A Combination of Single-Nucleotide Polymorphisms Is Associated with Interindvidual Variability in Cholecalciferol Bioavailability in Healthy Men1–4

Charles Desmarchelier,5,10 Patrick Borel,5,10* Aurélie Goncalves,9 Rachel Kopec,6,7 Marion Nowicki,5 Sophie Morange,8 Nathalie Lesavre,9 Henri Portugal,5 and Emmanuelle Reboul5
5NORT Nutrition Obesity and Thrombotic Risk, Aix-Marseille University, INRA National Institute for Agricultural Research, INSERM National Institute of Health and Medical Research, Marseille, France; 6National Institute for Agricultural Research, Avignon, France; 7Security and Quality of Plant Products, University of Avignon, Avignon, France; 8Clinical Investigation Centre, Conception Hospital, Marseille, France; and 9Clinical Investigation Centre, North Hospital, Marseille, France

Abstract

Background: Most people require dietary vitamin D to achieve the recommended concentration of 25-hydroxyvitamin D [25(OH)D] in the blood. However, the response to vitamin D supplementation is highly variable among individuals.

Objective: We assessed whether the variability in cholecalciferol bioavailability was associated with single-nucleotide polymorphisms (SNPs) in candidate genes.

Methods: In a single-group design, 39 healthy adult men with a mean ± SD age of 33 ± 2 y and mean ± SD body mass index (in kg/m²) of 22.9 ± 0.3 were genotyped with the use of whole-genome microarrays. After an overnight fast, plasma 25(OH)D status was measured, and the subjects then consumed a meal that provided 5 mg cholecalciferol as a supplement. Plasma chylomicron cholecalciferol concentration was measured over 8 h, and cholecalciferol response was assessed by calculating the postprandial area under the curve. Partial least squares regression was used to test the association of SNPs in or near candidate genes (61 genes representing 3791 SNPs) with the postprandial cholecalciferol response.

Results: The postprandial chylomicron cholecalciferol concentration peaked at 5.4 h. The cholecalciferol response was extremely variable among individuals (CV: 47%). It correlated with the chylomicron triglyceride (TG) response (r = 0.60; P < 0.001) but not with the fasting plasma 25(OH)D concentration (r = 0.04; P = 0.83). A significant (P = 1.32 × 10−4) partial least squares regression model that included 17 SNPs in 13 genes (including 5 that have been associated with chylomicron TG response) was associated with the variance in the cholecalciferol response.

Conclusion: In healthy men, there is a high interindividual variability in cholecalciferol bioavailability that is associated with a combination of SNPs located in or near genes involved in both vitamin D and lipid metabolism. This trial was registered at clinicaltrials.gov as NCT02100774.

Keywords: vitamin D, chylomicrons, dietary lipids, intestine, kinetics, nutrigenetics, 25-hydroxycholecalciferol, absorption, postprandial, genetic polymorphisms

Introduction

Vitamin D is the generic name for compounds that exhibit the biological activity of cholecalciferol (vitamin D₃). Vitamin D is essential for bone health and for regulating blood calcium and phosphate concentrations, but it is also involved in other biological functions such as immunity, cell proliferation, and apoptosis. Cholecalciferol needs 2 hydroxylations to become hormonally active. In the first hydroxylation, 25-hydroxyvitamin D [25(OH)D]11 is produced in the liver upon 25-hydroxylase action (cytochrome
DHCR7 (known as vitamin D–binding protein) (18); vitamin D (1,25-GC)

common variants in cytochrome P450, family 2, subfamily R, member 1 (2, 16, 17, 19, 21, 22). Moreover, as stated previously, at least a portion of vitamin D absorption requires protein transport (6, 7, 9, 23). Several groups have previously shown that genetic variants are associated with the variability in the postprandial chylomicron TG response (24–26), and chylomicrons are responsible for the transport of newly absorbed cholecalciferol into the peripheral circulation. Moreover, we have also shown that the variation in the postprandial response to other lipid-soluble micronutrients (i.e., tocopherol, lutein, lycopene, and β-carotene) (27–30) that share common absorption processes with cholecalciferol was modulated by single-nucleotide polymorphisms (SNPs) in genes involved in both the intestinal transport of these micronutrients and chylomicron metabolism. This set of arguments allows us to suggest that cholecalciferol bioavailability could be at least partly genetically modulated.

