Interindividual variability of lutein bioavailability in healthy men: characterization, genetic variants involved, and relation with fasting plasma lutein concentration

Patrick Borel, Charles Desmarchelier, Marion Nowicki, Romain Bott, Sophie Morange, and Nathalie Lesavre

ABSTRACT

Background: Lutein accumulates in the macula and brain, where it is assumed to play physiologic roles. The bioavailability of lutein is assumed to display a high interindividual variability that has been hypothesized to be attributable, at least partly, to genetic polymorphisms.

Objectives: We characterized the interindividual variability in lutein bioavailability in humans, assessed the relation between this variability and the fasting blood lutein concentration, and identified single nucleotide polymorphisms (SNPs) involved in this phenomenon.

Design: In a randomized, 2-way crossover study, 39 healthy men consumed a meal that contained a lutein supplement or the same meal for which lutein was provided through a tomato puree. The lutein concentration was measured in plasma chylomicrons isolated at regular time intervals over 8 h postprandially. Multivariate statistical analyses were used to identify a combination of SNPs associated with the postprandial chylomicron lutein response (0–8 h area under the curve). A total of 1785 SNPs in 51 candidate genes were selected.

Results: Postprandial chylomicron lutein responses to meals were very variable (CV of 75% and 137% for the lutein-supplement meal and the meal with tomato-sourced lutein, respectively). Postprandial chylomicron lutein responses measured after the 2 meals were positively correlated (r = 0.68, P < 0.0001) and positively correlated to the fasting plasma lutein concentration (r = 0.51, P < 0.005 for the lutein-supplement–containing meal). A significant (P = 1.9 × 10−4) and validated partial least-squares regression model, which included 29 SNPs in 15 genes, explained most of the variance in the postprandial chylomicron lutein response.

Conclusions: The ability to respond to lutein appears to be, at least in part, genetically determined. The ability is explained, in large part, by a combination of SNPs in 15 genes related to both lutein and chylomicron metabolism. Finally, our results suggest that the ability to respond to lutein and blood lutein status are related. This trial was registered at clinicaltrials.gov as NCT02100774.

INTRODUCTION

Lutein is present at high concentrations in the human macula lutea (1–6). The involvement of lutein in the prevention of age-related macular degeneration has been suggested (7–13). Lutein has recently been shown in the human brain where it has been suggested to have a beneficial role on cognitive function (14, 15).

Clinical studies that have provided dietary lutein have reported a high interindividual variability in blood and tissue lutein concentrations in response to dietary lutein (16). Both dietary factors (17) and genetic variations between individuals (18) have been proposed to explain this phenomenon. In support of the latter factor, recent studies have shown that blood and tissue concentrations of lutein are associated with genetic polymorphisms (16, 19, 20).

Lutein metabolism starts in the gastrointestinal lumen where digestive enzymes can modulate its bioaccessibility by allowing the release of this hydrophobic compound from its food matrix to mixed micelles (17, 21). Cell culture studies have shown that at least 2 apical membrane transport proteins [ie, scavenger receptor class B type I (SR-BI) (22, 23), which is encoded by SCARBI, and Niemann-Pick disease, type C1, gene-like 1 (NPC1L1) (24)] participate in the uptake of micellarized lutein. Candidate gene association studies have also suggested that other enterocyte membrane lipid transporters [ie, cluster determinant 36 (CD36) (19), ATP-binding cassette G5/G8 (ABCG5/G8) (19, 20), and ATP-binding cassette subfamily member 1 (ABCA1) (16, 20)] could participate in lutein absorption.

After its uptake at the apical side of the enterocyte, lutein is transported within the cell to the site where it is incorporated into chylomicrons. The precise mechanisms of this process are not known, although proteins involved in the intracellular transport of lipids might be involved (25).

