nmol·h)/L for total β-carotene were reached in the present study following the consumption of high-β-carotene sauce (containing 17 mg of β-carotene). Compared to our results, lower TRL-AUC$_{0-10h}$ responses of 35 (nmol·h)/L were reached when 15 mg of β-carotene in the form of boiled carrots was consumed. However, higher responses of 271 (nmol·h)/L were obtained following the consumption of the same amount of β-carotene (15 mg) when given in the form of a palm oil suspension (*40*). In a study investigating the amount of dietary fat (0, 6, 28 and g of canola oil) on β-carotene absorption from carrots in salad (containing 12 mg of β-carotene) AUC$_{0-9h}$ values of 6, 104, and 221 (nmol·h)/L were obtained after fat-free, reduced, and full-fat salad dressing consumption, respectively (*12*), highlighting the importance of fat present in the meal for β-carotene absorption. Similar high AUC results for β-carotene of 268 and 490 (nmol·h)/L were reported after consumption of 26 mg of β-carotene within a liquid emulsion of 60 g of sunflower oil and beef tallow, respectively. In summary, the dose-corrected β-carotene absorption from tomato sauce processed from high-β-carotene tomatoes in the present study seemed slightly higher than from carrots in earlier studies.

In addition to lycopene and β-carotene, tomatoes can also be rich sources of other carotenoids such as γ-carotene and precursors of lycopene such as phytoene, phytofluene, and ζ-carotene (*11*), which were not determined in the present study. As these compounds have also been suggested to possess health-beneficial properties such as lowering the risk of developing prostate cancer (*10*), future studies to characterize differences between tomato varieties and study absorption of additional carotenoids in human trials are merited.

In conclusion, this study supports the hypothesis of lycopene *cis*-isomers being highly bioavailable. The use of tangerine tomatoes provided a unique source to investigate absorption of *cis*-lycopene. The present results demonstrate high absorption of lycopene when consumed predominantly as *cis*-isomers, suggesting the consumption of this variety could be a way to increase lycopene absorption and to benefit from its health-associated effects. In addition, the high absorption of β-carotene from tomato sauce processed from a high-β-carotene tomato variety indicates that tomato products obtained from this unique variety are a good dietary source for β-carotene and therefore for vitamin A.

ABBREVIATIONS USED

AUC, area under the concentration vs time curve; ECD, electrochemical detection; GCRC, General Clinical Research Center; OSU, The Ohio State University; TRL, triacylglycerol-rich lipoprotein.

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