The main objectives of this study were to describe the postprandial chylomicron cholecalciferol response to a cholecalciferol-rich meal and its interindividual variability and to assess whether the interindividual variability in cholecalciferol bioavailability is associated with genetic variants located in or near candidate genes involved in vitamin D or lipid metabolism.

Methods

Subject number and characteristics. Forty healthy, nonobese, nonsmoking (31) men were recruited for the study (NCT02100774). 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 medications known to affect vitamin D 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 >13 g/dL was an inclusion criteria. The study was approved by a regional committee on human experimentation. The 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 beginning the study, and written informed consent was obtained from each subject. One subject left the study for personal reasons before he participated in the postprandial experiment, which left 39 subjects whose baseline characteristics are reported in Table 1.

DNA preparation and genotyping methods. DNA preparation and genotyping methods were performed as previously described (28). The whole genome was genotyped with the use of HumanOmniExpress BeadChip (Illumina), which allowed for the analysis of ~7.33 × 10⁷ SNPs per DNA sample. Subjects were also genotyped for 11 additional SNPs as described in the “Choice of Candidate Genes” section (32).

Postprandial experiments. To assess cholecalciferol bioavailability, we measured the postprandial chylomicron cholecalciferol response to a cholecalciferol-rich meal. This approach is commonly used to experimentally assess fat-soluble vitamin and carotenoid bioavailability (33–36). Because it has been shown that season is an important prediction factor for vitamin D response variation (37), the postprandial experiments were performed over a short period of time, more precisely between September and November. This postprandial experiment was part of a larger clinical research study in which we assessed the bioavailability of other lipid micronutrients and nutrients, i.e., lutein (30), tocopherol (27), lycopene (29), β-carotene (28), and cholesterol (unpublished

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Baseline characteristics of healthy men1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parameter</td>
<td>Value</td>
</tr>
<tr>
<td>Age, y</td>
<td>33 ± 2</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>73.5 ± 1.3</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.9 ± 0.3</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>TGs, g/L</td>
<td>0.78 ± 0.06</td>
</tr>
<tr>
<td>Total cholesterol, g/dL</td>
<td>1.65 ± 0.06</td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>15.1 ± 0.2</td>
</tr>
<tr>
<td>25-hydroxyvitamin D, nmol/L</td>
<td>51.4 ± 2.8</td>
</tr>
</tbody>
</table>

1 Values are means ± SEs, n = 39.
2 Fasting plasma value.
3 Fasting total blood value.
4 Concentrations ranged from 11.2 to 86.7 nmol/L; 3 subjects had concentrations >75 nmol/L.
data), in the same group of subjects. To avoid carryover and/or competition between the studied compounds, a washout period of ≥2 wk was respected in between 2 test meals. Subjects were asked to refrain from the consumption of vitamin supplements and vitamin D–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 abstain from any food or beverage consumption afterward with the exception of water. After the overnight fast, subjects arrived at the local clinic to have their baseline blood sample taken. Subjects then consumed the test meal together with 5 mg supplemental cholecalciferol (2 vials of 100-kIU UVEDOSE; Crinex). The meal also contained 70 g semolina cooked in 200 mL 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 half of the meal consumed in 10 min and the remainder consumed within 30 min (to diminish the variability because of different rates of intake and, thus, gastric emptying). No other food was permitted during the following 8 h. However, subjects were allowed to drink any remaining water from the meal. Additional blood samples were drawn 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 1620 × g) <2 h after collection.

**Chylomicron preparation.** Chylomicrons were prepared from plasma samples as previously described (28). Immediately after recovery, chylomicrons were stored at −80°C until analysis.

**Ability of Caco-2 cells to convert cholecalciferol to 25(OH)D.** Caco-2 clone TC7 cells were cultured as previously described (38). For each experiment, cells were seeded and grown on 6-well plates for 14 d to obtain confluent and highly differentiated cell monolayers. Twelve hours before each experiment, the complete medium was replaced with DMEM supplemented with 1% antibiotics and 1% nonessential amino acids. Cells were then incubated with DMEM supplemented with 1% antibiotics, 1% nonessential amino acids, 1% BSA, and 50 μM cholecalciferol up to 24 h. After incubation, the cell medium was harvested, and the cells were scraped in ice-cold PBS. The samples were then stored at −80°C until analysis.