1 From Institut National de la Recherche Agronomique (INRA), Unité Mixte de Recherche (UMR) INRA1260, Marseille, France (PB, CD, MN, and RB); the Institut National de la Recherche Médicale (INSERM), UMR_S 1062, Marseille, France (PB, CD, MN, and RB); Aix Marseille Université, Nutrition Obésité et Risque Thrombosté, Marseille, France (PB, CD, MN, and RB); the Centre d’Investigation Clinique (CIC) Hôpital de la Conception, Marseille, France (SM); and the CIC Hôpital Nord, Marseille, France (NL).
2 PB and CD are joint first authors.
3 This publication reflects only the views of the authors. The Lycocard community is not liable for any use that may be made of the results.
4 Supported by the European Community’s Sixth Framework Programme. The funding was attributed to the Lycocard project (no. 016213), which was an Integrated Project within the framework of the “Food Quality and Safety” programme. Lutein standard was a gift of DSM LTD. Lutein supplement was a gift of Pileje.
5 Address reprint requests and correspondence to P Borel UMR 1260 INRA/INRERAix-Marseille University, “Nutrition Obesity and Risk of Thrombosis,” Faculté de Médecine, 27, Boulevard Jean Moulin, 13005 Marseille, France. E-mail: patrick.borel@univ-amu.fr.
6 Received February 11, 2014. Accepted for publication April 15, 2014. First published online May 7, 2014. doi: 10.3945/ajcn.114.085720.
The enterocyte contains 2 enzymes that can be involved in carotenoid metabolism within the enterocyte β,β-carotene-15,15'-monooxygenase (BCMO1), which has been associated with blood lutein status (16, 19, 26–28), and β,β-carotene-9,10'-oxygenase (BCO2), the involvement of which in lutein metabolism has recently been shown (29). The fraction of carotenoid that is not metabolized by these enzymes is incorporated into chylomicrons, which are secreted into the lymph and enter the bloodstream. Chylomicrons are hydrolyzed to chylomicron remnants that are mainly taken up by liver cells (30).

Although it is known that there is high interindividual variability in lutein bioavailability, no study, to our knowledge, has attempted to comprehensively characterize this phenomenon. Furthermore, consequences of this variability with regard to chronic blood lutein status are not known. Thus, the aims of the current study were to 1) better characterize the interindividual variability of lutein bioavailability in healthy subjects, 2) assess whether this variability in lutein bioavailability can affect fasting concentrations of lutein, and 3) identify the interplay between the multiple genetic variants that are involved in this phenomenon.

SUBJECTS AND METHODS

Subject number and characteristics

Forty healthy, nonobese, nonsmoker men were recruited for the study. This group of subjects had allowed for the identification if a combination of single nucleotide polymorphisms (SNPs) that significantly explains the postprandial chylomicron triacylglycerol response to dietary fat (31). Subjects presented normal energy consumption (ie, 2500 kcal/d). Subjects either drank no alcohol or drank <2% alcohol as total energy. Subjects had no history of chronic disease, hyperlipidemia, or hyperglycemia and were not taking any medication known to affect lutein or lipid metabolism during the month before 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 an inclusion criterion. The study was approved by the regional committee on human experimentation (no. 2008-A01354-51; Comité de Protection des Personnes Sud Méditerranée I, France). Procedures followed were in accordance with the Helsinki Declaration of 1975 as revised in 1983. 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. One subject left the study for personal reason before he participated in the first postprandial experiment, which left 39 subjects whose baseline characteristics are reported in Table 1.

DNA preparation and genotyping methods

An average of 25 μg DNA was isolated from a saliva sample from each subject by using the Oragene kit (DNA Genotek Inc) as described in detail previously (32). The DNA concentration and purity were checked by using spectrophotometry at 260 and 280 nm (Nanodrop ND1000; Thermo Scientific). All genotyping procedures were carried out by the Integragen company. As concerns the whole genome genotyping, the procedure was as follows: 200 ng DNA was hybridized overnight to HumanOmniExpress BeadChips (Illumina), which allowed the analysis of ~7.33 × 10^5 SNPs/DNA sample. Unhybridized and nonspecifically hybridized DNA was washed out. BeadChips were then stained and scanned on an Illumina iScan scanner (Illumina). Detailed methods are provided in the Infinium HD Assay Ultra Protocol Guide (Illumina). As concerns the 43 other SNPs (see Choice of candidates genes), they were genotyped as previously described (33).

