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A Combination of Single-Nucleotide Polymorphisms Is Associated with Interindividual Variability in Dietary β-Carotene Bioavailability in Healthy Men[1–3]

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Abstract

Background: The bioavailability of β-carotene, the main dietary provitamin A carotenoid, is very variable among individuals. It is not known whether this variability can affect long-term β-carotene, and hence vitamin A, status.

Objectives: We hypothesized that variations in genes involved in β-carotene absorption and postprandial metabolism could at least partially explain the high interindividual variability in β-carotene bioavailability. Thus, the main objectives of this study were to identify associated single-nucleotide polymorphisms (SNPs), and to estimate whether populations with different allele frequencies at these SNPs could have a different ability to absorb provitamin A carotenoids.

Methods: In this single-group design, 33 healthy, nonobese adult men were genotyped with the use of whole-genome microarrays. After an overnight fast, they consumed a test meal containing 100 g tomato puree providing 0.4 mg β-carotene. The postprandial plasma chylomicron β-carotene concentration was then measured at regular time intervals over 8 h. Partial least squares (PLS) regression was used to identify the best combination of SNPs in or near candidate genes (54 genes representing 2172 SNPs) that was associated with the postprandial chylomicron β-carotene response (incremental β-carotene area-under-the-curve concentration over 8 h in chylomicrons).

Results: The postprandial chylomicron β-carotene response was highly variable (CV = 105%) and was positively correlated with the fasting plasma β-carotene concentration ($r = 0.78$; $P < 0.0001$). A significant ($P = 6.54 \times 10^{-3}$) multivalidated PLS regression model, which included 25 SNPs in 12 genes, explained 69% of the variance in the postprandial chylomicron β-carotene response, i.e., β-carotene bioavailability.

Conclusions: Interindividual variability in β-carotene bioavailability appears to be partially modulated by a combination of SNPs in 12 genes. This variability likely affects the long-term blood β-carotene status. A theoretic calculation of β-carotene bioavailability in 4 populations of the international HapMap project suggests that populations with different allele frequencies in these SNPs might exhibit a different ability to absorb dietary β-carotene. This trial was registered at clinicaltrials.gov as NCT02100774. *J Nutr* doi: 10.3945/jn.115.212837.

Keywords: SNP, genetic polymorphisms, genetic variations, chylomicrons, absorption, vitamin A, nutrigenetics, postprandial metabolism

Introduction

Vitamin A deficiency is still a serious public health problem in developing countries, causing blindness and death in hundreds of thousands of children and adults (1). Several strategies have been developed to fight against this deficiency, e.g., vitamin A supplementation, increasing consumption of local dietary sources of vitamin A, and engineering of vitamin A synthesis pathways in plants (2), as for example with golden rice, which has been genetically modified to synthesize bioavailable β-carotene (3, 4). There is still an ongoing debate on how to fight vitamin A deficiency (5–7).

Vitamin A is present in the human diet either as preformed vitamin A (i.e., mostly retinyl palmitate found in foods from animal origin) or as provitamin A carotenoids (i.e., β-carotene, α-carotene, and β-cryptoxanthin), found predominantly in fruits

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[3] Supplemental Tables 1–5 and Supplemental Methods are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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and vegetables. Provitamin A carotenoids are usually the main natural source of vitamin A in developing countries. Nevertheless, absorption efficiency of this source of vitamin A is low and affected by numerous factors (8, 9), and the effectiveness of provitamin A rich foods in improving vitamin A status has been questioned (10–12). Furthermore, there is great interindividual variability with regard to provitamin A carotenoid bioavailability (13) and its conversion into vitamin A (14, 15).

It is assumed that during digestion, provitamin A carotenoids are extracted from the food matrix in which they are embedded and then incorporated into mixed micelles. After their uptake by the enterocyte, provitamin A carotenoids have 2 possible fates. They can be incorporated as such in chylomicrons and then secreted into the bloodstream, or they can be cleaved by β-carotene 15,15′oxygenase-1 (BCO1)[8] (16–18), or to a lesser extent by β-carotene 9,10′oxygenase-2 (BCO2) (19), to form retinal or β-apo-10′-carotenol (18). Retinal can be reduced to retinol and then esterified, mostly as retinyl palmitate, and incorporated into chylomicrons and secreted in the bloodstream. Vitamin A delivery from provitamin A carotenoids depends on 2 principal factors: absorption efficiency and efficiency of conversion to one of the main biologically active vitamin A metabolites (20), i.e., retinal, retinol, and retinoic acid (21).