**Vitamin D extraction and analysis by HPLC.** Up to 2 mL of the samples were deproteinized by adding 1 volume of ethanol that also contained ergocalciferol (vitamin D2) as an internal standard and vortexed for 30 s. After adding 2 volumes of hexane, the mixture was vortexed for 10 min and centrifuged at 5000 × g for 10 min at 4°C. The upper phase (containing the different vitamin D forms) was collected, and the sample was extracted a second time with hexane following the same procedure. The hexane phases were pooled and completely evaporated under nitrogen gas. The dried residue from the chylomicron samples was dissolved in 200 μL acetonitrile:methanol-water (60:38:2) that contained 20 mM sodium perchlorate and 10 mM perchloric acid, whereas the dried residue from cell samples was dissolved in 200 μL acetonitrile. A volume of 200 μL was used for the HPLC analysis.

Cholecalciferol and ergocalciferol from chylomicron samples were separated with the use of a 250 × 4.6-mm reversed-phase C18 5-μm Zorbax column (Intercim) and a guard column and analyzed as previously described (9).

Cholecalciferol, 25(OH)D, and ergocalciferol from cell samples were separated with the use of a 100 × 3-mm ACE 3 C18-AR column (ATI). The mobile phase consisted of a gradient of acetonitrile and water (pH 10). The flow rate was 0.22 mL/min, and the column temperature was 20°C. The gradient profile of the mobile phase (acetonitrile:water) was set at 60:40 from 0 to 1.5 min. It then changed linearly to 100:0 for 6 min. This ratio was maintained for 12 min before the mobile phase was changed back to 60:40 from 18 to 18.5 min. This last ratio was finally maintained for 11.5 min.

**Plasma and chylomicron 25(OH)D analysis by chemiluminescence.** 25(OH)D from fasting plasma and chylomicron samples was assessed with the use of chemiluminescent immunoassay technology (LIASON analyzer; DiaSorin) according to the manufacturer’s instructions.

**Choice of candidate genes.** Candidate genes included those for which the encoded protein has previously been shown to be involved in cholecalciferol uptake by the enterocyte in vitro (9), genes that have been suggested to be involved (directly or indirectly) in the metabolism of fat-soluble micronutrients in the enterocyte (23), and genes that have been associated with circulating blood 25(OH)D concentration in genome-wide (15, 39–41) or candidate gene (18, 20, 21, 42–45) association studies. Consequently, 61 genes were selected (Supplemental Table 1), representing 4608 SNPs. In addition, we added 27 SNPs from 14 genes associated with the postprandial chylomicron TG response in the same subjects (24). Indeed, chylomicrons are the carrier of both newly absorbed TGs and cholecalciferol (10) in the blood. Thus, we hypothesized that the genetic variants that affect the secretion and clearance of chylomicrons also likely affect cholecalciferol response. Finally, we added 11 SNPs that have been previously associated with lipid metabolism and that were not genotyped with BeadChip (Supplemental Table 2). After genotyping the subjects, SNPs for which the genotype call rate was <5% or SNPs that presented a significant departure from the Hardy-Weinberg equilibrium (P < 0.05; chi-squared test) were excluded from all subsequent analyses. A total of 855 SNPs were excluded, leaving 3791 SNPs for the partial least squares (PLS) regression analysis (Supplemental Figure 1).

**Calculation and statistical analysis.** The trapezoidal approximation method was used to calculate the AUC of the postprandial plasma chylomicron cholecalciferol concentration over 8 h (henceforth referred to as the cholecalciferol response). All data were expressed as means ± SEMs. Normality was assessed with the use of the Kolmogorov-Smirnov and the Shapiro-Wilk tests. Statistical dependence between 2 variables was assessed by Pearson’s correlation coefficient. Differences in the postprandial chylomicron 25(OH)D concentrations over time were tested by repeated-measures ANOVA. For all tests, the bilateral α risk was 0.05. Statistical analyses were performed with the use of StatView version 5.0 and SPSS version 20 (SAS Institute).

**PLS regression.** To identify SNPs associated with the variability observed in the cholecalciferol response, we used PLS regression following previously published rationale and model assumptions (24, 30). Among the 3791 candidate SNPs, PLS identified those that were predictive of the cholecalciferol response according to their variable importance in the projection (VIP) value. A general genetic model was assumed. The 3 genotypes of each SNP (i.e., most frequent homozygote allele, heterozygote, less frequent homozygote allele) were treated as separate categories, with no assumption made about the effect conferred by the variant allele on cholecalciferol response. Different PLS regression models were built with the use of increasing VIP threshold values. The selection of the PLS regression model was carried out as previously shown (29). Additional validation criteria and procedures of the PLS regression models are described in Supplemental Methods (Supplemental Figures 2 and 3). SIMCA-P13 software version 13.0.3 (Umetrics) was used for all multivariate data analyses and modeling.

