Lycopene from heat-induced cis-isomer-rich tomato sauce is more bioavailable than from all-trans-rich tomato sauce in human subjects

Nuray Z. Unlu¹, Torsten Bohn¹, David M. Francis², Haikady N. Nagaraja³, Steven K. Clinton⁴ and Steven J. Schwartz¹*¹

¹Department of Food Science and Technology, The Ohio State University, Columbus, 2015 Fyffe Road, OH 43210, USA
²Department of Horticulture and Crop Science, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA
³Department of Statistics, The Ohio State University, 1958 Neil Avenue, Columbus, OH 43210, USA
⁴Department of Internal Medicine, Division of Hematology and Medical Oncology, The Ohio State University, 320 West 10th Avenue, Columbus, OH 43210, USA

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Lycopene is present mainly as cis-isomers in human serum and tissues whereas all-trans-lycopene predominates in tomato products, suggesting that all-trans-lycopene is isomerised in the body or is less bioavailable. The objectives of the present study were to develop processing conditions for tomatoes to obtain products with different cis-trans-lycopene isomer distribution and to assess their bioavailability. Healthy adult subjects (n 12) were recruited for this randomised cross-over trial. Each intervention was preceded by a 2-week washout period. Two tomato sauces, one rich in all-trans-lycopene (32·5 mg total lycopene/100 g sauce; 5 % cis-isomers), the other high in cis-lycopene (26·4 mg total lycopene/100 g sauce; 45 % cis-isomers), were produced by different heat-processing techniques. Each sauce (150 g) was served in a standardised meal at 08.00 hours after overnight fasting. Plasma TAG-rich lipoprotein fractions over 9·5 h following test-meal consumption as a measure of lycopene absorption for lycopene bioavailability. Furthermore, processing parameters can be controlled to alter isomer patterns of tomato products and influence lycopene bioavailability.

Cis- and trans-lycopene: Heat processing: Postprandial absorption: Plasma triacylglycerol-rich lipoprotein fraction

Lycopene, the predominant carotenoid in tomatoes and tomato products responsible for the red colour, has attracted considerable attention as epidemiological evidence continues to suggest that it may provide protection against a number of cancers and other degenerative diseases (Giovannucci, 1999; Nguyen & Schwartz, 2000; Khachik et al. 2002). The observation that serum and tissue lycopene consists of between 50 and 90 % cis-lycopene isomers, whereas tomatoes and tomato-based products contain mainly all-trans-lycopene, has led to the hypothesis that cis-isomers of lycopene are produced during food processing, cooking, or digestion or are more bioavailable (Erdman, 2005). Improved absorption of lycopene cis-isomers is hypothesised to result from greater solubility in mixed micelles and a lower tendency to aggregate (Stahl & Sies, 1992; Boileau et al. 1999), a hypothesis supported by rodent studies (Boileau et al. 1999; Korytko et al. 2003). Although most commercially processed tomato products such as juice, paste, soup and ketchup contain a relatively low percentage of cis-isomers (< 10 %), aggressive food processing or cooking has been shown to increase cis-isomer content (Shi & Le Maguer, 2000). The greatest increase in cis-isomer formation was observed when tomato products were heated in the presence of oil (Stahl & Sies, 1992; Schirle et al. 1997; van het Hof et al. 1998). Thus, research conducted so far suggests that lycopene cis-isomer formation is usually not observed at the mild temperatures commonly employed in most commercial food processing but is enhanced by the presence of oil at medium or higher processing and cooking temperatures.

The presence of elevated levels of lycopene cis-isomers in human biological samples compared with fresh tomatoes may also have biological significance, but this remains speculative (Clinton et al. 1996). cis-isomers may show unique tissue biodistribution patterns or perhaps distribute differently within cellular compartments to influence cell functions and responses to physiological or toxic stimuli in the body.
Experimental methods

Subjects

Twelve healthy, non-pregnant, non-smoking subjects (six male and six female; age 19–43 (median 29) years) were recruited for the study. Eligibility was based on a screening test for normal blood lipid profiles (cholesterol, TAG) and a health and lifestyle questionnaire. Exclusion criteria were hyperlipidaemia, any history of chronic gastrointestinal disease, use of medications affecting lipid metabolism, regular use of carotenoid-containing supplements, and frequent alcohol consumption. The study was approved by the Biomedical Sciences Institutional Review Board of The Ohio State University (OSU) and written informed consent was obtained.

