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Lycopene absorption in humans after the intake of two different single-dose lycopene formulations

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ABSTRACT

Lycopene is the main carotenoid present in tomato and its derivatives. It can also be obtained from the fungus *Blakeslea trispora* and its use as novel food ingredient was recently approved.

The aim of the present study was to investigate the absorption of lycopene, in humans, after the intake of a single dose providing 15 mg of lycopene from tomato extract (oleoresin 6%) or *B. trispora* (oil suspension 6%).

Twelve female subjects were enrolled and divided into two groups: group 1 was assigned to the sequence tomato lycopene/wash-out/*B. trispora* lycopene, whereas group 2 followed the sequence *B. trispora* lycopene/wash-out/tomato lycopene. The formulations were consumed early in the morning with 5 mL of sunflower oil, 100 g of bread and 150 mL of water. Blood was collected before consumption and after 2, 4, 6, 8, 10, 12 and 24 h. Plasma lycopene concentrations were determined by HPLC analysis. On the whole, statistical analysis of data did not demonstrate a different effect of the type of lycopene source on the carotenoid absorption. The maximum increase in plasma lycopene concentration was about 40 nmol/L for both products at 10–12 h ($p < 0.05$) post-consumption and decreased to basal values at 24 h. A transient higher increase in lycopene concentration at 4–6 h ($p < 0.05$) after tomato lycopene with respect to *B. trispora* lycopene intake was observed.

In conclusion, the intake of a single dose of the two liposoluble lycopene formulations revealed a comparable, small, but significant increase in plasma lycopene concentrations.

1. Introduction

Lycopene is a lipid soluble carotenoid pigment naturally occurring in red fruits and vegetables even if the main dietary sources are tomato and its derivatives.

Several studies demonstrated that regular intake of tomato products can have a significant impact on human health [1–3]. The specific compounds responsible for the protection afforded by tomatoes and derivatives have not been clearly demonstrated, even if lycopene seems to have an important role, probably through a synergy with other bioactive components present in these foods [1,4]. It has been demonstrated that lycopene is a powerful antioxidant able to protect lipids, DNA and protein from oxidative damage. It may also stimulate the modulation of cell growth, connexin 43 expression, IGF-1 and/or IGF binding protein circulating levels, inflammatory processes, immune function and other [5,6].

Lycopene intake varies depending on the population considered [7]; moreover many factors may affect lycopene bioavailability such as the amount introduced, the interaction with other compounds, the presence of effectors of absorption (e.g. fat), food processing, the nature of the food matrix as well as host related factors [8–12].

In this regard, several pharmacokinetic studies have evaluated the bioavailability of a number of different lycopene formulations. However, to our knowledge no data are available about the amount of lycopene coming from foods in which lycopene is added as an ingredient by the food industries.

For these reasons, the study of lycopene bioavailability from different food sources and also from novel ingredients may help us understand their nutritional contribution in terms of lycopene availability. At the present time there is considerable debate on the role and the safety of novel food ingredients as sources of bioactive compounds. In this regard, in 2009, the European Food Safety Authority (EFSA) authorized the placing on the market of lycopene from *Blakeslea trispora* as a novel food ingredient [13]. *B. trispora* is a fungus that received attention in recent years, as it can synthesize large quantities of lycopene. In humans, the absorption of lycopene from new formulations is not well documented; our purpose was to investigate and compare the absorption of lycopene from two commercial products of different origin like tomato and *B. trispora*.

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Plasma lycopene concentrations were evaluated after consumption of a single dose of each formulation, providing 15 mg of lycopene in oil suspension, in human volunteers.

2. Materials and methods

2.1. Chemicals

Standard of lycopene was from Sigma (Sigma Chemical Co, St Louis); echinenone standard was from La Roche (Hoffman–La Roche, Basel, Switzerland). Methanol (MetOH), tetrahydrofuran, ethanol and hexane were from VWR international (VWR Zurich, Switzerland); water was obtained from a MilliQ apparatus (Millipore, Milford, MA).

2.2. Dietary ingredients

The products used in the study were commercially available in the market and consisted of two formulations obtained from *B. trispora* and from tomato:

Formulation A: Oil suspension 6% in sunflower oil from *B. trispora*.
Formulation B: Oleoresin 6% – from tomato.