With the knowledge of a subject’s genotype at the SNPs associated with the variability observed in the cholecalciferol response, it was possible to calculate the subject’s ability to respond to cholecalciferol according to the following equation:

\[
RP = \alpha + \sum_i b_i \times \text{genotype(SNP)}_i
\]

where \(RP\) is the responder phenotype (i.e., the cholecalciferol response), \(\alpha\) as a constant (equal to the mean cholecalciferol response), \(b\) the number of SNPs in the selected PLS regression model, \(r\), 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.

In a second approach, we performed univariate analyses to compare the cholecalciferol 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 with the Benjamini-Hochberg correction with QVALUE software version 1.0 (46) and R software version 3.0.2. For all tests, an adjusted \(P\) value <0.05 was considered significant.
Results

Vitamin D status of the subjects. As shown in Table 1, most subjects displayed a fasting plasma 25(OH)D concentration lower than the recommended concentration of 75 nmol/L (47), with 16 of the 39 subjects exhibiting a concentration <50 nmol/L. The fasting plasma 25(OH)D concentration did not correlate with any of the reported characteristics of the subjects (all r values <0.11 and all P values >0.1; data not shown).

Chylomicron vitamin D responses to the cholecalciferol-rich meal. The mean cholecalciferol response after the consumption of the test meal is shown in Figure 1. In total, 37 of the 39 subjects had cholecalciferol concentrations below our limit of detection in their fasting chylomicron fraction, and the remaining 2 subjects had extremely low cholecalciferol concentrations (0.27 ± 0.01 nmol/L). The maximal chylomicron cholecalciferol concentration was obtained 5.4 ± 0.3 h after meal intake. Note that the subject with the lowest response exhibited an increase in his chylomicron cholecalciferol concentration only 8 h after meal intake. The individual responses ranked by increasing AUC are shown in Figure 2. They followed a normal distribution (P > 0.1 after the Kolmogorov-Smirnov and Shapiro-Wilk tests, respectively; skewness = 0.16; kurtosis = 0.394). The CV of the cholecalciferol response was 47%, and the ratio between the highest and lowest responder was 34. The cholecalciferol response correlated relatively well with the chylomicron TG response (r = 0.60; P < 0.001) and with fasting TG (r = 0.478; P = 0.002). Note that the cholecalciferol response was not related to the vitamin D status of the subjects [as estimated by their fasting plasma 25(OH)D concentrations] (r = 0.04; P > 0.1).

The postprandial chylomicron 25(OH)D concentrations are shown in Figure 3. These concentrations did not vary significantly over the postprandial period (P > 0.1).

Genetic variants associated with the cholecalciferol response to the cholecalciferol-rich meal. The PLS regression model that included all 3791 candidate SNPs (used as qualitative variables) in 61 candidate genes (Supplemental Table 1) described the cholecalciferol response with good accuracy (R2 = 0.34), only TG concentration, age, and BMI significantly contributed to the PLS regression model (with VIP values of 7.4, 4.0, and 2.1 and regression coefficients of 1.32, −0.2, and −0.07, respectively).

The association of the 17 selected SNPs with the cholecalciferol response was further evaluated with the use of univariate statistics by comparing the cholecalciferol response of subjects who bore different genotypes for each SNP (Table 2). For 11 of these 17 SNPs, subjects who bore different genotypes exhibited a significantly different cholecalciferol response (adjusted P value <0.05).

Genetic score to calculate the cholecalciferol response of a genotyped subject. With the knowledge of a subject’s genotype at the 17 aforementioned loci, it was possible to calculate the subject’s ability to respond to cholecalciferol according to Equation 1 with a = 114.18 and b = 17. A list of regression coefficients calculated by SIMCA-P13 can be found in Supplemental Table 4.

Conversion of cholecalciferol to 25(OH)D by Caco-2 cells. After a 24 h-incubation with 50 μM cholecalciferol, only ~2.3% of vitamin D recovered in the apical medium was found as 25(OH)D (Figure 4A), and only ~0.2% of the vitamin D taken up by the cells was recovered as 25(OH)D (Figure 4B) (secondary outcomes).