Tomato sauce processing

High-lycopene-variety tomatoes, FG99-218 (Department of Horticulture and Crop Science, OSU, Wooster, OH, USA), were used to produce tomato sauces. FG99-218 was homozygous for the dark green (dg) gene and for the old-gold crimson (og) gene. Plants were grown with 30cm within-row spacing and 1.5 m between-row spacing at OSU North Central Agricultural Research Station (near Fremont, OH, USA), following standard practices (Precheur, 2000). Following harvest, tomatoes were immediately processed (104°C, 30 min) into canned tomato juice. Afterwards, tomato juice was concentrated in the pilot plant scale rotary evaporator, at mild temperature (60°C) and under vacuum. After adding small amounts of an Italian seasoning mix (dried oregano, marjoram, basil and black pepper, prepared at the OSU pilot plant), 7% high-fructose maize syrup (both added to improve taste and palatability), and 10% maize oil to the concentrate on the basis of weight, the mixture was heated to 75°C (about 15–20 min) in an agitated steam-jacketed vessel and was hot-filled into small cans (type 300 x 303). After sealing and cooling in an upside-down position, this product was stored to be served as hot-filled tomato sauce rich in all-trans-lycopene (sauce A; 95% all-trans-lycopene). The second sauce, rich in lycopene cis-isomers (sauce B; 55% all-trans-lycopene), was obtained by applying the same procedure to the same tomato juice concentrate followed by further processing in a steriotort at 127°C for 40 min.

Test meals

Test meals consisted of tomato sauce (150 g) served with spaghetti (200 g) along with a slice (25 g) of fat-free white wheat bread to absorb the tomato sauce leftover on the plate, and 240 ml bottled water. The energy and macronutrient content of all meals were determined by Food Processor™ version 8.1 (ESHA Reach, Salem, OR, USA). The test meals provided 2·19 MJ energy, with 29% of the energy coming from fat, with the remainder being almost entirely carbohydrates. Both tomato sauces were kept in the refrigerator until served to the subjects. They were served at room temperature and added over warm, not hot spaghetti, in order not to cause any possible changes in the carotenoid composition.

Experimental design

Subjects presented at the clinic on two separate occasions to consume the two tomato sauce meals and were instructed to avoid foods rich in lycopene (especially tomatoes and tomato-containing food products such as pizza, ketchup and lasagne) for 2 weeks as a washout period before each visit. Tomato sauces were served in a randomised cross-over design, each subject acting as his/her own control. Subjects arrived at the OSU General Clinical Research Center after overnight fasting for 12 h. Following a baseline blood draw (0 h), test meals were served at 08.00 hours and consumed under supervision within 20 min. Consecutive blood samples were collected at 2, 3, 4, 5, 6, 8 and 9·5 h following test-meal consumption. At 4·5 h, subjects consumed a standardised lunch (120 ml reduced-fat cream of mushroom soup, 15 g fat-free salad dressing, 45 g chicken meat, 95 g fat-free bread, 50 g mixed vegetables, 30 g cheese, 240 ml water) to provide a constant supply of fat, protein and carbohydrates. Both sauce meals were kept in the refrigerator until served to the subjects. They were served at room temperature and added over warm, not hot spaghetti, in order not to cause any possible changes in the carotenoid composition.

