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Influence of olive oil on carotenoid absorption from tomato juice and effects on postprandial lipemia

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ABSTRACT

The potential benefits of tomato-rich diets for the cardiovascular system have been related to plasma concentrations of carotenoids. In addition, the bioavailability of carotenoids from foods depends on their chemical structure, processing and the food matrix. Our aim was to evaluate the effect of adding oil to tomato juice (not treated with heat) on the bioavailability of plasma carotenoids and postprandial lipid response. In a randomized, controlled, crossover feeding trial, eleven healthy volunteers were assigned to receive a single ingestion of 750 g of tomato juice (TJ) containing 10% of refined olive oil/70 kg body weight (BW) and 750 g of TJ without oil/70 kg BW on two different days. All lycopene isomers increased significantly in subjects consuming TJ with oil, reaching the maximum concentration at 24 h. LDL cholesterol and total cholesterol decreased significantly 6 h after the consumption of TJ with oil, which significantly correlated with an increase of *trans*-lycopene and 5-*cis*-lycopene, respectively.

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1. Introduction

Several studies have observed that a regular intake of tomato products may have a significant impact on human health (Burton-Freeman, Talbot, Park, Krishnankutty, & Edirisinghe, 2012; Ghavipour et al., 2012). The consumption of $\geqslant 7$ servings/week of tomato-based products has been associated with a 30% relative risk reduction for cardiovascular disease (CVD) (Sesso, Liu, Gaziano, & Buring, 2003) and a reduction of coronary and inflammatory biomarkers (Riso et al., 2006; Sesso, Wang, Ridker, & Buring, 2012). These potential benefits of tomato-rich diets on vascular health have been attributed to the high concentrations of carotenoids, mainly lycopene, reached in plasma (Mordente et al., 2011).

The bioaccessibility of carotenoids can be affected by many factors, including the food matrix, processing and cooking methods, and the interactions, during digestion and absorption, with other dietary compounds, such as fibre, lipids, phytosterols and other carotenoids (Yonekura & Nagao, 2007; Svelander et al., 2010). Among dietary factors, heat and mechanical treatments of foods and the presence of a certain amount of fat in the meal appear to be critical factors for carotenoid bioaccessibility and bioavailability *in vivo* (Fielding, Rowley, Cooper, & O'Dea, 2005). Structure also plays an important role in carotenoid bioaccessibility. The *trans* isomer is the most usual form of carotenoids found in fresh tomatoes, mainly *trans*-lycopene, since it is the most stable thermodynamically (Erdman, 2005). By contrast, human plasma and tissues contain more than 50% of *cis*-isomers, the most common and bioavailable forms of these compounds (Unlu et al., 2007).

Most studies on the bioavailability of carotenoids from tomato products are focussed only on lycopene (Frohlich, Kaufmann, Bitsch, & Bohm, 2006; Lee et al., 2009; Richelle et al., 2012). However, very little information is available, in the literature, regarding the absorption and bioavailability of different lycopene isomers and other carotenoids, and their possible effects on human lipid metabolism.

Abbreviations: AUC_{0-24}, area under the concentration–time curve from time zero (0) to 24 h; BMI, body mass index; BP, blood pressure; BW, body weight; CVD, cardiovascular disease; C_{max}/AUC_{0-24}, absorption rate; HDL-c, high density lipoprotein cholesterol; HPLC-MS/MS, high pressure liquid chromatography–mass spectrometry in tandem; LDL-c, low density lipoprotein cholesterol; MTBE, methyl tert-butyl ether; PTFE, polytetrafluoroethylene; SD, standard deviation; TJ, tomato juice; TC, total cholesterol.

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The aim of this study was to evaluate the effect of the addition of oil to a tomato juice (TJ) preparation, without cooking, on postprandial absorption and bioavailability of carotenoids from TJ and tentatively evaluate the plausible effect on lipid metabolism. For this purpose an open, controlled, randomized, crossover feeding trial was carried out in 11 healthy adults.

2. Materials and methods

2.1. Samples

The TJ was elaborated with raw tomato fruits (*Lycopersicum esculentum L.*, Royalty variety) at commercial maturity, supplied by CASI Cooperative (Almeria, Spain). TJ preparation was processed at the Torribera campus, University of Barcelona (UB, Barcelona, Spain) by a standardised industrial scale-like manufacturing process. Refined olive oil was kindly furnished by the Juan Ballester Rosés Company (Tortosa, Spain). Briefly, tomatoes were cleaned and sanitized with chlorinated water to remove any impurities and chemical compounds on the surface. Afterwards, the tomatoes were cleaved and weighed to obtain the desired amount of juice by crushing in a blender texturiser at high speed. Then the refined olive oil or the corresponding amount of water (10%) was added and the resulting TJ was packaged under vacuum to be frozen at −20 °C.