Postprandial experiments

For the clinical research study, we used a randomized, 2-way crossover design with a minimum washout period of 3 wk between each meal. Subjects were asked to refrain from the consumption of lutein-rich foods (an exclusion list was provided by a dietician) for 48 h before each postprandial clinic visit. In addition, the day before the postprandial clinic visit, subjects were asked to eat dinner between 1900 and 2000 without any alcohol intake and, afterward, abstain from the consumption of any food or beverage other than water until their clinic visit. After the overnight fast, subjects arrived at the local Center for Clinical Investigation (la Conception Hospital, Marseille, France) and consumed either a meal that provided lutein as a supplement or a meal that provided lutein in a food matrix (ie, tomato puree). Both meals consisted of semolina (70 g) cooked in 200 mL hot water, white bread (40 g), egg whites (60 g), peanut oil (50 g), and mineral water (330 mL). The lutein supplement was provided in 3 pills, each of which contained 5 mg free lutein (Visiobane Protect). The source of free lutein in the pills was FloraGLO (Kemin Industry Inc). The pills also contained Porphyra, B vitamins, vitamins C and E, fish oil, bee wax, and gelatin. The tomato puree (100 g/meal), which was purchased from a local supermarket, provided 0.1 mg free lutein as measured by using HPLC. The tomato puree was chosen as a source of food lutein because it has been shown that a postprandial chylomicron lutein response can efficiently be measured after tomato-puree consumption (34), and we used this food in a parallel study dedicated to lycopene bioavailability (data presented in a future article on tomato lycopene bioavailability). Thirty-three of 39 subjects participated in the study on tomato lycopene bioavailability and, thus, consumed the tomato puree. Subjects were asked to consume the meals at a steady pace, with one-half of the meal consumed in 280 nm (Nanodrop ND1000; Thermo Scientific). All genotyping procedures were carried out by the Integragen company. As concerns the whole genome genotyping, the procedure was as follows: 200 ng DNA was hybridized overnight to HumanOmniExpress BeadChips (Illumina), which allowed the analysis of ~7.33 × 10^5 SNPs/DNA sample. Unhybridized and nonspecifically hybridized DNA was washed out. BeadChips were then stained and scanned on an Illumina iScan scanner (Illumina). Detailed methods are provided in the Infinium HD Assay Ultra Protocol Guide (Illumina). As concerns the 43 other SNPs (see Choice of candidates genes), they were genotyped as previously described (33).

Postprandial chylomicron lutein response (nmol/L/h)^4

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>32.3 ± 2.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.5 ± 1.3</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.0 ± 0.3</td>
</tr>
<tr>
<td>Glucose (mmol/L)^2</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides (g/L)^2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Total cholesterol (g/L)^2</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)^2</td>
<td>15.1 ± 0.1</td>
</tr>
<tr>
<td>Lutein (μmol/L)^2</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>Fasting plasma variables.</td>
<td></td>
</tr>
<tr>
<td>2 Because the fasting plasma lutein concentration can be affected by recent dietary intakes of lutein, blood concentrations were measured on 4 occasions for each subject with a ≥3-wk interval between each measurement.</td>
<td></td>
</tr>
<tr>
<td>4 Incremental AUC of chylomicron lutein concentration measured during the postprandial period (0–8 h) after the intake of the meal providing the lutein supplement.</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 1

Characteristics of subjects included in the statistical analysis of results^1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>73.5 ± 1.3</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>23.0 ± 0.3</td>
</tr>
<tr>
<td>Glucose (mmol/L)^2</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>Triglycerides (g/L)^2</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>Total cholesterol (g/L)^2</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)^2</td>
<td>15.1 ± 0.1</td>
</tr>
<tr>
<td>Lutein (μmol/L)^2</td>
<td>0.30 ± 0.01</td>
</tr>
<tr>
<td>Postprandial chylomicron lutein response (nmol/L/h)^4</td>
<td>69.0 ± 8.3</td>
</tr>
</tbody>
</table>

^1 n = 39.