Blood sampling and analysis

Blood samples (12 ml) drawn from a forearm vein into EDTA tubes were immediately centrifuged for plasma separation at 1250 g for 10 min at 4°C (Baman/IEC Division, Needham, MA, USA). Measurements of plasma cholesterol and TAG levels were based on a spectrophotometric enzymatic method (Beckman-Coulter, 2006), using a Synchron LX 20 system (Beckman Coulter Inc., Fullerton, CA, USA). TRL fractions were isolated from plasma by ultracentrifugation at 155 000 g for 30 min at 20°C (Beckman L8-M ultracentrifuge, SW 50·1 swinging bucket rotor) following a method described by van Vliet et al. (1995).
Carotenoid extractions and quantification

Carotenoids were extracted from tomato sauces (Ferruzzi et al. 1998) and from the plasma-TRL (Ferruzzi et al. 2001) fraction with acetone–hexane as described previously. All procedures were conducted under red light. Standards of lycopene and β-carotene were purchased from Sigma Chemicals (St Louis, MO, USA). Stock solutions of carotenoids were prepared in hexane and their concentrations were determined spectrophotometrically, using their specific absorption coefficients (Weast, 1973). All-trans standards were used for the quantification of both trans- and cis-isomers. Analysis of all-trans-lycopene and all-trans-β-carotene standards indicated no degradation during simulated extraction and on-column during HPLC analysis. Identification of cis-isomers was based on the comparison with previously reported UV-visible spectrophotometry and electrochemical methods (Ferruzzi et al. 1998, 2001; Hadley et al. 2003). Carotenoids were analysed using a Hewlett Packard 1050 HPLC system (Foster City, CA, USA) connected to an electrochemical detector (5600 Coullarray™; ESA, Chelmsford, MA, USA) with cell potentials set from 200 to 620 mV in 60 mV increments. A C30 column (YMC™, 150 × 4.6 mm, 5 μm; Waters, Milford, MA, USA) was used for the analysis of carotenoids in conjunction with a gradient method (Ferruzzi et al. 2001).

Statistical analyses

Postprandial absorption for each carotenoid in the plasma-TRL fraction, expressed as baseline-corrected area under the concentration v. 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, USA). Statistical analyses were performed using SAS JMP version 5.0 (SAS Institute, Cary, NC, USA). ANOVA and residuals were examined for model compliance. Normality of residuals was checked using the Shapiro–Wilk test. Statistical modelling of AUC data was made by dividing through the total amount of lycopene consumed. Log-transformation (decadic) of AUC data was used to improve the statistical modelling potential, set from 200 to 620 mV in 60 mV increments. A C30 column (YMC™, 150 × 4.6 mm, 5 μm; Waters, Milford, MA, USA) was used for the analysis of carotenoids in conjunction with a gradient method (Ferruzzi et al. 2001).

Fractional absorption = \((\ln 2)/t_2 \times (\text{calculated oral AUC})/\text{oral dose}\)

assuming \(t_2\) of chylomicrons to be 0.192 h, plasma volume (ml) being 927 + 31.47 × body weight in kg, mass being the molecular weight of lycopene in g/mol, and oral dose being the amount of lycopene consumed in g.

### Results

All recruited subjects (six males, six females; Table 1) completed the study and consumed both test meals. Age, BMI, plasma cholesterol and TAG levels were not significantly different between men and women. Fasting plasma TAG and cholesterol levels were all within the normal reported range (<2.3 mmol/l for TAG, and <5.2 mmol/l for cholesterol (National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, & Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), 2002). Effects of sex, age, BMI and plasma cholesterol values were found not to affect carotenoid absorption significantly, while plasma TAG values had a significant effect on carotenoid absorption when these variables were used in the model as covariate, i.e. higher TAG concentrations were significantly associated with higher carotenoid absorption.

Total lycopene (sum of all-trans- and cis-isomers identified as 5-cis-, 9-cis-, 13-cis-, and other unidentified cis-isomers) and β-carotene (sum of all-trans- and the 9-cis-isomer) content of both sauces per 150 g serving size are given in Table 2. The additional heat treatment (sterirotart) of sauce A elevated the percentage of lycopene cis-isomers approximately 9-fold in sauce B, resulting in about 45 % cis-isomers of total lycopene. Higher temperatures were also tested; however, setting temperature and time above the chosen parameters of 127°C and 40 min resulted in an unacceptable brown-coloured product in preliminary trials, which was of a burnt taste. Because the two tomato sauces were processed in two different batches, DM content was slightly different, Brix values* for sauce A and B being 20.8 (SEM 0.09) and 19.6 (SEM 0.07) (*Conversion factor to mg/l is × 885.7).