Discussion

To our knowledge, this study is only the second report of the chylomicron cholecalciferol response to a cholecalciferol-rich meal after cross-validation). Therefore, to improve the model and to find an association of SNPs more predictive of the cholecalciferol 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, we selected a model with a VIP threshold value of 2.1 that included 18 SNPs, of which 17 were not in linkage disequilibrium. The 17 SNPs were located in or near 13 genes (Table 2) and described 63.5% of the variance (R2), with a prediction index Q2 of 47.6%. The measured and predicted cholecalciferol responses were positively correlated (r = 0.80; P < 0.001). The robustness and stability of the model were validated by 3 additional methods (Supplemental Methods). It is interesting to note that when the quantitative X variables BMI (in kg/m2), age, fasting plasma TGs, cholesterol, glucose, and hemoglobin concentrations were included, only TG concentration, age, and BMI significantly contributed to the PLS regression model (with VIP values of 7.4, 4.0, and 2.1 and regression coefficients of 1.32, −0.02, and −0.07, respectively).

The association of the 17 selected SNPs with the cholecalciferol response was further evaluated with the use of univariate statistics by comparing the cholecalciferol response of subjects who bore different genotypes for each SNP (Table 2). For 11 of these 17 SNPs, subjects who bore different genotypes exhibited a significantly different cholecalciferol response (adjusted P value <0.05).

Genetic score to calculate the cholecalciferol response of a genotyped subject. With the knowledge of a subject’s genotype at the 17 aforementioned loci, it was possible to calculate the subject’s ability to respond to cholecalciferol according to Equation 1 with a = 114.18 and b = 17. A list of regression coefficients calculated by SIMCA-P13 can be found in Supplemental Table 4.

Conversion of cholecalciferol to 25(OH)D by Caco-2 cells. After a 24 h-incubation with 50 μM cholecalciferol, only ~2.3% of vitamin D recovered in the apical medium was found as 25(OH)D (Figure 4A), and only ~0.2% of the vitamin D taken up by the cells was recovered as 25(OH)D (Figure 4B) (secondary outcomes).
in humans (48). The increased subject number and longer duration of time points taken postprandially provide a more complete picture of cholecalciferol bioavailability in humans than previous work.

The subjects enrolled in our study were apparently healthy. However, most presented subdeficient vitamin D concentrations, as classified by a fasting plasma 25(OH)D concentration <75 nmol/L (47). This observation was not surprising because the prevalence of vitamin D insufficiency is high in France, as it is elsewhere (49, 50).

The first major observation of this study was that the interindividual variability in cholecalciferol bioavailability was quite large, with a 34-fold difference in the AUC observed between the highest and lowest cholecalciferol responders. The lack of a significant correlation between the chylomicron cholecalciferol response and fasting plasma 25(OH)D concentration in our subjects could suggest that the variability in cholecalciferol bioavailability does not significantly affect long-term vitamin D status in these individuals. This is perhaps because of the fact that the fasting plasma 25(OH)D concentration is the result of interactions between several factors, including vitamin D synthesis by the skin, dietary vitamin D intake, vitamin D absorption efficiency, hepatic vitamin D metabolism to 25(OH)D, and rates of renal conversion to 1,25-dihydroxyvitamin D.

To identify genetic variants that are associated with the interindividual variability in the chylomicron cholecalciferol response, we employed a statistical approach that previously allowed us to identify combinations of candidate SNPs involved in the variability of the postprandial chylomicron response to other fat-soluble vitamins (i.e., vitamins E and A) (27, 28). This approach revealed that 63.5% of the interindividual variability in the postprandial cholecalciferol response was explained by 17 SNPs in or near 13 genes. Five of these genes (ATP-binding cassette, subfamily A, member 1; apolipoprotein B; blocked early in transport 1 homolog; lipoprotein lipase; and N-acetyltransferase 2) have been associated with the postprandial chylomicron TG response in the same group of subjects (24). Because it has been assumed that most newly absorbed cholecalciferol is carried from the intestine to peripheral organs and the liver via chylomicrons (48), it is plausible that SNPs in these genes have an indirect effect on the cholecalciferol response by modulating chylomicron metabolism. Indeed, 3 of these SNPs (blocked early in transport 1 homolog reference SNP rs10464587, lipoprotein lipase rs10096561, and N-acetyltransferase 2 rs4921920)

