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Original Contribution

Lycopene bioavailability is associated with a combination of genetic variants

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ABSTRACT

The intake of tomatoes and tomato products, which constitute the main dietary source of the red pigment lycopene (LYC), has been associated with a reduced risk of prostate cancer and cardiovascular disease, suggesting a protective role of this carotenoid. However, LYC bioavailability displays high interindividual variability. This variability may lead to varying biological effects following LYC consumption. Based on recent results obtained with two other carotenoids, we assumed that this variability was due, at least in part, to several single nucleotide polymorphisms (SNPs) in genes involved in LYC and lipid metabolism. Thus, we aimed at identifying a combination of SNPs significantly associated with the variability in LYC bioavailability. In a postprandial study, 33 healthy male volunteers consumed a test meal containing 100 g tomato puree, which provided 9.7 mg all-*trans* LYC. LYC concentrations were measured in plasma chylomicrons (CM) isolated at regular time intervals over 8 h postprandially. For the study 1885 SNPs in 49 candidate genes, i.e., genes assumed to play a role in LYC bioavailability, were selected. Multivariate statistical analysis (partial least squares regression) was used to identify and validate the combination of SNPs most closely associated with postprandial CM LYC response. The postprandial CM LYC response to the meal was notably variable with a CV of 70%. A significant ($P=0.037$) and validated partial least squares regression model, which included 28 SNPs in 16 genes, explained 72% of the variance in the postprandial CM LYC response. The postprandial CM LYC response was also positively correlated to fasting plasma LYC concentrations ($r=0.37$, $P<0.05$). The ability to respond to LYC is explained, at least partly, by a combination of 28 SNPs in 16 genes. Interindividual variability in bioavailability apparently affects the long-term blood LYC status, which could ultimately modulate the biological response following LYC supplementation.

Introduction

Lycopene (LYC) is the red pigment found in tomatoes and tomato products. It is the carotenoid found at the highest levels in the blood of Americans and the second one in the blood of Europeans. Its protective role against the development of prostate cancer and cardiovascular diseases has been suggested [1–6] but the mechanisms involved have yet to be fully elucidated. Indeed, although LYC's potential as an antioxidant is well characterized in vitro [7], there is evidence that its biological effects could also be mediated by metabolic products of LYC [8–11], a hypothesis supported by the results of recent molecular studies [11–14].

LYC digestion begins in the gastrointestinal lumen where digestive enzymes can modulate its bioaccessibility by facilitating its release from the food matrix to micelles [15–17]. Micelles then carry LYC to the apical side of the enterocyte. LYC uptake is not only passive, as previously thought [18], but its transport is also facilitated by two membrane proteins, SR-BI (scavenger receptor class B type I) [19], encoded by *SCARB1*, and CD36 (cluster determinant 36) [20]. Following uptake, it is possible that a portion of LYC may be metabolized. Indeed, enterocytes contain two enzymes with the potential to cleave LYC. The first one, BCO1

Abbreviations: ABCB1, ATP-binding cassette, subfamily B (MDR/TAP), member 1; AUC, area under the curve; BCO1, β-carotene 15,15′-oxygenase-1; BCO2, β-carotene 9,10′-oxygenase-2; CD36, cluster determinant 36; CM, chylomicron; ELOVL2, ELOVL fatty acid elongase 2; HPLC, high performance liquid chromatography; ISX, intestine specific homeobox; L-FABP, liver–fatty acid binding protein; LYC, lycopene; MTTP, microsomal triglyceride transfer protein; PLS, partial least squares; SCARB1, scavenger receptor class B, member 1; SNPs, single nucleotide polymorphisms; SOD2, superoxide dismutase 2, mitochondrial; SR-BI, scavenger receptor class B type I; VIP, variable importance in the projection

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(β-carotene 15,15′-oxygenase-1), is a dioxygenase [21] that catalyzes the oxidative cleavage of LYC with an efficiency similar to that of β-carotene [22], and which has been associated with blood LYC status [23]. The second enzyme, BCO2 (β-carotene-9,10′-oxygenase), has been previously suggested to be the main LYC-cleaving enzyme [10]. Regardless of possible metabolism, the fraction of parent LYC remaining in the enterocyte is transported within the cell to the site where it is incorporated into chylomicrons (CM). The mechanism of this transport is not known, but proteins involved in intracellular transport of lipids have been suggested to be involved [24]. LYC-containing CM are secreted into the lymph, and then enter the bloodstream. LYC is assumed to stay within these lipoproteins and to follow the fate of CM which is ultimately taken up by the hepatocytes [25].

Clinical trials dedicated to the study of LYC bioavailability have reported high interindividual variability in blood and tissue LYC concentration in response to LYC intake [18,26–30]. Genetic variations between individuals may provide a partial explanation of this phenomenon [31,32]. Some genetic polymorphisms have been shown to be associated with the variability in fasting blood LYC concentrations [23,30,33–36], and yet only one study has attempted to assess the role of genetic polymorphisms involved in the variability in blood LYC concentration following a LYC load [30]. Only two SNPs in *BCO1* have been previously shown to be associated with the variability in LYC bioavailability [30]. While promising, the small number of candidate genes previously investigated calls for more studies to explain the variation in a phenotype that is likely affected by numerous environmental and genetic factors [31]. In fact, we have recently demonstrated that the bioavailability of the carotenoid lutein is a complex phenotype that is not modulated by any single gene, but by the additive effects of several gene SNPs [37]. Thus, the aim of this study was to identify the combination of SNPs associated with the variability in LYC bioavailability.