^2 Because the fasting plasma lutein concentration can be affected by recent dietary intakes of lutein, blood concentrations were measured on 4 occasions for each subject with a ≥3-wk interval between each measurement.

^4 Incremental AUC of chylomicron lutein concentration measured during the postprandial period (0–8 h) after the intake of the meal providing the lutein supplement.
10 min and the remainder of the meal consumed within 30 min to diminish the variability because of different rates of intake and, thus, gastric emptying. No other food was permitted over the next 8 h. Subjects were only allowed to finish the remaining bottled water they had not drunk during the meal. A baseline blood sample was drawn before administration of meals (ie, in the fasted state) 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) ≈ 2 h after collection.

Chylomicron 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 by using a SW41Ti rotor (Beckman Coulter) in a Thermo Sorvall WX100 ultracentrifuge (Thermo Scientific). The upper phase, which contained mainly chylomicrons and large chylomicron remnants (35, 36), was collected. Immediately after recovery, chylomicrons were stored at −80°C before lutein analysis.

Chylomicron lutein extraction and analysis

Chylomicron lutein was extracted and analyzed as follows. Briefly, ≤2 mL chylomicrons was deproteinated by adding one volume of ethanol that contained apo 8'-carotenal as an internal standard. After the addition of 2 vol hexane, the mixture was vortexed for 10 min and centrifuged at 500 × g for 10 min at 4°C. The upper phase (which contained lutein) was collected, and the sample was extracted a second time with hexane following the same procedure. Hexane phases were pooled and evaporated completely under nitrogen gas. The dried extract was redissolved in 200 μL dichloromethane:methanol mixture (65:35; vol:vol). All extractions were performed at room temperature under yellow light to minimize light-induced damage. A volume of 90 μL was used for the HPLC analysis. Separation was achieved by using a 10 × 4.0-mm, 2-μm Modulo-Cart QS guard column (Interchim) followed by a 250 × 4.6-mm internal diameter, 5-μm particle size YMC C30 column (Interchim) held at 35°C. The mobile phase was composed of HPLC-grade methanol (A), methyl tert-butyl ether (B), and water (C) (Carlo Erba–SDS). A linear gradient from 96% A, 2% B, and 2% C at t = 0 to 18% A, 80% B, and 2% C at t = 27 min at a flow rate of 1 mL/min was used. The HPLC system consisted of a pump (Waters 2690) associated with a photodiode-array detector (Waters 2996) (Waters). Lutein was identified via spectra and a retention time coincident with an authentic standard (DSM LTD) and quantitated at 450 nm. Peaks were integrated by using Chromeleon software (version 6.80; Dionex), and quantitation was performed by comparing the sample peak area with lutein calibration curves and corrected by extraction efficiency on the basis of the recovery of the internal standard.

Calculations

A trapezoidal rule was used to calculate the postprandial plasma chylomicron lutein response (ie, the AUC of the postprandial plasma chylomicron lutein concentration over 8 h). Calculated AUCs were incremental (ie, baseline-corrected by using the fasting plasma chylomicron lutein concentration measured in each subject).