2.3. Recruitment of volunteers

Twelve female subjects, 25.2 ± 2.2 years old with body mass index (BMI) within the normal range ($20.2 \pm 1.6\,kg/m^2$) were recruited from the student population of the University of Milan by advertisements displayed in the University Bulletin Boards. Preliminary selection of the subjects was performed on the basis of their eating behaviour evaluated by means of a questionnaire about food intake and their preferences, specifically evaluating the frequency of fruit and vegetable intake, targeting tomato products. A medical history questionnaire was also used to exclude subjects with health problems such as dyslipidemia, hepatic, renal, or gastrointestinal dysfunction and obesity. Subjects were specifically excluded from the study if they were smokers or were taking drugs, supplements or medications at least 1 month before the beginning of the experimentation Moreover volunteers who did not eat fruits and vegetables, those who followed a specific diet such as vegetarian, vegan or macrobiotic, and those who had a specific aversion to tomato or tomato products were excluded. Subject selection was also determined on the basis of the results of blood analysis performed, in order to exclude those with high basal levels of plasma lycopene (>600 nmol/l as previously observed in groups of young Italian subjects), high levels of triglycerides (>1.69 mmol/l), total cholesterol (>5.2 mmol/l), LDL cholesterol (>3.34 mmol/l) and low HDL cholesterol (<1.3 mmol/l). Volunteers enrolled declared to consume from 3 to 5 portions per week of tomato products. The protocol and the purpose of the study were exhaustively explained to the participants and informed written consent was signed by volunteers. The study protocol was approved by the Ethic Committee of the University of Milan.

2.4. Experimental design

Subjects selected for the study were deprived of food sources of lycopene 2 days before experimentation. Moreover, each volunteer received a complete list of foods to be avoided; the list included lycopene-rich foods such as raw tomatoes and tomato products like ketchup, paste, sauce, juice and soup. Vegetables and fruits rich in carotenoids, as well as supplements, were not allowed.

A randomised, repeated measures crossover design study was performed. Subjects were randomly divided in two different groups: group 1 was assigned to the sequence tomato lycopene/wash-out/*B. trispora* lycopene, whereas group 2 followed the sequence *B. trispora* lycopene/wash-out/tomato lycopene. On the scheduled day, fasting overnight subjects consumed one single dose of the two different formulations (formulation A: 250 mg of oil suspension 6% in sunflower oil from *B. trispora*; formulation B: 250 mg of Oleoresin 6% – from tomato) providing 15 mg of lycopene. The formulations were consumed early in the morning with 5 ml of sunflower oil (rich in oleic acid), 100 g of bread and 150 ml of water. One ml of blood was collected from each volunteer at time 0, and after the product intake at 2, 4, 6, 8, 10, 12 and 24 h.

Lunch was provided 4.5 h after the intake of the product; it contained a negligible amount of carotenoids and consisted of two sandwiches (one with cooked ham and cheese and one with raw ham), approximately 50 g of ice cream and 500 ml of water. The dinner was self-selected and subjects were asked to exclude all carotenoid-containing foods.

To check the compliance to the dietary instructions, 1 day-food record was kept by subjects in each experimental session, 2 days before and 1 day after the intake of formulations.

2.5. Lycopene determination

Plasma for lycopene determination was obtained by centrifugation for 10 min at $1000 \times g$ and samples were stored at $-80\,^{\circ}C$ until analysis.

Lycopene was extracted from plasma according to a method previously described [8]. Briefly, plasma (100 μL), in triplicate, was extracted with 100 μL ethanol containing internal standard (echinenone) and 200 μL hexane. Samples were vortexed for 1 min and centrifuged for 5 min at $1000 \times g$ at $4\,^{\circ}C$. The supernatant (150 μL) was evaporated under nitrogen in the dark and dissolved in 100 μL of the mobile phase for the HPLC injection (50 μL). The HPLC analysis [14] was performed with a system equipped with an Alliance mod 2695 (Waters, Milford, MI, USA) and a mod 2996 photodiode array detector (Waters). Chromatographic data were acquired using an Empower workstation (Waters). The analytical column was a 5 μm C18 Vydac 201TP54 (250 mm × 4.6 mm, i.d.; Esperia, CA, USA) with biocompatible frits. The eluent was methanol:THF (95:5) at a flow rate of 1 mL/min. Visible detection was achieved at 472 nm. Lycopene standard (Sigma Chemical Co., St Louis, MO) was prepared daily to avoid degradation. Data were corrected by the recovery of the internal standard. The recovery was between 90 and 100%.

2.6. Lipid profile

Serum total cholesterol, HDL-C, triglyceride and glycemia levels were measured by routine validated laboratory methods based on immunoenzymatic and colorimetric tests. LDL-C was determined by using Friedenwald's formula.

2.7. Statistical analysis

Sample size was calculated on the basis of data obtained in a previous study evaluating the absorption of about the same amount of lycopene (16.5 mg) from two tomato products [8]. Moreover, the use of a homogeneous group of subjects (for anthropometric characteristics and tomato intake) and of the repeated measure crossover design was chosen as the optimal approach to limit inter-individual variability thus allowing the demonstration of differences between treatments with a small sample size. Statistical analysis was performed by means of STATISTICA software (Statsoft Inc., Tulsa, OK, US). The Shapiro–Wilk test was applied to verify the normal distribution of the variables. Data obtained were examined by two ways ANOVA for repeated measures design, using the type of product and time as dependent factors. Differences between means