### Table 1. Subject characteristics at the onset of the study

<table>
<thead>
<tr>
<th></th>
<th>Women (n 6)</th>
<th>Men (n 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.3</td>
<td>3.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Plasma TAG (mmol/l)</td>
<td>4.70</td>
<td>0.40</td>
</tr>
<tr>
<td>Plasma cholesterol (mg/l)</td>
<td>4.07</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Conversion factor to mg/l is × 885.7.

* Brix values are the measurement of the specific density of a solution. A solution has 1° Brix if the density is equal to 100g water containing 1g sucrose.
test-meal intake in the plasma-TRL fraction was relatively low, and is therefore not reported.

When comparing the percentage of cis-isomers of total lycopene in the sauce vs. the plasma-TRL fraction, an average increase from approximately 5 to 42% was observed for sauce A. For sauce B, this percentage increased from 45 to 52%. A strong significant correlation (P<0.0001) between the shortened approach to determine lycopene TRL AUC based on four blood samples (AUC<sub>4–6</sub>) vs. AUC<sub>0–9.5</sub> was found for total lycopene, lycopene cis-isomers and all-trans-lycopene (log-transformed (decadic) data, dose adjusted and baseline corrected). A linear model was developed to estimate the AUC<sub>0–9.5</sub> by using AUC<sub>4–6</sub> as:

\[
\text{Log (total lycopene AUC<sub>0–9.5</sub>)} = 0.537 + 0.834 \\
\times (\text{total lycopene AUC<sub>4–6</sub>}) \quad (r^2=0.912);
\]

\[
\text{Log (total cis – lycopene AUC<sub>0–9.5</sub>)} = 0.477 + 0.790 \\
\times (\text{cis – lycopene AUC<sub>4–6</sub>}) \quad (r^2=0.875);
\]

\[
\text{Log (all – trans – lycopene AUC<sub>0–9.5</sub>)} = 0.504 + 0.860 \\
\times (\text{all – trans – lycopene AUC<sub>4–6</sub>}) \quad (r^2=0.920);
\]

\[
\text{Log (5 – cis – lycopene AUC<sub>0–9.5</sub>)} = 0.380 + 0.680 \\
\times (5 – cis – lycopene AUC<sub>4–6</sub>) \quad (r^2=0.755);
\]

\[
\text{Log (9 – cis – lycopene AUC<sub>0–9.5</sub>)} = 0.506 + 1.035 \\
\times (9 – cis – lycopene AUC<sub>4–6</sub>) \quad (r^2=0.999);
\]

\[
\text{Log (13 – cis – lycopene AUC<sub>0–9.5</sub>)} = 0.487 + 1.015 \\
\times (13 – cis – lycopene AUC<sub>4–6</sub>) \quad (r^2=0.995).
\]
Values are means (trans-rich in all-lycopene and lycopene cis-

Fig. 2. Dose-adjusted plasma TAG-rich lipoprotein concentrations of total lycopene and lycopene cis-isomers following the consumption of sauce A (rich in all-trans-lycopene) and sauce B (rich in cis-lycopene) over 9.5 h. Values are means (n 12), with standard errors represented by vertical bars. (→), Total lycopene in sauce A; (→•→), total lycopene in sauce B; (→-•, dotted line), cis-lycopene in sauce A; (→-••, dotted line), cis-lycopene in sauce B.

Discussion

The results of the present study demonstrate a more than 55 % increase in fractional total lycopene absorption following the consumption of a cis-lycopene-rich tomato sauce v. a very similar all-trans-rich tomato sauce. In addition, the study shows an enhanced cis- compared with all-trans-lycopene plasma-TRL response when taking into account the amount of isomer consumed. These findings are in line with the hypothesis of preferential absorption of lycopene cis-isomers. To our knowledge, this is the first human study demonstrating enhanced cis-lycopene-isomer response compared with its all-trans-isomer from tomato products with a common food matrix, similar to those commercially available.