Our group and others have demonstrated that there is great variability in the absorption of provitamin A carotenoids (22, 23) and their conversion into vitamin A (24, 25). This interindividual variability at least partially has been attributed to variations in genes involved in provitamin A carotenoid absorption and metabolism. In support of this hypothesis, several single-nucleotide polymorphisms (SNPs) have been associated with fasting blood β-carotene concentration (26, 27), as well as with the efficiency of β-carotene conversion into vitamin A (14, 15). Thus far, no study has attempted to assess which SNPs are involved in the first step of provitamin A carotenoid metabolism within the body, i.e., their intestinal absorption. This might have important consequences with regard to the fight against vitamin A deficiency. Indeed, we hypothesize that populations with different allele frequencies at loci involved in provitamin A carotenoid bioavailability might exhibit differential abilities to absorb provitamin A carotenoids. Thus, the main objectives of this study were to *1*) better characterize interindividual variability in β-carotene bioavailability in healthy subjects, *2*) assess whether this variability might affect long-term blood β-carotene status, *3*) identify the best combination of SNPs associated with the variability in β-carotene bioavailability, and *4*) estimate whether populations that have different allele frequencies in these SNPs might exhibit a different ability to absorb provitamin A carotenoids.

Methods

Subject number and characteristics. Thirty-five healthy, nonobese, nonsmoking men were recruited for the study. This number was close to the number of subjects that allowed us to identify combinations of SNPs that were associated with both lutein (28) and vitamin E (29) bioavailability. Subjects reported normal energy consumption (i.e., ~2500 kcal/d) with <2% alcohol as total energy intake. Subjects had no history of chronic disease, hyperlipidemia, or hyperglycemia and were not taking any medication known to affect β-carotene or lipid metabolism the month before the study or during the study period. Because of the relatively large volume of blood collected during the study, a blood hemoglobin concentration >1.3 g/L was an inclusion criteria. The study was approved by the regional committee on human experimentation (No. 2008-A01354–51, Research Ethics Committee Sud Méditerranée I, France). Procedures followed were in accordance with the Declaration of Helsinki of 1975 as revised in 1983. Objectives and requirements of the study were fully explained to all participants before they began the study, and written informed consent was obtained from each subject. Two subjects left the study for personal reasons before they participated in the postprandial experiment, which left 33 subjects whose baseline characteristics are reported in **Table 1**.

DNA preparation and genotyping methods. A mean of 25 μg of DNA was isolated from a saliva sample from each subject with the use of the Oragene kit (DNA Genotek), as described in detail by Hansen et al. (30). DNA concentration and purity were determined by spectrophotometry (Nanodrop ND1000, Thermo Scientific) at 260 nm and 280 nm, respectively. All genotyping procedures were outsourced to Integragen. The whole genome was genotyped as follows: 200 ng of DNA was hybridized overnight to HumanOmniExpress BeadChips (Illumina), which allowed for the analysis of $\sim 7.33 \times 10^5$ SNPs/DNA sample. Unhybridized and nonspecifically hybridized DNA was then washed away. Afterward, the BeadChips were stained and scanned on an Illumina iScan scanner. Detailed methods are provided in the Infinium HD Assay Ultra Protocol Guide (Illumina). Subjects were further genotyped for 40 additional SNPs (**Supplemental Table 1**), as described below (see "Choice of candidate genes") (31).

Postprandial experiments. In order to assess β-carotene bioavailability, we measured the postprandial chylomicron β-carotene response to a β-carotene–containing meal. This approach is widely used for experimental assessment of β-carotene bioavailability (13–15, 32). Subjects were asked to refrain from the consumption of vitamin supplements and β-carotene rich foods 48 h before the postprandial experiment (an exclusion list was provided by a dietitian). In addition, subjects were asked to eat dinner between 1900 and 2000 the day before the postprandial experiment, and to then abstain from any food or beverage consumption with the exception of water. After the overnight fast, subjects arrived at the local center for clinical investigation (Hôpital de la Conception, Marseille, France) and consumed the test meal, which included 100 g tomato puree (containing 0.4 mg β-carotene/100 g wet weight as determined by HPLC). The tomato puree was purchased from a local supermarket, and was chosen for 2 reasons. First, we wanted β-carotene to be delivered via a food matrix rather than in a supplement so that our results could be extrapolated to β-carotene in other foods. Second, we were also interested in studying lycopene bioavailability (33),

TABLE 1 Characteristics of the healthy men included in the statistical analysis of the results[1]

Variable	Value
Age, y	32.6 ± 2.3
Weight, kg	72.6 ± 1.4
BMI, kg/m^2	22.8 ± 0.4
Glucose, mmol/L	4.7 ± 0.1[2]
TG, g/L	0.7 ± 0.1[2]
Total cholesterol, g/L	1.7 ± 0.1[2]
Hemoglobin, g/dL	15.0 ± 0.2[2]
β-Carotene, μmol/L	0.32 ± 0.04[2]

[1] Values are means ± SEMs, n = 33.
[2] Fasting plasma concentrations.