Choice of candidate genes

Candidate genes included those of which their encoded proteins have been shown by in vitro methods to be involved in lutein uptake by the enterocyte [ie, scavenger receptor class B, member 1 (SCARB1) and NPC1LI (22, 24)], genes that are suspected to be involved, directly or indirectly, in enterocyte lutein metabolism, [ie, liver-fatty acid binding protein (L-FABP), intestinal-fatty acid binding protein (I-FABP) (25), and microsomal triglyceride transfer protein (MTTP)], and genes that have been associated in genome-wide association studies (26) or candidate gene association studies (18, 28, 37) with blood lutein concentrations. This choice resulted in the selection of 28 genes (see Supplementary Table 1 under “Supplemental data” in the online issue), which represented 2091 SNPs on arrays. In addition, we added 30 SNPs in 23 genes that we have recently shown to be associated with the postprandial chylomicron triacylglycerol response in the same group of subjects (31). Indeed, chylomicrons are the main blood carrier of newly absorbed lutein, and we hypothesized that genetic variants that affect the secretion and clearance of chylomicrons in the postprandial period likely affect the postprandial blood response in lutein. We also added 43 SNPs that have been associated, in previous publications, with lipid metabolism (see Supplementary Table 2 under “Supplemental data” in the online issue). After the genotyping of subjects (see DNA preparation and genotyping methods), SNPs for which the genotype call rate was <95% or SNPs that presented a significant departure from the Hardy-Weinberg equilibrium (P < 0.05; chi-square test) were excluded from all subsequent analysis (336 SNPs excluded, which left 1785 SNPs for the partial least-squares (PLS) regression analysis).

Statistics: PLS regression

To identify SNPs associated with the variability in the postprandial chylomicron lutein response, we used PLS regression. PLS is a multivariate statistical tool often used for chemometric (38) and spectrometric modeling and has recently been applied to SNP-based predictions by our group (31) and other researchers (19, 31, 39, 40). Because of the large number of SNPs compared with the low number of subjects and the multicollinearity between SNPs, PLS regression was chosen to identify SNPs (of the 1785 candidate SNPs) that were predictive of the postprandial chylomicron lutein response according to their variable importance in the projection (VIP) value. A general genetic model was assumed (ie, the 3 genotypes of each SNP were treated as separate categories with no assumption made about the effect conferred by the variant allele for homozygotes or heterozygotes on the postprandial chylomicron lutein response). Different PLS regression models were built by using increasing VIP threshold values. The model that maximized the explained variance (R²) and predicted variance (Q²) and validated the following cross-validation ANOVA was selected. See Supplementary Methods under “Supplemental data” in the online issue for additional validation procedures of PLS regression models (41, 42) that were also performed. SIMCA-P12 software (Umetrics) was used for all multivariate data analyses and modeling.
which is known to be a marker of lutein status, was related to the plasma lutein concentrations. Correlations between lutein responses to meals and fasting showed that there was a significant positive relation (Pearson’s $r = 0.68, P < 0.0001$; Spearman’s rank correlation coefficient = 0.45, $P = 0.011$) between the 2 responses.

Correlation between lutein responses measured after consumption of the 2 meals
To assess whether the ability to respond to dietary lutein was an intrinsic characteristic of subjects, we calculated the correlation between lutein responses measured after the 2 meals. Results showed that there was a significant positive relation (Pearson’s $r = 0.51, P < 0.003$; Spearman’s rank correlation coefficient = 0.49, $P = 0.006$). The relation between the lutein response to the meal that contained tomato puree as a source of lutein and the fasting plasma lutein concentration was also positive (Pearson’s $r = 0.3$, Spearman’s $\rho = 0.3$) although nonsignificant ($P = 0.09$ and $P = 0.07$, respectively), which was likely because of the higher variability of the lutein response observed after the meal (see Interindividual variability in chylomicron lutein responses to meals).