### TABLE 2 Genes and SNPs associated with the postprandial chylomicron cholecalciferol response in healthy men

<table>
<thead>
<tr>
<th>Genes</th>
<th>SNP rs number</th>
<th>Biological role of the encoded protein</th>
<th>VIP value</th>
<th>SNP minor allele frequency</th>
<th>Adjusted P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL</td>
<td>rs5686874</td>
<td>Chylomicron clearance</td>
<td>3.85</td>
<td>0.173</td>
<td>0.013</td>
</tr>
<tr>
<td>LPL</td>
<td>rs10096561</td>
<td>Chylomicron clearance</td>
<td>3.71</td>
<td>0.128</td>
<td>0.013</td>
</tr>
<tr>
<td>ISX</td>
<td>rs5754862</td>
<td>Transcriptional regulation</td>
<td>2.95</td>
<td>0.253</td>
<td>0.025</td>
</tr>
<tr>
<td>SLC10A2</td>
<td>rs9558203</td>
<td>Apical transport</td>
<td>2.78</td>
<td>0.198</td>
<td>0.016</td>
</tr>
<tr>
<td>GC</td>
<td>rs6945026</td>
<td>Plasma transport</td>
<td>2.67</td>
<td>0.133</td>
<td>0.032</td>
</tr>
<tr>
<td>SCARB1</td>
<td>rs12598083</td>
<td>Apical transport</td>
<td>2.64</td>
<td>0.150</td>
<td>0.032</td>
</tr>
<tr>
<td>PNLP</td>
<td>rs2915775</td>
<td>Dietary fat hydrolysis</td>
<td>2.75</td>
<td>0.257</td>
<td>0.046</td>
</tr>
<tr>
<td>DHC7</td>
<td>rs11604724</td>
<td>Conversion of 7-dehydrocholesterol to cholesterol</td>
<td>2.44</td>
<td>0.197</td>
<td>0.036</td>
</tr>
<tr>
<td>ABCA1</td>
<td>rs7043894</td>
<td>Basolateral efflux transport</td>
<td>2.41</td>
<td>0.240</td>
<td>0.040</td>
</tr>
<tr>
<td>ABCB1</td>
<td>rs2235023</td>
<td>Apical efflux transport</td>
<td>2.31</td>
<td>0.106</td>
<td>0.040</td>
</tr>
<tr>
<td>ABCB1</td>
<td>rs10260862</td>
<td>Apical efflux transport</td>
<td>2.29</td>
<td>0.215</td>
<td>0.062</td>
</tr>
<tr>
<td>PNLP</td>
<td>rs3010494</td>
<td>Dietary fat hydrolysis</td>
<td>2.26</td>
<td>0.293</td>
<td>0.062</td>
</tr>
<tr>
<td>APOB</td>
<td>rs2854725</td>
<td>Chylomicron formation</td>
<td>2.26</td>
<td>0.087</td>
<td>0.042</td>
</tr>
<tr>
<td>MAPRE2</td>
<td>rs1125425</td>
<td>Unknown</td>
<td>2.23</td>
<td>0.279</td>
<td>0.055</td>
</tr>
<tr>
<td>BET1</td>
<td>rs10464587</td>
<td>Transit of chylomicrons through the enterocyte</td>
<td>2.21</td>
<td>0.297</td>
<td>0.062</td>
</tr>
<tr>
<td>NAT2</td>
<td>rs4921920</td>
<td>Unknown</td>
<td>2.19</td>
<td>0.101</td>
<td>0.062</td>
</tr>
<tr>
<td>SLC10A2</td>
<td>rs9555166</td>
<td>Apical transport</td>
<td>2.17</td>
<td>0.197</td>
<td>0.055</td>
</tr>
</tbody>
</table>

1. ABCA1, ATP-binding cassette, subfamily A, member 1; ABCB1, ATP-binding cassette, subfamily B, member 1; BET1, blocked early in transport 1 homolog; DHC7, 7-dehydrocholesterol reductase; GC, group-specific component; ISX, intestine-specific homeobox; LPL, lipoprotein lipase; MAPRE2, microtubule-associated protein RP/EB family member 2; NAT2, N-acetyltransferase 2; PNLP, pancreatic lipase; rs, reference SNP; SCARB1, scavenger receptor class B, member 1; SLC10A2, solute carrier 353, family 10 (sodium/bile acid cotransporter), member 2; SNP, single-nucleotide polymorphism; VIP, variable importance in the projection.