[8] Abbreviations used: *ABCA1*, ATP-binding cassette, sub-family A (ABC1), member 1; *ABCG5*, ATP-binding cassette, sub-family G (WHITE), member 5; BCO1, β-carotene 15,15′oxygenase 1; BCO2, β-carotene 9,10′oxygenase 2; *CD36*, CD36 molecule (thrombospondin receptor); *CXCL8*, chemokine (C-X-C motif) ligand 8; *ELOVL2*, ELOVL fatty acid elongase 2; *ISX*, intestine-specific homeobox; HapMap, an international project to develop a haplotype map of the human genome; *LIPC*, lipase, hepatic; *PKD1L2*, polycystic kidney disease 1-like 2; PLS, partial least squares; *RPE65*, retinal pigment epithelium-specific protein 65kDa; *SCARB1*, Scavenger receptor class B, member 1; SR-BI, Scavenger receptor class B, member 1; *TCF7L2*; Transcription factor 7-like 2 (T-cell specific, HMG-box); SNP, single-nucleotide polymorphism; VIP, variable importance in the projection.

and tomato puree is a rich dietary source thereof (the tomato puree used provided 9.7 mg all-trans lycopene as determined by HPLC). The meal also contained 70 g semolina cooked in 200 mL of hot water, 40 g white bread, 60 g cooked egg whites, 50 g peanut oil, and 330 mL mineral water. Subjects were asked to consume the meal at a steady pace, with one-half of the meal consumed in 15 min and the remainder of the meal consumed within 30 min (to diminish variability from different rates of intake and, thus, gastric emptying). No other food was permitted over the following 8 h. However, subjects were allowed to consume any remaining bottled water from the meal. A baseline blood sample was drawn before administration of the meal (i.e., in the fasted state) as well as 2, 3, 4, 5, 6, and 8 h after meal consumption. Blood was collected via evacuated purple-top glass tubes containing potassium-EDTA. Tubes were immediately placed on ice 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. Chylomicrons were prepared from plasma samples as previously described (28, 29, 33, 34). Immediately after recovery, chylomicrons were stored at −80°C before β-carotene analysis.

Plasma and chylomicron β-carotene extraction and analysis. Chylomicron β-carotene was extracted and analyzed as previously described (28, 33). β-Carotene was identified via spectra and retention time coincident with authentic standard and quantitated at 450 nm. Peaks were integrated with the use of Chromeleon software (version 6.80, Dionex), and quantitation was performed by comparing sample peak area with external β-carotene calibration curves, and corrected for extraction efficiency based on the recovery of the internal standard.

Other analytic determinations. Serum TGs (35), total cholesterol (36), and glucose (37) were determined by enzymatic procedures with commercial kits (Boehringer). Hemoglobin concentrations were measured with a calibrated laboratory machine (ADVIA 2120 hematology system, Siemens Healthcare) immediately after blood sample collection.

Calculations. The trapezoidal approximation method (38) was used to calculate the AUC of the postprandial plasma chylomicron β-carotene concentration over 8 h, henceforth referred to as "β-carotene response."

Choice of candidate genes. Candidate genes included those for which the encoded protein has previously been shown to be involved in β-carotene uptake by the enterocyte in vitro (39–42), genes that have been suggested to be involved (directly or indirectly) in enterocyte metabolism of fat-soluble micronutrients (43, 44), and genes that have been associated with circulating β-carotene or retinol concentration in genome-wide (27, 45) or candidate gene (46, 47) association studies. Consequently, 31 genes were selected (**Supplemental Table 2**), representing 2570 SNPs. In addition, we added 42 SNPs in 16 genes that we have previously shown were associated with the postprandial chylomicron TG response in the same subjects (34). Indeed, chylomicrons are the main carriers of both newly absorbed TGs and β-carotene in the blood. Thus, we hypothesized that genetic variants that affect the secretion and clearance of chylomicrons also likely affect the postprandial blood response of β-carotene. Finally, we added 11 SNPs that previously have been associated with lipid metabolism and that were not genotyped with the BeadChips (Supplemental Table 1). After genotyping of the 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 [451 SNPs excluded, leaving 2172 SNPs for the partial least squares (PLS) regression analysis].

