The proportion of lycopene isomers in human plasma is modulated by lycopene isomer profile in the meal but not by lycopene preparation

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Running title: Fate of lycopene isomers during absorption in humans

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Abstract

Dietary lycopene consists mostly of (all-E) isomer. Upon absorption, (all-E) lycopene undergoes isomerisation into various (Z)-isomers. Because these isomers offer potentially better health benefits than the (all-E) isomer, the aim of the present study was to investigate if the profile of lycopene isomers in intestinal lipoproteins is affected by the profile of lycopene isomers in the meal and by the tomato preparation. Six post-prandial, cross-over tests were performed in healthy men. Three meals provided about 70% of the lycopene as (Z)-isomers either mainly as 5-(Z), or 13-(Z), or a mixture of 9-(Z) and 13-(Z) lycopene, while three tomato preparations provided lycopene mainly as (all-E) isomer. Consumption of the 5-(Z) lycopene rich meal led to a high (60%) proportion of this isomer in triacylglycerol-rich lipoproteins indicating a good absorption and/or a low intestinal conversion of this isomer. By contrast, consumption of meals rich in 9-(Z) and 13-(Z) lycopene isomers resulted in a low level of these isomers but high amounts of the 5-(Z) and (all-E) isomers in TRL. This indicates that the 9-(Z) and 13-(Z) isomers were less absorbed or were converted into 5-(Z) and (all-E) isomers. Dietary (Z)-lycopene isomers were, therefore, differently isomerised and released in TRL during their intestinal absorption in men. Consuming the three meals rich in (all-E) lycopene resulted in similar proportions of lycopene isomers in TRL: 60% (all-E), 20% 5-(Z), 9% 13-(Z), 2% 9-(Z), and 9% unidentified (Z)-isomers. These results show that the tomato preparation has no impact on the lycopene isomerisation occurring during human absorption.
Introduction

Lycopene is an acyclic C₄₀ non-polar carotenoid, present in several dietary sources such as tomato, watermelon, guava and apricot. Although being not a provitamin A, lycopene has been shown to have multiple biological activities including decrease of risk of prostate cancer(1), inhibition of cell proliferation, migration and invasion in breast of endometrial and liver carcinoma cells(2-7), and prevention of mutagenesis and chromosome instability(8, 9). In addition, a variety of epidemiological trials indicated that high intakes of lycopene containing foods (primarily tomato products) or elevated blood lycopene concentrations are associated with a decreased risk of cardiovascular diseases and prostate cancer(10-13).

Lycopene has eleven conjugated double bonds and each of them could be either in an (E) or (Z) configuration. (All-E)-lycopene is the predominant isomer in plants, representing about 80–97% of total lycopene in tomatoes and related products(14). In human body fluids and tissues such as plasma, prostate, testis and skin, 25–70% of lycopene is found in various (Z) forms(15-20). The high concentrations of (Z)-isomers in vivo trigger the hypothesis that they are more bioavailable and/or that (all-E) lycopene is transformed into (Z)-isomers within the human body. Moreover, when a tomato-based meal rich in the (all-E)-isomer is consumed daily over a few weeks, plasma lycopene concentration increases and its main part (60%) is present as (Z)-isomers(21). Recently, we have demonstrated that lycopene isomerisation takes place in the enterocytes and not in the gastrointestinal lumen(22).

In terms of bioactivity, Shi & Le Maguer(14) indicated that the biological potency of (Z)-lycopene isomers is different from that of the (all-E) form. Böhm et al.(23) reported that some (Z)-isomers have a stronger in vitro antioxidant activity than the (all-E) form. For these reasons, (Z)-lycopene isomers are regarded as offering potentially better health benefits than the (all-E)-isomer.

In the present study, we investigated, in humans, the effect of meals enriched in lycopene on the lycopene isomer profile of plasma triacylglycerol-rich lipoprotein (TRL) fractions. The main
objective was to compare the profile of lycopene isomers appearing in TRL following the ingestion of tomato preparations rich in (Z)-lycopene isomers. Three different tomato preparations rich in Z-lycopene (about 70% of total lycopene) were produced. One preparation was rich in 5-(Z) (65%), one in 13-(Z) (42%) and one in a mixture of 9-(Z) (31%) and 13-(Z) (24%) isomers. Another objective was to determine if the tomato preparation can affect the intestinal isomerisation of (all-E) lycopene during absorption.
Experimental methods