Correlations between lutein responses to meals and fasting plasma lutein concentrations
To assess whether the fasting plasma concentration of lutein, which is known to be a marker of lutein status, was related to the ability to respond to dietary lutein, we calculated the correlation between the lutein responses of subjects and their fasting plasma lutein concentrations. To estimate the fasting plasma lutein concentration, we averaged 4 fasting plasma lutein concentrations measured ≥3 wk apart. Results showed that there was a significant positive relation between the lutein response to the meal that contained the lutein supplement and the fasting plasma lutein concentration (Pearson’s $r = 0.51, P < 0.003$; Spearman’s rank correlation coefficient = 0.49, $P = 0.006$). The relation between the lutein response to the meal that contained tomato puree as a source of lutein and the fasting plasma lutein concentration was also positive (Pearson’s $r = 0.3$, Spearman’s $\rho = 0.3$) although nonsignificant ($P = 0.09$ and $P = 0.07$, respectively), which was likely because of the higher variability of the lutein response observed after the meal (see Interindividual variability in chylomicron lutein responses to meals).

Genetic variants associated with the lutein response
As stated previously, we used PLS regression to examine whether the 1785 candidate SNPs (used as qualitative X variables) could explain a significant part of the variability in the lutein response of 39 subjects after consumption of the meal that contained the lutein supplement. We focused on the lutein supplement-containing meal because of the higher number of subjects and the higher, more accurate lutein response measured because of the higher dose of lutein in the meal. As shown in Table 2, the model that included all SNPs described the group variance with good accuracy ($R^2 = 0.92$) but was not predictive of the variance ($Q^2 = -0.10$). Therefore, to improve the model and find an association of SNPs more predictive of the lutein response, we filtered out SNPs that made no important contribution (ie, those that displayed the lowest VIP value). After the application of several thresholds of the VIP value (Table 2), we showed that the best model obtained included 39 SNPs (see Supplementary Table 3 under “Supplemental data” in the online issue for the 29 SNPs not in linkage disequilibrium plus 10 SNPs in linkage disequilibrium). The 29 SNPs were located in or near 15 genes (Table 3) and described 73% of the group variance with a prediction index $Q^2$ of 56% (Table 2). The robustness and stability of the model were validated by 3 additional methods (see Supplementary Methods under “Supplemental data” in the online issue).
TABLE 2  
Performances of different partial least-square regression models that explained the postprandial chylomicron lutein response to the meal that contained the lutein supplement.

<table>
<thead>
<tr>
<th>VIP threshold</th>
<th>$R^2$</th>
<th>$Q^2$</th>
<th>SNP no.</th>
<th>$P$ (CV-ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No selection</td>
<td>0.92</td>
<td>−0.10</td>
<td>1785</td>
<td>1</td>
</tr>
<tr>
<td>&gt;0.5</td>
<td>1.00</td>
<td>0.59</td>
<td>505</td>
<td>0.99</td>
</tr>
<tr>
<td>&gt;1.0</td>
<td>0.95</td>
<td>0.61</td>
<td>148</td>
<td>0.14</td>
</tr>
<tr>
<td>&gt;1.5</td>
<td>0.76</td>
<td>0.49</td>
<td>69</td>
<td>$2.7 \times 10^{-3}$</td>
</tr>
<tr>
<td>&gt;1.6</td>
<td>0.74</td>
<td>0.50</td>
<td>62</td>
<td>$2.2 \times 10^{-3}$</td>
</tr>
<tr>
<td>&gt;1.7</td>
<td>0.73</td>
<td>0.47</td>
<td>55</td>
<td>$2.2 \times 10^{-3}$</td>
</tr>
<tr>
<td>&gt;1.8</td>
<td>0.73</td>
<td>0.56</td>
<td>39</td>
<td>$1.9 \times 10^{-4}$</td>
</tr>
<tr>
<td>&gt;1.9</td>
<td>0.71</td>
<td>0.52</td>
<td>31</td>
<td>$1.8 \times 10^{-4}$</td>
</tr>
<tr>
<td>&gt;2.0</td>
<td>0.69</td>
<td>0.52</td>
<td>26</td>
<td>$1.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>&gt;2.1</td>
<td>0.61</td>
<td>0.47</td>
<td>18</td>
<td>$2.0 \times 10^{-4}$</td>
</tr>
<tr>
<td>&gt;2.2</td>
<td>0.62</td>
<td>0.48</td>
<td>17</td>
<td>$1.2 \times 10^{-4}$</td>
</tr>
<tr>
<td>&gt;2.3</td>
<td>0.58</td>
<td>0.46</td>
<td>13</td>
<td>$3.1 \times 10^{-4}$</td>
</tr>
<tr>
<td>&gt;2.4</td>
<td>0.58</td>
<td>0.46</td>
<td>13</td>
<td>$3.1 \times 10^{-4}$</td>
</tr>
<tr>
<td>&gt;2.5</td>
<td>0.54</td>
<td>0.42</td>
<td>11</td>
<td>$7.0 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