Multivariate analysis with PLS regression. To identify SNPs associated with the variability observed in the β-carotene response, which is likely a phenotype modulated by the additive/synergistic effects of several genetic variants, we used PLS regression, which is particularly well suited when the number of independent variables exceeds that of observations and when multicollinearity occurs (48, 49). The independent variables were the 2172 candidate SNPs and the predicted variable was the β-carotene response of the 33 subjects. A general genetic model was assumed. The 3 genotypes of each SNP (i.e., homozygous for the most frequent allele, heterozygous, and homozygous for the least frequent allele) were treated as separate categories, with no assumption made about the effect conferred by the variant allele on β-carotene response. Different PLS regression models were built with the use of increasing variable importance in the projection (VIP) threshold values. We first tested the validity of the model with the use of the permutation technique (see **Supplemental Methods** for more details). We considered a PLS regression model as valid when the mean of the R^2 values of 100 permuted models was less than half of the R^2 value of the original model, indicating that ≥50% of the variability was not due to chance. Of all the validated models generated, the one presenting the highest Spearman's ρ between the measured and the predicted β-carotene response was selected. Additional validation criteria and procedures of the PLS regression models (50, 51) are described in Supplemental Methods. SIMCA-P12 software (Umetrics) was used for all multivariate data analyses and modeling.

Univariate analyses. In a second approach, we performed univariate analyses to compare the β-carotene response between subgroups of subjects who bore different genotypes for the SNPs present in the selected PLS model. Differences obtained between the various genotype subgroups were analyzed with the use of a Student's *t* test using the Benjamini–Hochberg correction with QVALUE software (version 1.0, designed by researcher) (52) and R software (version 3.0.2). For all tests, an adjusted P value < 0.05 was considered significant.

Other statistics. Because we did not know whether the possible relation between the fasting plasma β-carotene concentrations and the postprandial β-carotene responses to the test meal was linear or monotonic, both Pearson product-moment and Spearman rank were used to measure the correlation between the 2 variables. Fasting plasma β-carotene concentrations measured the day of the postprandial experiment and at 2 other times (either 3 wk before the postprandial experiment or 3 wk after) were compared by repeated-measures ANOVA. P values < 0.05 were considered significant.

Results

Interindividual variability in the β-carotene response to the tomato puree meal. The β-carotene response after consumption of the test meal is shown in **Figure 1**. The mean postprandial secretion of β-carotene was biphasic for most subjects, with the maximum concentration appearing at 3 h, and

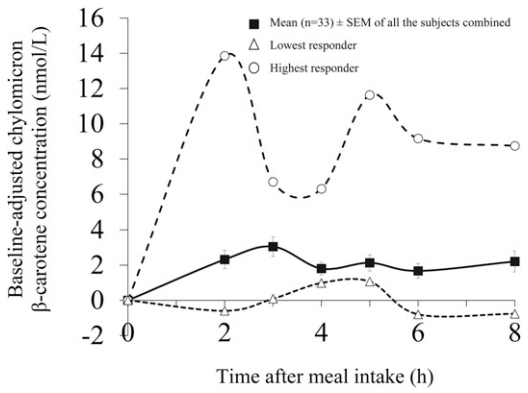

FIGURE 1 Baseline-adjusted chylomicron β-carotene concentrations over 8 h after consumption of the meal that provided β-carotene in tomato puree in the group of healthy men and in the lowest and highest responders. For each subject, postprandial chylomicron β-carotene concentrations were baseline-adjusted by using fasting chylomicron β-carotene concentration.

a second smaller maximum appearing at 5 h. However, it should be noted that some subjects had a monophasic response, as demonstrated by the lowest responder (denoted in Figure 1 with the dotted line). Note that the 2 subjects who had the lowest β-carotene responses had no increase in chylomicron β-carotene concentration, as illustrated by the plotting of the AUC of the β-carotene response after the test meal (**Figure 2**). The CV of the β-carotene response was 105%.

Correlation between the β-carotene response to the tomato puree meal and the fasting plasma β-carotene concentration. To determine whether the fasting plasma β-carotene concentration, as a marker of β-carotene status, was related to the ability to respond to dietary β-carotene, we calculated the correlation between the β-carotene response of the subjects after the test meal and their fasting plasma β-carotene concentrations. Results showed a positive correlation between the β-carotene response and the fasting plasma β-carotene concentration (Pearson's r = 0.78, P < 0.0001; Spearman's rank correlation coefficient = 0.65, P = 0.0003). A significant positive correlation was also observed between fasting plasma β-carotene concentration and the β-carotene response normalized to chylomicron TG response (Pearson's r = 0.64, P < 0.0001; Spearman's rank correlation coefficient = 0.68, P = 0.0002). Finally, it should be noted that the fasting plasma β-carotene concentrations of each subject measured the day of the postprandial experiment and at 2 other times (either 3 wk before the postprandial experiment or 3 wk after) were not significantly different (P = 0.82, repeated-measures ANOVA).