LYCOPENE PREPARATIONS

Tomato preparations rich in (Z)-lycopene isomers

A tomato preparation rich in 5-(Z) lycopene was produced by mixing 10 g of tomato oleoresin in 500 mL dichloromethane with 580 μL Iodine solution (5.6 mg Iodine in 596 μL methylene chloride). The mixture was photoisomerised for 10 hours at room temperature. Photoisomerised tomato oleoresin was further enriched in 5-(Z) lycopene by successive fractionation in ethanol and in ethyl acetate. The solid fraction was then re-suspended in ethyl acetate (1:80 w/w) and the suspension centrifuged at 16’900 x g for 5 min at room temperature. The solid fraction was discarded and the tomato preparation enriched in 5-(Z) lycopene was obtained by distilling ethyl acetate under reduced pressure at 30°C. This preparation provided 65% of total lycopene as 5-(Z) isomer (Table 1).

Tomato preparation rich in 13-(Z) lycopene isomer: A suspension of tomato oleoresin in ethyl acetate (1:10 v/v) was refluxed for 1 h under stirring. After cooling to room temperature the precipitate (~10 % w/w of initial raw material) was filtered off. This precipitate contained mainly (all-E) lycopene isomer. The isomerised tomato preparation was obtained by evaporating ethyl acetate from the filtrate under reduced pressure at 30°C and by azeotropic distillation after addition of water and ethanol (water/ethanol ratio: 8:2). This preparation provided 70% of total lycopene as (Z)-isomers with 42% as 13-(Z) lycopene (Table 1).

Tomato preparation rich in 9- and 13-(Z) lycopene isomers: a similar process as the one described for the tomato preparation rich in 13-(Z) lycopene was performed with the exception that the heating duration has been increased up to 24 h. This preparation provided 72% of total lycopene as (Z)- isomers with 31% as 9-(Z) lycopene and 24% as 13-(Z) lycopene (Table 1).
Tomato preparations rich in (all-E) lycopene

Three tomato products, *i.e.* a commercial tomato paste packed in a 300-g tube (Thomy, Vevey, Switzerland), a tomato oleoresin (Indena, Milan, Italy) and a lactolycopene™ preparation (Indena, Milan, Italy), contained about 95% of total lycopene as (all-E) isomer.

The lycopene isomer profiles of the tomato supplements are presented in Figure 1 and Table 1. With the exception of tomato paste, the daily doses of test products were packed separately as aliquots in light-protected containers, sealed under nitrogen and stored at −80°C during the course of the study. The lycopene content and profile of isomers were shown to be stable for 6 months at -40 °C in the tomato products indicating that the storage conditions were adequate.

**Subjects**

Thirty healthy men were enrolled in the study. A total of twenty-seven subjects, aged 24 (SEM 1) years, completed the study. Their mean starting body weight was 70 (SEM 1) kg and BMI 22.5 (SEM 0.3) kg/m². Subjects were normolipidemic, *i.e.* they had a ratio of plasma cholesterol to HDL cholesterol < 5.0 and plasma triacylglycerol concentrations < 1.5 mmol/L. Because of the large amount of blood that was drawn during the study, subjects were required to have a blood hemoglobin concentration > 1.3 g/L as inclusion criteria. The subjects did not use medication, neither they had history of gastrointestinal disease or lipid metabolic disorders. Three volunteers abandoned the trial before the end for the following reasons: unavailability, medical treatment related to an eye injury, nausea related to the consumption of fatty meals.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the ethical committee of Marseille (Marseille, France). All subjects received information on background and design of the
study and gave written informed consent before participation. They were free to withdraw from the study at any time.

**STUDY DESIGN**

The study was a randomized, 6-periods, 6-treatments cross-over clinical trial with a washout period of 3 weeks minimum. Subjects and technicians were blinded while the investigator was blinded for 5 tomato products but not for the tomato paste which was a commercial product. Subjects were asked to refrain for 48 h before the post-prandial tests from eating lycopene rich products: tomatoes (crude, cooked and tomato sauces including ketchup and harissa), pizza, ratatouille, lasagna, pasta including tomato sauce, watermelon, pink grapefruits and guava. In addition to this dietary restriction, the subjects ate a standard meal the evening before the post-prandial tests consisting of green vegetables, a source of cereals (paste, bread or rice), lean meat or fish, one low-fat natural yoghurt, one fruit and one mineral water. They should have consumed this dinner in the evening between 19:00 and 20:00. These recommendations were checked by the investigator on the post-prandial test day.