In the present study, cis-lycopene formation was controlled in the all-trans-lycopene-rich tomato sauce by applying a mild processing temperature of 75°C, resulting in 5 % cis-isomer formation. cis-Lycopene formation was induced to 45 % of total lycopene in a sauce by adjusting the temperature to 127°C (40 min). The published literature regarding the effects of tomato processing on the formation of lycopene cis-isomers remains somewhat controversial. Nguyen & Schwartz (1998), Nguyen et al. (2001) and Re et al. (2002) have shown that modest thermal treatment did not cause a considerable shift in the distribution of trans:cis-lycopene isomers. However, the addition of fat, together with applying higher temperatures, such as may occur during the cooking of tomato products, has been suggested to cause an increase in lycopene cis-isomer formation during tomato processing. Agarwal et al. (2001) observed an increase from 6 to 29 % cis-isomers when heating tomato juice (1 h) with 10 % added maize oil. Similarly, van het Hof et al. (1998) found elevated levels of cis-isomers (about 55 %) when tomato paste was heated to 100°C for 30 min in the presence of 10 % maize oil, whereas it was lower with 5 % oil addition (about 35 %) and low (about 5 %) in the absence of oil. Together, these studies suggest that most processed food products contain primarily all-trans-lycopene, but that additional processing or cooking methods could increase cis-lycopene content. In the present study, the addition of 10 % maize oil to both sauces before processing resulted in the formation of cis-lycopene isomers with concentrations comparable with the former studies. It can be speculated that a certain amount of fat needs to be present in the product to solubilise and release lycopene from the matrix to enhance isomerisation by thermal treatment.

Although estimated total lycopene and isomer content of the two tomato sauces differed, we understand that the HPLC method used to quantify isomers is limited by the fact that pure authentic standards of isomers are not readily available for calibration. Measurements for all detected lycopene isomers are based on response factors for the all-trans compound. When using UV-visible spectrophotometry methods of detection, this would result in an underestimation of lycopene isomer content because of the shift in absorbance spectra when isomerisation occurs. Electrochemical methods of detection were found to be similar in detector response to all-trans-lycopene but not for all lycopene isomeric forms (Ferruzzi et al. 2001). As such, further refinements in methodology are necessary to quantify lycopene cis-isomers including other cis-carotenoid geometrical forms.

Previous studies (Stahl & Sies, 1992; Gartner et al. 1997) have suggested that heat treatment alone might improve lycopene bioavailability without significantly altering the isomer composition within foods. In the present study, it is therefore possible that the higher lycopene absorption from the cis-isomer-rich product was due to the higher temperature treatment. The observation that not only the cis-lycopene plasma-TRL response, but also the all-trans-lycopene
response increased following consumption of the cis-lyco-
pen e-rich product as compared with the all-trans-rich product, we would support this hypothesis. However, in another study by our group (Unlu et al. 2007), we found a significantly higher plasma-TRL response from tomato sauce obtained from tangerine tomatoes rich in cis-lycopene (about 97% cis-isomers) produced under similar mild processing conditions as compared with the all-trans-rich tomato sauce in the present study. Thus, both preferred absorption of lycopene cis-isomers per se, and improved absorption of total lycopene due to heat treatment could have contributed to the present results, as the effect of cis-lycopene isomerisation could not be separated from the heat processing. In addition to heat treatment, the lower dose of lycopene served within the cis-isomer-rich sauce could have added to an increased fractional lycopene absorption, as lycopene absorption has been reported to follow a curvilinear absorption pattern, as tested with doses ranging between 10 and 120 mg (Gustin et al. 2004). However, in the present study, even the non-dose-adjusted AUC suggested higher lycopene bioavailability from the cis-isomer-rich lycopene product.