Genetic variants associated with the β-carotene response to the tomato puree meal. The PLS regression model that included all 2172 candidate SNPs (used as qualitative X variables) described the β-carotene response with good accuracy (explained variance R^2 = 0.90), but was not predictive of this response (predicted variance after crossvalidation Q^2 = −0.1) as shown in **Table 2**. Therefore, to improve the model and find an association of SNPs more predictive of the β-carotene response, we filtered out those that displayed the lowest VIP value (i.e., those that made no important contribution to the PLS regression model). After the application of several increasing VIP value thresholds (Table 2), we selected a model (see the Table 2 legend) that included 30 SNPs, of which 25 were not in linkage disequilibrium (**Supplemental Table 3**). The 25 SNPs were located in or near 12 genes (**Table 3**) and described 69% of the variance (R^2), with a predicted variance Q^2 = 49%. The Spearman's ρ value of this model was 0.67 (P < 0.001). The robustness and the stability of the model were validated by 4 additional methods including the leave-k-out procedure (Supplemental Methods and **Supplemental Table 4**).

TABLE 2 Performances of different PLS regression models to explain the variability in the postprandial chylomicron β-carotene responses of healthy men[1]

VIP threshold	R^2	Q^2	SNP no.	P^2	Mean R^2 of 100 permuted models	Spearman's ρ
No selection	0.90	−0.10	2172	1	0.90	0.84
>0.5	0.91	0.47	602	0.78	0.88	0.84
>1.0	0.86	0.73	165	2.98×10^{-2}	0.68	0.87
>1.5	0.76	0.59	45	2.65×10^{-3}	0.43	0.75
>1.55	0.76	0.58	42	2.10×10^{-3}	0.42	0.77
>1.6	0.73	0.57	39	3.36×10^{-3}	0.40	0.71
>1.65	0.74	0.57	38	2.35×10^{-3}	0.41	0.74
>1.7	0.69	0.50	32	7.54×10^{-3}	0.35	0.68
>1.75	0.69	0.49	30	6.54×10^{-3}	0.34	0.67
>1.8	0.66	0.46	28	7.87×10^{-3}	0.33	0.65
>1.85	0.65	0.46	25	9.78×10^{-3}	0.29	0.63
>1.9	0.64	0.46	24	8.70×10^{-3}	0.29	0.63
>1.95	0.61	0.41	22	1.57×10^{-2}	0.27	0.61
>2.0	0.58	0.38	17	1.93×10^{-2}	0.23	0.60

[1] Different PLS models were built with the use of increasing VIP threshold values. The model with VIP >1.75 shown in the table above was chosen based on the selection criteria previously detailed in the Methods section under the subheading Multivariate analysis with PLS regression. Validation procedures for the selected PLS regression model are described in Supplemental Methods. PLS, partial least squares; Q^2, predicted variance; R^2, explained variance; SNP, single-nucleotide polymorphism; VIP, variable importance in the projection.
[2] Crossvalidation ANOVA.

The association between the 25 selected SNPs and β-carotene response was further evaluated with the use of univariate statistics by comparing the β-carotene response of subjects who bore different genotypes for each SNP (Table 3).

Genetic score to calculate the β-carotene response of a genotyped subject or a genotyped population. With the knowledge of a subject's genotype at the 25 aforementioned loci, it was possible to calculate his ability to respond to β-carotene according to the following equation:

$$\mathrm{RP} = a + \sum_{1}^{25} r_i \; X \; \mathrm{genotype.(SNPi)} \qquad (1)$$

with RP as the responder phenotype (i.e., the β-carotene response), a as a constant (15.18), r_i as the regression coefficient of the i^{th} SNP included in the PLS regression model, and "genotype.(SNP_i)" as a Boolean variable indicating the subject's genotype at the i^{th} SNP. A list of regression coefficients calculated by the SIMCA-P12 software can be found in **Supplemental Table 5**. The Spearman's ρ between the measured and the calculated β-carotene response was 0.67 (P < 0.001).

We then applied this genetic score to calculate theoretic β-carotene bioavailability in the 4 populations of the first phase of the international HapMap project (53) with the use of the allele frequencies retrieved from the Single Nucleotide Polymorphism Database (54). Interestingly, the theoretic β-carotene bioavailability in the population of Utah residents with Northern

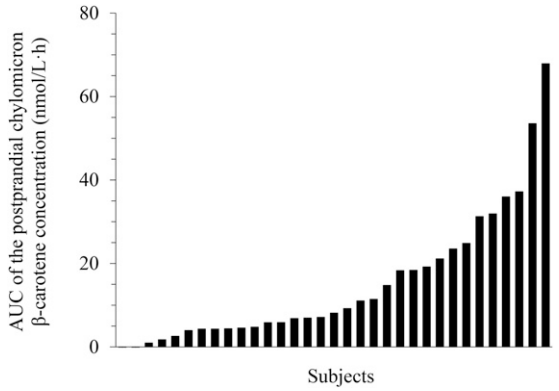

FIGURE 2 Individual AUCs of the postprandial chylomicron β-carotene response after consumption by 33 healthy men of the meal that provided β-carotene in tomato puree. Subjects were sorted by increasing postprandial chylomicron β-carotene response (i.e., 0–8 h AUC).

