CD36 and SR-BI Are Involved in Cellular Uptake of Provitamin A Carotenoids by Caco-2 and HEK Cells, and Some of Their Genetic Variants Are Associated with Plasma Concentrations of These Micronutrients in Humans1–3

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Abstract
Scavenger receptor class B type I (SR-BI) and cluster determinant 36 (CD36) have been involved in cellular uptake of some provitamin A carotenoids. However, data are incomplete (e.g., there are no data on α-carotene), and it is not known whether genetic variants in their encoding genes can affect provitamin A carotenoid status. The objectives were 1) to assess the involvement of these scavenger receptors in cellular uptake of the main provitamin A carotenoids (i.e., β-carotene, α-carotene, and β-cryptoxanthin) as well as that of preformed vitamin A (i.e., retinol) and 2) to investigate the contribution of genetic variations in genes encoding these proteins to interindividual variations in plasma concentrations of provitamin A carotenoids. The involvement of SR-BI and CD36 in carotenoids and retinol cellular uptake was investigated in Caco-2 and human embryonic kidney (HEK) cells. The involvement of scavenger receptor class B type I (SCARB1) and CD36 genetic variants on plasma concentrations of provitamin A carotenoids was assessed by association studies in 3 independent populations. Cell experiments suggested the involvement of both proteins in cellular uptake of provitamin A carotenoids but not in that of retinol. Association studies showed that several plasma provitamin A carotenoid concentrations were significantly different (P < 0.0083) between participants who bore different genotypes at single nucleotide polymorphisms and haplotypes in CD36 and SCARB1. In conclusion, SR-BI and CD36 are involved in cellular uptake of provitamin A carotenoids, and genetic variations in their encoding genes may modulate plasma concentrations of provitamin A carotenoids at a population level. J. Nutr. 143: 448–456, 2013.

Introduction

Vitamin A is found in the human diet either as preformed vitamin A in foods from animal origin or as provitamin A carotenoids in foods from plant origin. The main dietary provitamin A carotenoids are, in decreasing order of provitamin A activity, as follows: β-carotene, α-carotene, and β-cryptoxanthin. Both sources of vitamin A have led to the development of different

1 The SUVIMAX study was funded by Direction Générale de la Santé (Ministry of Health) and supported by Médéric, Ipsen, Mutuelle Générale de l’Éducation Nationale, Sodexho, and Pierre Fabre. Work on the subset population of SUVIMAX was granted by Agence Nationale de la Recherche (no. ANR-05-FNRA-010). The HELENA Study was funded by the European Union’s Sixth RTD Framework Programme (grant no. FOOD-CT-2006-007034); Universidad Politécnica de Madrid (grant no. CH018/2008); Axia-Shell Diagnostics, Ltd. (Oslo, Norway); Abbott Scientific S.A. (Spain); the Spanish Ministry of Education (EX-2007-1124); and Cognis GmbH (Germany). The Fish Oil Intervention and Genotype (FINGEN) study was supported by contract no. RRD7/N02/A from the UK Food Standards Agency.
3 Supplemental Tables 1–3 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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strategies to fight vitamin A deficiency in developing countries where it is still a serious public health problem affecting mostly pregnant or lactating women and preschool children, with an estimated 250 million risk of developing vitamin A deficiency disorders (1). The latest strategies to increase the availability of provitamin A carotenoids are either through the breeding of new varieties of staple food crops with higher concentrations of provitamin A carotenoids (biofortification) or through genetic modification of existing crops (i.e., Golden Rice) (2–4).