After an overnight fast, subjects arrived at the Clinical Pharmacology and Therapeutic Trial Center of University of Marseille and consumed a standard meal consisting of semolina (70 g) cooked in 200 mL hot water, white bread (40 g), egg whites (60 g), groundnut oil (40 g), natural yoghurt (125 g), sugar (5 g) and water (330 g). This standard meal provided 842 kcal (3520 kJ) with the following nutrient composition: protein (11.7 %), carbohydrates (39.3 %) and lipids (49.0 %). A portion of one of the six lycopene preparations adapted to provide 25 mg of lycopene was incorporated into the test meal just before its consumption. This meal was consumed within 30 min. No other food was allowed over the following 6 h, but the subjects were allowed to drink bottled water with a maximum of 330 mL. Fasting blood was drawn from an anticubital vein by venipuncture into an evacuated tube containing potassium EDTA/K₃ that was immediately placed in
an ice-water bath and covered with an aluminium foil to avoid light exposure. Blood samples were collected 20 min and 5 min before consumption of the standard meal as well as after 2h, 3h, 4h, 5h and 6h post-absorption.

This short post-prandial time frame, *i.e.* 6h, was chosen to assess lycopene isomerization within the intestine with as low contamination by lycopene isomers coming from other organs (mainly the liver) as possible. Indeed, during the post-prandial period, chylomicrons secreted by the intestine, which contained lycopene coming from the meal, are transformed into chylomicron remnants following triacylglycerol hydrolysis by lipoprotein lipase. These chylomicron remnants are taken up by the liver. A fraction of dietary lycopene is then incorporated in VLDL and released in the blood. It is not known, but possible, that an isomerisation of lycopene also occurs in the liver leading to increase blood concentration of lycopene isomers. Because liver VLDL are starting to be produced some hours after a fat meal intake, the longer the post-prandial period, the higher the level in the blood of VLDL coming from the liver. As, technically, it is quite complicated to isolate the chylomicrons remnants from the VLDL, we decided to collect the TRL during the initial post-prandial period so that they contain mostly lipoproteins from intestinal origin.

**BLOOD SAMPLE PREPARATION**

Tubes containing the blood were protected from light, stored at 4°C and then centrifuged within 2h (10 min, 4°C, 878 x g) to separate the plasma. On the test day, plasma (6 mL) was overlaid with 0.9% NaCl solution and centrifuged for 28 min at 130’000 x g at 10°C in a SW41Ti rotor (Beckman, Fullerton, CA, USA) in a L7 ultracentrifuge (Beckman). The upper phase containing TRL, *i.e.* mainly chylomicrons and chylomicron remnants, was collected. Immediately after recovery, TRL were divided into aliquots and immediately stored at -80°C prior to lycopene isomer profile determination.
185 **Analytical Procedures**

The profile of lycopene isomers was determined according to the method described previously by Schierle et al.\(^{18}\) for lycopene products (Figure 1), and according to the method of Ferruzzi et al.\(^{24}\) for TRL. The main lycopene isomers identified were 5-(Z), 9-(Z), 13-(Z) and (all-E) lycopene. Minor compounds eluting between 24 and 48 min (Figure 1) were identified as (Z)-lycopene by LC–MS/MS using an Applied Biosystems APCI 4000 LC–MS/MS (Foster City, CA, USA). Following conditions were used: isocratic flow 1 mL/min; declustering potential, 130 V; 60 psi (414 kPa) N\(_2\); capillary voltage 22 V; vaporiser temperature, 400\(^\circ\)C; corona needle 5mA; the fragmentation conditions used were those described by dos Anjos Ferreira et al.\(^{25}\). The peak areas of unidentified (Z)-lycopene isomers were summed up and reported as x-(Z) lycopene (Table 1).

**Data Analyses**

The raw data consisted in TRL-lycopene isomer concentrations (expressed in mmol/L) of five lycopene-isomers i.e. x-(Z), 5-(Z), 9-(Z), 13-(Z) and (all-E) measured at 6 time points (before and, 2, 3, 4, 5 and 6 hours after ingestion of the standardized meal containing lycopene) for each of the 6 different test meals and each of the 27 subjects. These raw data were averaged for the 27 subjects and presented as mean +/- SEM for each time point (Figure 2). The baseline was the average of the two lycopene concentrations measured in plasma samples collected before consumption of the meal. As our goal was to determine lycopene isomerisation during the absorption phase, we voluntary have limited the measurement of lycopene isomer profiles to short period of time after lycopene intake. Thus, we measured mainly lycopene present in chylomicrons in the TRL fraction with only few lycopene present in the VLDL fraction coming from the liver. These purely descriptive analyses confirmed that lycopene concentrations did not return to baseline 6 hours after ingestion of test meal. In consequence, no formal kinetics modeling (e.g. area under the curve, T\(_{max}\) or C\(_{max}\)) was further considered. In order to complete this descriptive data analysis and to simplify...
the discussion of results, individual lycopene isomers were expressed as relative percentage of total lycopene calculated as the sum of the five lycopene isomers, \textit{i.e.} $x$-(Z), 5-(Z), 9-(Z), 13-(Z) and (all-E) (Figure 3).
Results

Following the ingestion of the six standardized meals supplemented with lycopene, TRL-lycopene concentrations increased promptly, reaching a first maximum concentration between 2 and 4 h and started to increase again after 5 h (Figure 2). Since lycopene isomer profiles were determined over a short period of time only, the second maximum was not well characterized.