*Different partial least-square regression models were built by using increasing VIP-threshold values. The model that maximized the $R^2$ and $Q^2$ and was validated after using a cross-validation ANOVA plus 3 other validation methods (see Supplemental Material under “Supplemental data” in the online issue for descriptions) was selected. This is the model with VIP >1.8 shown in the table. CV-ANOVA, ANOVA after cross-validation; $Q^2$, predicted variance; $R^2$, explained variance; SNP, single nucleotide polymorphism; VIP, variable importance in the projection.*

With the use of univariate statistics, the association of the 29 SNPs with the lutein response was further evaluated by comparing for each SNP the lutein response of subjects who bore different genotypes (Table 3). For 11 of 29 SNPs, subjects who bore different genotypes exhibited a significantly different lutein response ($q < 0.05$).

Genetic score to predict the lutein response of a genotyped subject

With the knowledge of a subject’s genotype at the 29 afore-mentioned loci, it was possible to predict the subject’s ability to respond to lutein according to the following equation

$$\hat{RP} = a + \sum_{i=1}^{29} \gamma_i \times \text{genotype} \cdot (\text{SNPi})$$  \hspace{1cm} (I)

with RP as the responder phenotype (ie, the lutein response), $a$ as a constant, $\gamma_i$ as the regression coefficient of the $i$th SNP included in the PLS regression model, and genotype (SNPi) as a Boolean variable indicating the subject’s genotype at the $i$th SNP. See Supplementary Table 4 under “supplemental data” in the online issue for a list of regression coefficients calculated by the SIMCA-P12 software.

**DISCUSSION**

The first noteworthy observation was the high interindividual variability observed in the lutein response to the 2 lutein-containing meals (CVs of 75% and 137% after the meal with the lutein supplement and the tomato meal, respectively). This result is in agreement with the high interindividual variability reported for β-carotene (44–48), lycopene (48), and lutein (48). The higher interindividual variability observed after intake of the tomato meal compared with the meal that contained the lutein supplement was likely attributable to the lower amount of lutein provided by the tomato puree (0.1 compared with 15 mg), which likely led to a lesser accuracy in the chylomicron lutein measure. Nevertheless, several of the following additional factors could also have participated in this higher interindividual variability: 1) the lower number of subjects who consumed the tomato meal compared with the lutein-supplement meal (33 compared with 39, respectively); 2) the tomato matrix in which lutein was embedded and which contained fibers that may have impaired lutein absorption (49); 3) a competition with other carotenoids present in the tomato matrix (eg, lycopene and β-carotene) (34);
4) a higher variability in the gastric emptying rate that was attributed to the 100-g tomato puree; and 5) a higher variability in chylomicron clearance rates.