The efficiency of vitamin A and carotenoid absorption is determined by the regulation of a number of proteins involved in the process (5). Free retinol enters intestinal cells by either an unknown facilitated process and/or a simple diffusion process (6), whereas its secretion may require a facilitated transport at physiologic doses via ATP binding cassette A1 (ABCA1)14 (7–9). Conversely, although the intestinal absorption of provitamin A carotenoids was thought to be a passive diffusion process (5,10), it is now becoming clear that the absorption of lutein (11), β-carotene (7,12,13), β-cryptoxanthin (13), and lycopene (14) involves scavenger receptor class B type 1 (SR-BI), an enterocyte apical membrane transporter. It has further been suggested that the cluster determinant 36 (CD36), also known as FAT (fatty acid transporter), is involved in cellular uptake of β-carotene (12). Importantly, the involvement of CD36 in the cellular uptake of carotenoids was confirmed in a recent study showing that Cameo2, which is considered homologous to mammalian CD36, is implicated in carotenoid transport in the silk gland in Bombyx mori (15) and in uptake of carotenoids by adipose tissue (16). To date, the involvement of these scavenger receptors in the intestinal uptake of retinol has been studied only for SR-BI (7). However, it is difficult to draw definite conclusions from these results because the small interfering RNA (siRNA) strategy used led to only partial (40%) knockdown of SR-BI expression. Furthermore, there are no data on the involvement of SR-BI in the intestinal uptake of α-carotene, 1 of the 3 main dietary provitamin A carotenoids or on the involvement of CD36 in the intestinal uptake of retinol, α-carotene, and β-cryptoxanthin.

The involvement of proteins in cellular absorption of provitamin A carotenoids (5,6) led us to hypothesize that genetic variations in the genes encoding these proteins could affect the uptake efficiency of these compounds (17). In turn, this raises the possibility that genetic variants in these key genes could affect the provitamin A carotenoid status of affected individuals and, in turn, explain some of the large observed interindividual variations (17,18).

The objectives of this study were to provide evidence in cell lines to support the involvement of SR-BI and CD36 in the cellular uptake of β-carotene, α-carotene, β-cryptoxanthin, and retinol. Furthermore, the study assessed whether genetic variants in genes that encode these transporters are associated with plasma concentrations of provitamin A carotenoids at a population level using data derived from 3 independent populations.

### Participants and Methods

#### Products and reagents

All-trans-carotenoids (α-carotene, β-carotene, β-cryptoxanthin, echinone; all ≥95% pure) were a generous gift from DSM Ltd., Basel, Switzerland. All-trans-retinol (≥99% pure), all-trans-retinyl palmitate (≥85% pure, retinol-free), retinyl-acetate, 2-oleoyl-1-palmitoyl-sn-glycero-3-phosphocholine (phosphatidylcholine), 1-palmitoyl-sn-glycero-3-phosphocholine (lysophosphatidylcholine), monoolein, free cholesterol, oleic acid, sodium taurocholate, and pyrogallol were purchased from Sigma-Aldrich. Blocks lipid transport 1 (BLT1), which is a highly specific inhibitor of SR-BI that does not interfere with receptor-mediated endocytosis or other forms of intracellular vesicular traffic (19), was purchased from Chembridge. Succinimidy sulfonyl oleate (SSO), which is a specific chemical inhibitor of CD36 (20), was synthesized as previously published (21). Its purity was checked by ultra performance liquid chromatography coupled to an ion trap mass spectrometer operated in the negative electrospray ionization mode and 1H-NMR analyses and was ≥99%. DMEM containing 4.5 g/L glucose and trypsin–EDTA (500 and 200 mg/L, respectively) were purchased from Bio-Whittaker, FBS was from Biomedia, and nonessential amino acids and penicillin/streptomycin were purchased from Gibco BRL. The protease-inhibitor cocktail was from Roche. All solvents used were HPLC grade and purchased from Carlo Erba-SDS.

#### Preparation of retinol and carotenoid-rich micelles

For the delivery of retinol or carotenoids to human intestinal Caco-2 cell monolayers, mixed micelles were prepared as published previously (11) to obtain the following final concentrations: 0.04 mmol/L phosphatidylcholine, 0.16 mmol/L lysophosphatidylcholine, 0.3 mmol/L monolein, 0.1 mmol/L free cholesterol, 0.5 mmol/L oleic acid, 0.5 to 5 μmol/L carotenoid or retinol (22,23) and 5 mmol/L taurocholate. The concentrations of retinol and carotenoids in the micellar solutions were checked before each experiment.