TABLE 3 Genes and SNPs associated with the postprandial chylomicron β-carotene responses of healthy men[1]

Gene and SNP rs no.	VIP value	SNP minor allele frequency	Adjusted *P*
ABCA1 rs2791952	2.98	0.148[2]	0.004[3]
ELOVL2 rs9468304	2.88	0.309	0.065
ELOVL2 rs3798709	2.72	0.250	0.065
CXCL8 rs1247620	2.53	0.129	0.007
ABCA1 rs10991408	2.43	0.127	0.008
ELOVL2 rs911196	2.43	0.251	0.083
ISX rs5755368	2.42	0.234	0.083
RPE65 rs4926340	2.41	0.083	0.022
TCF7L2 rs946199	2.32	0.173	0.083
RPE65 rs12139131	2.31	0.102	0.083
APOB rs1042031	2.26	0.153	0.083
ISX rs16994824	2.20	0.196	0.083
LIPC rs1869138	2.03	0.117	0.065
APOB rs4643493	2.02	0.103	0.103
BCO1 rs7196470	1.98	0.244	0.083
ABCG5 rs2278357	1.98	0.214	0.103
ABCA1 rs3887137	1.97	0.140	0.083
ISX rs202313	1.96	0.142	0.103
CXCL8 rs6834586	1.93	0.177	0.083
LIPC rs11857380	1.91	0.154	0.119
APOB rs35364714	1.87	0.127	0.127
CXCL8 rs1358594	1.83	0.254	0.083
LIPC rs12185072	1.81	0.192	0.116
PKD1L2 rs8043708	1.77	0.227	0.138
SOD2 rs2501175	1.77	0.298	0.103

[1] SNPs present in the selected PLS regression model shown in Table 2. SNPs are ranked by decreasing VIP value. Five out of the 30 SNPs present in the selected model were in linkage disequilibrium. Because these SNPs provided redundant information to the model, we randomly kept one of each SNP (those presented in this table) in the final selected PLS regression model. See Supplemental Table 3 for SNPs in linkage disequilibrium. Refer to Supplemental Table 1 for definitions of gene symbols in Table 3. PLS, partial least squares; SNP, single-nucleotide polymorphism; VIP, variable importance in the projection.

[2] Minor allele frequency in percentage of the frequency of the 3 alleles.

[3] Student's *t* test with the Benjamini–Hochberg correction was carried out to test differences between the postprandial chylomicron β-carotene response according to genotype groups for each SNP.

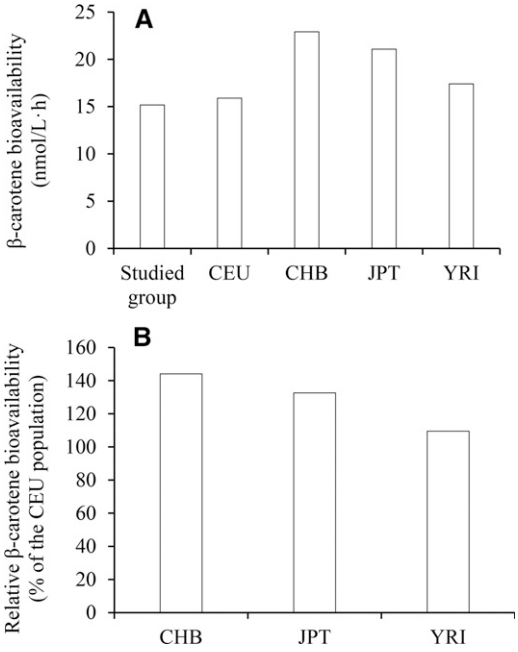

FIGURE 3 Theoretic β-carotene bioavailability in different populations. β-Carotene bioavailability (expressed as the chylomicron β-carotene response to a tomato puree meal providing 0.4 mg β-carotene) of the studied group (n = 33 subjects) and theoretic β-carotene bioavailability of the same dose of β-carotene in the 4 HapMap Phase I populations (53) (A). Theoretic relative β-carotene bioavailability of the 3 non-CEU HapMap populations compared with the CEU HapMap population (B). The theoretic β-carotene bioavailability of each HapMap population was calculated by using the regression coefficients of the genetic score (Supplemental Table 5) and the genotype frequencies at each SNP with the use of the Single Nucleotide Polymorphism Database (54). CEU, Utah residents with Northern and Western European ancestry; CHB, Han Chinese in Beijing, China; HapMap, haplotype map; JPT, Japanese in Tokyo, Japan; YRI, Yoruba in Ibadan, Nigeria.

and Western European ancestry was very close to that measured in our group of Western European subjects (15.90 mmol/L · h vs. 15.18 ± 2.59 mmol/L · h, respectively) (**Figure 3**A). It was also lower than the theoretic β-carotene bioavailability calculated in the Chinese from Beijing and Japanese populations (31% and 25%, respectively) (Figure 3B).