These two rises of lycopene concentrations are in agreement with the known lycopene absorption processes and show a first appearance of dietary lycopene in chylomicrons (before 4 hours) and a subsequent incorporation of dietary lycopene chylomicrons into newly released VLDL from the liver (after 5 hours). In consequence, our choice for a short post-prandial time frame (0-6 hours), which accounts for the appearance of lycopene in chylomicrons, is ideal to determine lycopene isomerization within the intestine.

Interestingly, the profile of lycopene isomers expressed in percentage (Figure 3) did not vary over the 6h post-absorption. Moreover, the inter-individual variability of concentrations (Figure 2) and percentages of lycopene (Figure 3) was very small.

PROPORTION OF LYCOPENE ISOMERS IN TRL AFTER CONSUMPTION OF TOMATO PREPARATIONS RICH IN (Z)-LYCOPENE

Consumption of a tomato preparation rich (65%) in 5-(Z) lycopene resulted in a close proportion (60%) of this isomer in TRL suggesting a good absorption and a low isomerisation of this isomer in the enterocytes (Figures 2A and 3A). The other lycopene isomers observed in TRL consisted of 20% of (all-E), 9% of x-(Z), 7% of 13-(Z) and 2% of 9-(Z). Since, these (Z)-isomers were not present in the diet, their appearance in TRL suggests that they were formed during absorption by isomerisation of the (all-E) lycopene.

Only a low amount (9%) of the 13-(Z) isomer was measured in TRL following the intake of a tomato preparation which contained 42% of this isomer (Figures 2B and 3B). Other lycopene
isomers measured in TRL were mainly the (all-E) (about 50%) and the 5-(Z) (25%) isomers,
suggesting that part of the 13-(Z) lycopene was isomerised into these isomers during absorption.

Eating the tomato preparation rich in a mixture of 9-(Z) and 13-(Z) lycopene induced a
release of lycopene in TRL containing mostly the (all-E) (42%) and 5-(Z) (27%) isomers (Figures
2C and 3C), suggesting that the 9-(Z) and 13-(Z) isomers were isomerised into the (all-E) and 5-(Z)
isomers.

**PROPORTION OF Lycopene ISOMERS IN TRL AFTER CONSUMPTION OF TOMATO PREPARATIONS RICH IN (ALL-E) Lycopene**

Consumption of the tomato paste rich in (all-E) lycopene (about 95%) resulted in observing
various lycopene isomers in TRL, *i.e.* roughly 60% (all-E) and 40% (Z)-isomers, with about 18% of
5-(Z), 10% of 13-(Z) and x-(Z), and 2% of 9-(Z) (Figures 2D and 3D). The appearance of isomers
that were not detected in the tomato paste, *i.e.* 9-(Z), 13-(Z) and x-(Z), strongly suggests that part of
the (all-E) was isomerised during absorption.

The same profile of lycopene isomers in TRL was also observed with the two other tomato
preparations, *i.e.* lactolycopene and oleoresin, containing about 95% (all-E) lycopene (Figures 2E,
2F, 3E, 3F). These results indicate that the three tomato preparations rich in (all-E) isomer were
similarly isomerised during their absorption in humans and, therefore, that the lycopene food matrix
did not influence it.
Discussion

We have recently demonstrated that, during absorption, a significant isomerisation of (all-E) lycopene into various (Z)-lycopene isomers takes place in the human enterocytes (22). The first objective of the present study was to determine if the lycopene isomer profile in the blood circulation was affected by the isomer profile in the meal.

Consumption of the tomato preparation rich in 5-(Z) lycopene led to a very high proportion of 5-(Z) lycopene isomer in TRL (around 60%), which indicates either a good absorption, or a greater thermodynamical stability (27) of this isomer, or both.