The second noteworthy observation was that lutein responses measured after the 2 meals in the same subjects were significantly correlated. This result suggested that, as previously observed for β-carotene (44, 45, 48), the ability to absorb dietary lutein was an intrinsic, likely genetic characteristic of subjects. This possibility, together with the fact that several proteins have been associated with lutein absorption and transport within the body (25), led us to hypothesize that polymorphisms in genes involved in lutein metabolism might be involved in the lutein response (18, 50). Because the lutein response is a complex phenotype that likely involves several genes, a thorough study of the association between this phenotype and candidate genetic variants should simultaneously include SNPs in all genes that are assumed to be involved in this phenotype. The results of the multivariate analysis, the validity of which was checked by using several tests (see Subjects and Methods; also see Supplementary Methods under “Supplemental data” in the online issue), showed that a significant part (75%) of the interindividual variability in the lutein response could be assigned to 29 SNPs in or near 15 of 51 candidate genes. Seven out of these 29 SNPs (Table 3) have recently been shown to be involved in the postprandial chylomicron triacylglycerol response in the same group of subjects (31). This observation was not surprising because it was assumed that most newly absorbed lutein is carried from the intestine to peripheral organs via chylomicron, which are mainly constituted of triacylglycerol.

Because this study aimed to identify genes that are specifically associated with the lutein response, we will only discuss the potential role of the 4 genes [ie, intestine specific homebox (ISX), ELOVL fatty acid elongase 2 (ELOVL2), ATP-binding cassette, subfamily G, member 2 (ABCG2), and MTTP] that had not been associated with the chylomicron triacylglycerol response (31) and displayed a significant P value after univariate statistics (Table 3). First, it was striking to observe that the SNPs in these 4 genes were the most important in the PLS model (ie, they had the highest VIP value and, thus, were the main contributors in lutein variability). ISX has been shown to act as a transcriptional repressor of SR-BI expression in the intestine (51). Because SR-BI is involved in lutein uptake (22), we hypothesize that SNPs in ISX may affect its expression and activity. In turn, this outcome would affect the SR-BI expression and, thus, lutein uptake efficiency. ABCG2 encodes for a breast cancer resistance protein (BCRP), which is a multidrug transporter (52). As far as we know, there is currently no study on the involvement of this protein in lutein transport. However, we selected it as a candidate gene because flavonoids have been shown to be inhibitors of ABCG2/BCRP (53), and the flavonoid naringenin impairs lutein uptake in Caco-2 cells (54). Thus, the association of SNPs in ABCG2/BCRP with the lutein response suggested that this protein participates in lutein absorption. Additional studies are required to verify this hypothesis. MTTP encodes for the microsomal triglyceride transfer protein, which is involved in the packaging of triacylglycerol within the chylomicron. Therefore, the association between an SNP in MTTP and the lutein response is not surprising to us because lutein is incorporated into chylomicron in the enterocyte. ELOVL2 catalyzes the elongation of EPA to docosapentaenoic acid, and, subsequently, DHA. This association was also observed in a previous study in which the rs1150561 SNP near ELOVL2 was associated with a blood lutein response to lutein supplementation (16). The mechanism of this relation is uncertain and requires additional study.

The third noteworthy observation was that subjects’ fasting plasma lutein concentrations and their lutein responses were positively correlated. This relation has been previously reported by Norkus et al (55) who observed that higher initial (baseline) serum lutein concentrations predicted greater serum lutein responses after lutein supplementation. This effect suggests that the ability to respond to dietary lutein is a key factor that governs blood and likely tissue lutein concentrations.

To conclude, results of this study show that the interindividual variability in lutein bioavailability is at least partially genetically controlled. They also show that the ability to absorb dietary lutein is an important determinant of circulating fasting blood concentrations of lutein. Finally, our results suggest that a significant portion of the interindividual variability in lutein bioavailability can be explained by a combination of SNPs, most of which are located in or near genes that have been associated with the chylomicron triacylglycerol response. We believe that this study will be the starting point of a series of studies aiming to genetically predict lutein bioavailability in individuals or, more probably, a group of individuals carrying key SNPs involved in lutein bioavailability. The objective of these kinds of studies will be to give nutritionists an accurate and validated genetic tool to predict the lutein response for future supplementation studies. Ultimately, this approach could be used to help optimize lutein intake for individuals who may be at risk of developing diseases that may benefit from lutein consumption (eg, macular degeneration and cognitive decline).

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