2.2. Subjects

Eleven healthy subjects (6 men and 5 women), with mean age, 28 ± 3 years and mean BMI 23 ± 2 kg/m^2, were recruited. BMI was obtained by doing measurements of height and weight at the beginning of intervention. None reported any history of heart disease, homeostatic disorders or other medical conditions. All subjects were non-smokers and were not receiving medication or vitamin supplements. None had followed any special diet for at least 4 weeks prior to participating in the study.

2.3. Study design

The design was similar to previous acute studies performed by our research group with other foods (Roura et al., 2007, 2008).

The study was an open, controlled, randomized and crossover feeding trial. The eleven participants underwent a three-day washout period during which they were asked to consume their regular diet avoiding tomato or processed tomato-based products and foods containing carotenoids. A list showing permitted and forbidden foods was provided to the participants, as two sample menus. The subjects fasted for at least 8 h before the intervention intake. Using a computer-generated random list of numbers, each volunteer was assigned to receive: 750 g TJ with refined olive oil and 750 g TJ without refined olive oil/70 kg of body weight (BW) on two different days. The TJ portion was chosen according to data available in the literature (Gustin et al., 2004; Porrini, Riso, & Testolin, 1998) in order to achieve, after administration of a single dose, a sufficient carotenoid plasma level to observe a quantifiable effect response.

Blood samples were collected in plasma EDTA tubes before the test meal and at 3, 6 and 24 h after each intervention. During the 6 h interval, participants rested in the clinical ward in a quiet room and were allowed to drink only water. In the period from 6 to 24 h, participants were asked to consume their regular diet, avoiding tomato or processed tomato-based products and foods containing carotenoids. Blood samples were immediately centrifuged after collection at 1500*g* for 15 min at 4 °C and plasma was aliquotted and stored at −80 °C prior to analysis.

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee of Clinical Investigation of the University of Barcelona (Spain) and the Institutional Review Board of the Hospital Clinic in Barcelona. Written informed consent was obtained from all subjects before inclusion in the trial. This study has been registered at the London Controlled-trial register with ISRCTN99660610 as the number.

2.4. Dietary assessment

Before each intervention, we used a 24 h food recall questionnaire to assess compliance with the recommended diet. Dietary intake was converted into nutritional data, using the Professional Diet Balancer software (Cardinal Health Systems).

2.5. Blood pressure monitoring and biochemistry analysis

Blood pressure (BP), glucose and lipid profile were analysed at baseline and 6 h after each intervention. BP was measured in the non-dominant arm, using an automatic oscillometer (Omron 705 CP; Omron Matsusaka Co., Ltd., Matsusaka City, Japan) after 10 min resting in a seated position. Plasma concentration of total cholesterol (TC) and triglycerides was analysed by enzymatic procedures: HDL and LDL cholesterol after precipitation with phosphotungstic acid and magnesium chloride and blood glucose by the glucose oxidase method.

2.6. Extraction and isolation of carotenoid compounds from TJ

The extraction of carotenoids was carried out very quickly, avoiding exposure to light, oxygen, high temperatures and pro-oxidant metals such as iron or copper, in order to minimise autoxidation and *cis*/*trans* isomerisation. TJ samples (0.5 g) were homogenised with 5 ml of ethanol/hexane (4:3 v/v), following a procedure described elsewhere by Vallverdu-Queralt, Martinez-Huelamo, Arranz-Martinez, Miralles and Lamuela-Raventos (2012). The homogenate was sonicated for 5 min and centrifuged at 2140*g* for 15 min at 4 °C. The supernatant was transferred into a flask and the extraction was repeated. The two supernatants were combined and evaporated under nitrogen flow. Finally, the residue was reconstituted with methyl tert-butyl ether (MTBE) up to 1 ml and filtered through a 13 mm, 0.45 μm polytetrafluoroethylene (PTFE) filter (Waters, Milford, MA, USA) into an insert-amber vial for HPLC analysis. Samples were stored at −80 °C prior to analysis. Extractions were performed in triplicate.

2.7. Extraction and isolation of carotenoid compounds from human plasma

Briefly, 800 μl of ethanol was added to 800 μl of plasma. After vortex-mixing for 45 s (s), the plasma was extracted twice with hexane (2 ml, stabilized with 0.1 g/l of butylated hydroxytoluene), and the extracts were vortex-mixed for 1 min and centrifuged at 2140*g* for 5 min at 4 °C, following the procedure described by Olmedilla et al. (1997). The organic phases were removed, pooled, and evaporated under nitrogen flow. Finally, they were reconstituted with 300 μl of a solution of MTBE, filtered through a 13 mm, 0.22 μm PTFE filter (Waters, Milford, MA, USA) into an insert-amber vial for HPLC analysis and injected into the HPLC–UV system.

2.8. HPLC separation and HPLC–MS/MS identification of plasma carotenoids

Chromatographic analysis was performed, using an HP 1100 HPLC system (Hewlett–Packard, Waldbronn, Germany) equipped with a quaternary pump and an autosampler. The analytes were

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