2. See Supplemental Table 1 for a complete list of gene names and symbols.

3. SNPs present in the selected partial least squares regression model. SNPs are ranked by decreasing variable importance by the VIP values. Note that ABCB1 rs2235015 and ABCB1 rs10260862 were in linkage disequilibrium. We kept one, chosen at random (ABCB1 rs10260862), in the final selected partial least squares regression model and removed the other because it provided redundant information.

4. The given biological role of the protein is that in relation to vitamin D bioavailability.
have been previously associated with the variability in the postprandial chylomicron TG response in the same group of subjects (24). This is further illustrated by the significant correlation between the cholecalciferol and chylomicron TG responses.

However, this correlation was only partial, highlighting the fact that a significant considerable part of the variance of the cholecalciferol response can be attributed to factors other than the variance in the chylomicron TG response, such as processes specific to cholecalciferol metabolism. Indeed, several SNPs associated with the interindividual variability in the cholecalciferol response were located in genes involved more directly in vitamin D metabolism and transport. Intestine-specific homeobox is an intestinal transcription factor that modulates scavenger receptor class B, member 1 (SCARB1) expression (51). SCARB1 encodes scavenger receptor class B type 1, an apical membrane protein that is involved in cholecalciferol uptake by enterocytes (9). In addition, an SNP in SCARB1 was significantly associated with the cholecalciferol response in our PLS regression model. Solute carrier family 10 (sodium/bile acid cotransporter), member 2 encodes for the apical sodium-dependent bile acid transporter (ASBT), the main transporter involved in bile acid uptake in the distal ileum (52) and a candidate transporter for cholecalciferol uptake. Indeed, data from our laboratory suggest that ASBT is involved in the cellular uptake of cholecalciferol by Caco-2 TC7 cells and by ASBT-transfected cells (data not shown). GC encodes for vitamin D-binding protein, which is responsible for the plasma transport of 25(OH)D, and several SNPs in this gene have been associated with plasma 25(OH)D concentration (20, 53). The association of an SNP in GC with the variability in cholecalciferol bioavailability could suggest the existence of a feedback loop that regulates the circulating 25(OH)D concentration. ATP-binding cassette, subfamily B, member 1 encodes for P-glycoprotein (also known as multidrug resistance protein 1), which is involved in the efflux of xenobiotic compounds with a broad substrate specificity. Unpublished data from our lab that have shown that ABCB1-transfected cells effluxed more cholecalciferol than control cells further support this apical membrane protein could also be involved in cholecalciferol efflux. The association of 2 SNPs in pancreatic lipase with the cholecalciferol response suggests that the hydrolysis efficiency of dietary TGs, in which cholecalciferol is assumed to be solubilized, is an important factor regarding cholecalciferol absorption efficiency. This could be because of either the fact that pancreatic lipase allows the release of cholecalciferol from TGs and its incorporation in micelles or that the lipolysis products of TGs by pancreatic lipase, i.e., FAs, facilitate cholecalciferol absorption (38). In support of this hypothesis, it has been shown that patients with cystic fibrosis with pancreatic insufficiency display lower cholecalciferol absorption (54).

To conclude, the results from this study have allowed us to describe the interindividual variability in cholecalciferol bioavailability. They also suggest for the first time to our knowledge that this variability is at least partly explained by a combination of SNPs in genes involved in cholecalciferol and chylomicron metabolism and that ASBT and ABCB1 are involved in cholecalciferol bioavailability, likely in its intestinal uptake. We acknowledge some limitations of the study. It is likely that SNPs that have a significant effect on cholecalciferol bioavailability...
were not entered in the PLS regression analysis because they were not present on the BeadChip, they were excluded from the analysis (for not following the Hardy-Weinberg equilibrium), or simply because they were not located in the candidate genes. Moreover, these findings need to be tested in other population groups (e.g., based on sex, age, or genetic backgrounds). Thus, future studies are required to confirm these associations and to identify other genes and/or SNPs involved in cholecalciferol bioavailability.

Acknowledgments
PB designed the research project; PB and ER designed the protocol; MN, SM, and NL conducted the clinical study; AG and ER analyzed chylomicron cholecalciferol; HP analyzed plasma and chylomicron 25(OH)D; ER conducted the cellular study from culture to the interpretation of the results; CD and PB analyzed the results; CD performed statistical analyses; CD and PB wrote the paper with consultation from RK and ER; and CD and PB had primary responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

References