The main mechanism by which cis-lycopene accumulates in blood or tissue remains controversial. The higher plasma-TRL responses of cis-lycopene and its isomers compared with all-trans-lycopene (when taking into account the amount isomer consumed) from both, but especially from the all-trans-lycopene-rich product, could be explained either due to higher absorption of cis-lycopene, increased in vivo isomerisation from all-trans-lycopene, or both. The observation, however, that following consumption of the cis-rich product, only a slightly decreased ratio of trans:cis-lycopene was found in the TRL fraction indicated that an enhanced uptake of cis-lycopene alone cannot explain the observed results. This less pronounced difference in isomer composition between the plasma-TRL fraction and the tomato sauce consumed indicated that isomerisation continued toward an equilibrium between all-trans- and cis-lycopene isomers in the plasma-TRL fraction. An earlier in vitro study investigating trans:cis-lycopene isomer distribution in organic solutions suggested a ratio of 50% all-trans- to 50% cis-lycopene to be the most stable (Nguyen & Schwartz, 1998), values which are close to the ones observed in the plasma-TRL fractions of the present study. The trans:cis-isomer ratio of the plasma-TRL fraction was 0.92 and 1.39 following the cis-lycopene and all-trans-lycopene-rich sauce consumption, respectively, suggesting that the higher the trans:cis-isomer ratio in the product consumed, the higher the trans:cis ratio in the TRL AUC response. Boileau et al. (1999) found significantly higher amounts of cis-lycopene isomers in mesenteric lymph (77%) compared with dose, stomach and intestinal contents (6–18%) in ferrets. It has thus been suggested that there is a continuous isomerisation of all-trans-lycopene to cis-lycopene isomers in the body, with higher proportions of cis-lycopene following the order tissue > serum > chylomicrons (Clinton et al. 1996). This is further supported by a previous human study by the observation that upon cessation of dietary (predomi-
nantly all-trans) lycopene intake, the percentage of cis-isomers of total lycopene increases in plasma (Frohlich et al. 2006). Interestingly, the present study additionally indicates in vivo isomerisation of either all-trans- or cis-isomers of lycopene to 5-cis-lycopene, as this was not detected in the tomato sauce, while it was found in high concentrations in the plasma-TRL fractions following sauce consumption. As formation of 5-cis from other cis-isomers would require several isomerisation steps, and all-trans-lycopene was present in much higher concentra-
tion in the product and plasma-TRL, it appears more likely that all-trans-lycopene was isomerised to 5-cis-lycopene in vivo. 9-cis- and 13-cis-lycopene were present in both sauce and plasma-TRL, and their presence can thus be explained by absorption.

The carotenoid response measured in the TRL-fraction of plasma was suggested to be a valuable measure of carotenoid bioavailability from single meals, as this fraction represents newly absorbed carotenoids (O’Neill & Thurnham, 1998). However, a large number of blood samples over a 10–12 h course are often employed. We found a significant correlation between TRL AUC4–6 and AUC0–9 following the consumption of test meals for total lycopene, lycopene cis-isomers and all-trans-lycopene, and a linear model was developed to estimate the AUC0–9.5 values based on the AUC4–6. No significant differences in the AUC were found when comparing the estimated AUC0–9.5 values (based on the AUC4–6) with the measured AUC0–9.5. Thus, in future studies comparing lycopene bioavailability from different test meals, it seems feasible to collect only four blood samples at 0, 4, 5 and 6 h following test-meal consumption. The limited data obtained within the present study suggest a similar kinetic behaviour of the cis-isomers and all-trans-lycopene within the plasma-TRL fraction during the observed 9.5 h time period, as the TRL appearance pattern, Cmax, and the similar regression curves for estimating the AUC0–9.5 based on the AUC4–6 values indicate. As a smaller number of samples are beneficial both for the researcher and subject, future studies to extend this method to additional carotenoids are merited.

In conclusion, we observed a significant increase in lycopene absorption when tomato sauce was enriched in cis-isomers by heat treatment, being in agreement with the hypothesis that cis-lycopene isomers are preferentially absorbed in man. Additional heat treatment of the tomato matrix, enhancing availability of lycopene to digestive and absorptive processes, could also have contributed to this result. The present results further suggest that preferred absorption of cis-lycopene isomers alone cannot explain the dramatically increased proportion of lycopene cis-isomers in biological samples, an observation that probably reflects an additional in vivo isomerisation from all-trans- to cis-isomers. However, based upon these findings, tomato-based food products could be manipulated by temperature processing to favour the formation of specific isomer patterns to improve lycopene bioavailability and possibly health benefits.

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