The consumption of either a preparation rich in a mixture of 9-(Z) and 13-(Z) or a preparation rich in 13-(Z) lycopene was associated with the release of TRL which contained lower proportions of these two isomers as compared to those present in the tomato preparations. These results indicate either a low absorption of these isomers or an isomerization of these isomers into more thermodynamically stable lycopene isomers, i.e. (all-E) and 5-(Z), during absorption. The second hypothesis seems the most likely as the proportion of the (all-E) and 5-(Z) isomers measured in TRL after intake of the preparation rich in 9-(Z) and 13-(Z) isomers was higher than in the preparation itself.

These results show that, although the human intestine is able to modify the profile of dietary lycopene isomers which is finally found in the body, the resulting profile is not the same for all isomer sources supplied but is depending on the initial relative proportions of these isomers existing in the meal. This suggests that the mechanism of (E) to (Z) conversion can be overwhelmed by offering high amounts of dietary lycopene (Z)-isomers e.g. 5-(Z). It is, therefore, possible to modulate the profile of (Z)-isomers which enter the human body via the intestinal lipoproteins by a lycopene isomer-rich diet.

Besides, it is well-known that the bioavailability of (all-E) lycopene is dependent on the tomato preparation (21). In agreement with results reported in the literature (15, 17, 26) we have shown that consuming a tomato paste which contained mainly (95%) (all-E) lycopene resulted in secretion...
of TRL containing 40% of lycopene (Z)-isomers\(^{(21, 22)}\), which suggests that part of the ingested (all-E) lycopene was converted into (Z)-isomers. The secondary objective of this work was to assess whether the preparation of all-E lycopene affects the isomerisation of the (all-E) lycopene during intestinal absorption. Present results show that consumption of the three tomato preparations rich in (all-E) isomer (tomato paste, (all-E) oleoresin and lactolycopene) resulted in a similar isomerisation of the (all-E) lycopene. This shows that lycopene isomerisation occurring in the enterocytes is not significantly affected by the lycopene preparation. Another key observation is that the main (Z)-isomer recovered in TRL was the 5-(Z) lycopene (about 20% of the total lycopene content). This suggests that either the 5-(Z) lycopene is specifically formed during isomerisation of the (all-E) lycopene, or the 5-(Z) isomer, being more stable than the others\(^{(27)}\), leading to its preferential accumulation during absorption, or both.

Interestingly, there is a low inter-individual variation of lycopene isomerisation during intestinal absorption, which is in agreement with previous results observed with tomato paste\(^{(22)}\). (Z)-lycopene isomers have been discussed for their specific bioactivity. Indeed, individual (Z)-lycopene isomers exhibit different antioxidant activities\(^{(23)}\) and some human organs like prostate\(^{(16)}\) and skin accumulate high amounts of (Z)-lycopene isomers. More research is needed to evaluate how chronic supplementation with a diet enriched in (Z)-lycopene isomers will affect their distribution in tissues and subsequently will modulate lycopene bioactivity in humans.
Acknowledgments

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M. R., P. L., C.J., P.B. and K. B. conceived and designed the present study. I. T., P. L., A-F. M. and K. B. achieved the design and production of formulations of the lycopene products. C.J. coordinated the trial and supervised the analytic aspects. C.J. and P.B. contributed to the development of analytic methods, lycopene analysis and data collection. M. R., P. L., C.J., P.B., K. B. and A.R. analyzed the data. M. R. wrote the manuscript, and all authors were involved in interpreting the results and in critical revision of the paper.

No author has any advisory board affiliations. There is no conflict of interest.
References


Figure legends

**Figure 1**: HPLC chromatograms of the lycopene isomer profiles in the tomato paste rich in (all-E) lycopene and in the three tomato preparations rich in (Z)-lycopene isomers.

**Figure 2**: TRL-lycopene isomer concentrations over 6h post-consumption of the standard meals providing 25 mg lycopene from various sources. Results are presented as mean ± SEM (nmol/L, n=27). x-(Z) lycopene (♦); 5-(Z) lycopene (■); 9-(Z) lycopene (▲); 13-(Z) lycopene (×); (all-E) lycopene (*).

**Figure 3**: TRL-lycopene isomer profiles over 6h post-consumption of the standard meals providing 25 mg lycopene from various sources. Results are expressed as percentage of the total lycopene (mean ± SEM, n=27). x-(Z) lycopene (♦); 5-(Z) lycopene (■); 9-(Z) lycopene (▲); 13-(Z) lycopene (×); (all-E) lycopene (*).
Table 1: Lycopene isomer profile of the six lycopene preparations. Results are expressed as percentage of total lycopene.

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<th>X-(Z)</th>
<th>5-(Z)</th>
<th>9-(Z)</th>
<th>13-(Z)</th>
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