Discussion

The first noteworthy observation of this study was the relatively large interindividual variability in β-carotene response after the test meal, as illustrated by a CV of 105%. This variability was strikingly higher than what we have reported previously, i.e., 61% CV in β-carotene response in 79 subjects after the consumption of 120 mg β-carotene with a test meal (13). We suggest that this lower variability was due to the pharmacologic dose of β-carotene used, which likely saturated the transport system and consequently led to a fraction of β-carotene being absorbed by passive diffusion (55), leading to a lessening of the effect of the genetic variants.

For reasons explained in the Methods section, we used PLS regression to identify the best combination of SNPs that was associated with the variability in the β-carotene response. The best PLS model that was obtained, which was validated by several tests to check its robustness and stability (see Multivariate analysis section and Supplemental Methods), showed that a significant 69% of the interindividual variability in β-carotene response could be explained by 25 SNPs in or near 12 genes. Four of these 12 genes [ATP-binding cassette, sub-family A (ABC1) (*ABCA1*); *APOB;* Transcription factor 7-like 2 (T-cell specific, HMG-box) (*TCF7L2*); and lipase, hepatic (*LIPC*)] have been shown to be involved in the postprandial chylomicron TG response in the same group of subjects (34). Because most newly absorbed β-carotene is carried from the intestine to peripheral organs and the liver via chylomicrons (56, 57), we postulate that SNPs in these 4 genes likely have an indirect effect on the β-carotene response by modulating chylomicron metabolism (34). The association of SNPs in *BCO1* with the β-carotene response was also anticipated, because BCO1 is the primary cleavage enzyme of β-carotene in the liver and in the intestine (20, 58–60), and SNPs in this gene modulate the β-carotene response (14, 15, 61). The lack of association of SNPs in *BCO2* with the β-carotene response can be explained by the minor role that this carotenoid oxygenase has toward provitamin A cleavage (18). Among the 8 remaining genes, intestine-specific homeobox (*ISX*), retinal pigment epithelium-specific protein 65kDa (*RPE65*), polycystic kidney disease 1-like 2 (*PKD1L2*), and ELOVL fatty acid elongase 2 (*ELOVL2*) have been associated with the postprandial chylomicron response of the

carotenoid lutein (28). We anticipated an association with *ISX*, because the gene product is a transcriptional repressor of Scavenger receptor class B, member 1 (*SCARB1*) expression in the intestine (44), and Scavenger receptor class B, member 1 (SR-BI), the product of *SCARB1* expression, is involved in β-carotene uptake by intestinal cells (39, 41, 42). The association with ATP-binding cassette, sub-family G (WHITE), member 5 (*ABCG5*) suggests that this apical membrane protein, which is involved in the efflux of phytosterols by the enterocyte (62), is also involved in the efflux of a fraction of newly absorbed β-carotene. The other gene associations are more difficult to explain with current knowledge of β-carotene metabolism. Thus, we will only venture some speculative hypotheses on the association with *RPE65* and chemokine (C-X-C motif) ligand 8 (*CXCL8*), the 2 remaining genes for which subgroups of subjects with different genotypes exhibited significantly different β-carotene responses after univariate analyses (Table 3). RPE65 functions primarily in the retinal pigment epithelium and humans with null mutations in RPE65 are totally blind. Nevertheless, it is not known whether this protein is expressed in the gastrointestinal tract and whether it is involved, directly or indirectly, in carotenoid metabolism. Both β-carotene and vitamin A metabolites of β-carotene attenuate the inflammatory response in various models, as recently summarized (63). *CXCL8* encodes the proinflammatory IL-8. The association with *CXCL8* could be related to a secondary effect of the metabolite vitamin A on inflammation (63), for reasons yet to be elucidated. Likewise, an in vitro study has demonstrated that β-carotene reduces hydrogen peroxide–induced IL-8 mRNA expression in gastric cells, but evidence of the reverse relation (i.e., the impact of IL-8 on β-carotene uptake, transport, and/or metabolism) has yet to be established (64).