Subjects and methods

Subject number and characteristics

Thirty-five healthy, nonobese, nonsmoking male subjects were recruited for the study. Subjects presented with normal energy consumption, i.e., $\approx$ 2500 kcal/d and drank $\leq$2% alcohol as total energy. They had no history of chronic disease, hyperlipidemia, hyperglycemia, and were not taking any medication that might affect LYC or lipid metabolism (e.g., tetrahydrolipstatin, ezetimibe, phytosterols, cholestyramine, fibrates) during the month prior to the study or during the study period. Because of the relatively large volume 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 study was approved by the regional committee on human experimentation (No. 2008-A01354-51, Comité de Protection des Personnes Sud Méditerranée I, France).[2] The procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. The objectives and requirements of the study were fully explained to all participants before beginning the study, and informed written consent was obtained from each subject. Two subjects left the study for personal reasons before participating in the postprandial experiment, leaving 33 subjects whose baseline characteristics are reported in Table 1. Note that the fasting plasma LYC concentration of these subjects was relatively high as compared to previous reported data [38], suggesting that these subjects regularly consumed tomatoes and tomato products.

DNA preparation and genotyping methods

An average of 25 μg of DNA was isolated from a saliva sample from each subject using the Oragene kit (DNA Genotek Inc., Kanata, ON, Canada) as described in detail previously [39]. DNA concentration and purity were checked by spectrophotometry at 260 and 280 nm (Nanodrop ND1000, Thermo Scientific, Villebon sur Yvette, France). All genotyping procedures were carried out by Integragen (Evry, France). Concerning the whole genome genotyping, the procedure was as follows: 200 ng of DNA was hybridized overnight to HumanOmniExpress BeadChips (Illumina, San Diego, CA, USA), allowing the analysis of approximately 7.33×10^5 SNPs per DNA sample. Unhybridized and nonspecifically hybridized DNA was washed out. The BeadChips were then stained and scanned on an Illumina iScan. Detailed methods are provided in the Infinium HD Assay Ultra Protocol Guide from Illumina. Concerning the 40 other SNPs (see "Choice of candidate genes"), they were genotyped as previously described [40].

Postprandial experiments

Subjects were asked to refrain from consuming LYC-rich foods (tomatoes, tomato products, foods rich in tomato, watermelon) for 48 h before the postprandial clinic visit. In addition, the day prior to the visit, they were asked to eat dinner between 7 and 8 p.m., without any alcohol intake. They were also asked to abstain from consuming any food or beverage other than water after the dinner and until the clinic visit. After the overnight fast, they arrived at the local Center for Clinical Investigation (la Conception Hospital, Marseille, France) and consumed a test meal including 100 g of tomato puree purchased from a local supermarket providing 9.7 mg all-*trans* LYC as measured by high performance liquid chromatography (HPLC). The puree also contained around 0.5 mg *cis* isomers of LYC as evaluated by the relative peak area of all the *cis* isomers (4 peaks identified) as compared to the peak area of the all-*trans* isomer in the HPLC chromatograms. This LYC dose allowed us to deliver about twice the mean dietary intake of LYC in France and a LYC dose close to that of the daily intake in the United States [30,38]. The remainder of the test meal consisted of semolina (70 g) cooked in 200 mL of hot water, white bread (40 g), egg whites (60 g), peanut oil (50 g), and mineral water (330 mL). The subjects were asked to consume the meal at a steady pace, with half of the meal consumed in the first 10 min, and the remainder of the meal consumed in the 20 next min. This pacing should have ultimately reduced any variability in gastric emptying due to variation in rates of intake. No other food was permitted over the following 8 h, but subjects were permitted to finish the remainder of the 330 mL of water they had not drunk during the meal. A fasting baseline blood sample was drawn before administration of the meal as well as at 2, 3, 4, 5, 6, and 8 h after meal consumption. Blood was taken up into evacuated tubes containing K-EDTA. The tubes were immediately placed into an ice-water bath and covered with aluminum foil to avoid light exposure. Plasma was isolated by centrifugation (10 min at 4 °C and 878*g*) within 2 h following collection.

CM preparation

Plasma (6 mL) was overlaid with 0.9% NaCl solution (4.5 mL) and centrifuged for 28 min at 130,000*g* at 10 °C using a SW41Ti rotor (Beckman Coulter, Villepinte, France) in a Thermo Sorvall WX100 ultracentrifuge (Thermo Scientific, Saint Herblain, France). The upper phase, containing mainly CM and large CM remnants

[2] This clinical trial is registered at http://www.clinicaltrials.gov/ct2/. The ID number is NCT02100774.