Surprisingly, no SNPs in gene coding for intestinal apical β-carotene transporters, namely *SCARB1* (39, 41, 42) and CD36 molecule (thrombospondin receptor) (*CD36*) (39, 41), were associated with the β-carotene response. We have 2 hypotheses to explain this. The first is that genotyped SNPs in these genes are indeed not associated with this phenotype, or their association is weaker than that of SNPs retained in the selected PLS regression model. The second is that some SNPs in these genes were not entered in the PLS regression analysis because either they were not expressed on the BeadChips or they were excluded from the analysis (for not respecting the Hardy–Weinberg equilibrium or because their genetic call rate was <95%). We acknowledge this limitation but it does not change the important finding of this study that a significant part of the variability in β-carotene bioavailability is associated with a combination of genetic variants, as revealed by PLS regression analysis. Furthermore, because there is evidence that the main provitamin A carotenoids share similar intestinal transport mechanisms, i.e., same transporters involved in cellular uptake (39), same main cleavage enzymes (16, 17), and same secretion in chylomicrons, we believe that the results presented herein can be extrapolated to other provitamin A carotenoids, i.e., α-carotene and β-cryptoxanthin.

The second noteworthy observation of this study was that a subject's fasting plasma β-carotene concentration was positively correlated with his postprandial β-carotene response ($r = 0.78$, $P < 0.001$). In addition, the fasting β-carotene concentration before the test meal was not significantly different from the fasting β-carotene concentration measured on 2 other occasions at 3 wk intervals. The fasting plasma β-carotene concentration is the result of interactions between several individual factors, e.g., dietary β-carotene intake, β-carotene absorption efficiency, and β-carotene catabolism rate, but the individual ability to respond to dietary β-carotene appears to be a key one. This likely affects tissue concentrations as well.

The fact that β-carotene bioavailability is at least partly genetically controlled led us to wonder whether different populations would exhibit a different theoretic ability to respond to dietary β-carotene, based on their allele frequencies at the SNPs associated with the variability in β-carotene bioavailability. To test this hypothesis, we calculated the theoretic β-carotene bioavailability in different populations genotyped in the international HapMap Phase I project (http://hapmap.ncbi.nlm.nih.gov/) (Figure 3). These calculations support the assumption that our group of 33 French subjects was representative of the population of Utah residents with Northern and Western European ancestry, as far as β-carotene bioavailability was concerned, and suggest that there could be variability in β-carotene bioavailability in different populations worldwide. Obviously, more work needs to be done to confirm whether our calculated theoretic postprandial β-carotene response would hold true experimentally in these various populations. Nevertheless, this finding raises a question as to whether certain populations that display a low β-carotene bioavailability should have modified provitamin A dietary recommendations to ensure vitamin A sufficiency.

It is likely that some of the absorbed β-carotene was cleaved by BCO1 in the intestine. This hypothesis is supported by the association of an SNP in *BCO1* with the variability in the β-carotene response and by numerous studies that have shown intestinal β-carotene conversion after consumption. Unfortunately, the amount of retinyl palmitate in the chylomicron fraction was clearly below our limit of quantitation. In fact, retinyl palmitate was close to the limit of quantitation in the chylomicron fraction after the intake of a much higher dose of β-carotene, i.e., 120 mg in the form of a β-carotene 30% oil suspension (13). Furthermore, the fact that β-carotene was embedded in a food matrix, and was likely in competition with tomato puree lycopene for its absorption, may have further decreased its bioavailability (8, 65, 66) and subsequent conversion. Nevertheless, it is unlikely that competition between lycopene and β-carotene can explain the interindividual variability in β-carotene bioavailability because all subjects received the same test meal. Thus, the fact that we did not detect a quantifiable amount of retinyl palmitate in the chylomicron fraction was likely due to the relatively low dose of β-carotene in the test meal, which was 300 times lower than that used in our previous study (13). Thus, it is possible that part of the β-carotene absorbed in our study was immediately converted to retinyl palmitate in the small intestine and was not measured.

In conclusion, there is high interindividual variability in dietary β-carotene bioavailability, which can be partly explained by a combination of SNPs. Furthermore, differences in β-carotene bioavailability apparently have direct implications for long-term β-carotene status. These findings, together with the fact that different populations exhibit significantly different allele frequencies at several of the SNPs that we identified, allow us to suggest that these populations might exhibit different β-carotene absorption efficiencies. Future studies are warranted to determine whether population-tailored β-carotene dietary recommendations are important to ensure sufficient β-carotene intake for vitamin A sufficiency.

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analyzed the tomato puree and chylomicron β-carotene by HPLC; PB and CD analyzed the data; CD performed the statistical analyses; and PB and CD wrote the paper and had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

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