#### Preparation of retinol and carotenoid-rich complete medium

For delivery of retinol or carotenoids to human embryonic kidney (HEK) cells, an appropriate volume of each micronutrient solvent stock solution was carefully evaporated under nitrogen in a glass tube. The dried residue was solubilized in FBS overnight, and DMEM was then added to reach a final PBS concentration of 10%. The concentration of retinol or carotenoids in the medium was checked before each experiment.

#### Cell culture

**Caco-2 cell culture.** Caco-2 clone TC-7 cells (24,25), which are human intestinal cells, were a gift from M. Rousset (UMR S 872, Paris, France). These cells express SR-BI (11) but not CD36 (11). Cells were cultured in the presence of DMEM supplemented with 20% heat-inactivated FBS, 1% nonessential amino acid, and 1% antibiotics (complete medium) as previously described (11). For each experiment, the cells were seeded and grown on trans-wells to obtain confluent differentiated cell monolayers. Twelve hours before each experiment, the medium used in the apical and basolateral chambers was changed to a serum-free complete medium, and cell monolayer integrity was checked by measuring the transepithelial electrical resistance as previously described (11).

**HEK cell culture and transfection.** HEK 293-T cells were purchased from the American Type Culture Collection. Cells were cultured in 10% FBS complete medium as previously described (21). For each experiment, cells were seeded at a 1:10 dilution in 6-well plates, and transfected after 24 h with 3 μg DNA [i.e., human SCARB1 in pcDNA3.1 plasmid and empty pcDNA3.1 plasmid, human CD36 in pRES plasmid (Clontech) and empty pRES plasmid] and 6 μL Jet Pei (QIagen) per well in NaCl 150 mmol/L according to the manufacturer's instructions. The medium was then changed after 10–12 h and cells were grown for an additional 24 h. Transfection efficiency was checked by Western blotting as previously published (11). Briefly, proteins were determined by using a bicinechonic acid kit (Pierce), and 50 μg was used for Western blot analysis. The blotting membrane was incubated with the mouse monoclonal immunoglobulin (Ig) G against the external domain of human SR-BI (anti-CLA-1; BD Transduction Laboratories) or the mouse monoclonal anti-human CD36 IgM (Sigma) at 1:1000 dilution. For visualization, monoclonal anti-mouse IgM or IgG conjugated to alkaline phosphatase (Sigma) was used as a secondary antibody at 1:5000 dilution.

**SCARB1 and CD36 and blood provitamin A carotenoids**

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Measurement of carotenoid and retinol uptake by cells

At the beginning of each experiment, the cell monolayers were washed twice with 0.5 mL PBS. The apical side of the Caco-2 cell monolayers received the micelles, and the other side received the serum-free complete medium. The HEK cells received the enriched complete medium. The cells were incubated for 1 h at 37°C. At the end of each experiment, media were harvested. The cells were washed twice with 0.5 mL ice-cold PBS, then scraped and collected into 0.5 mL PBS. Uptake was estimated from the concentration of retinol or carotenoids in the scraped cells (and in the basolateral medium if applicable). Because provitamin A carotenoids could be cleaved to retinol (26,27), that is reduced into retinol, which can in turn be esterified to produce retinyl esters (8), both retinol and retinyl esters were measured to avoid underestimation of the uptake of vitamin A. However, probably because of the short incubation time, neither retinol nor retinyl esters were detected in the scraped cells or in basolateral medium in any experimental condition.

Retinol and carotenoid apical transport inhibition by BLT1 and SSO

The effect of BLT1 and SSO on retinol and carotenoid uptake was assessed as follows. The cells were pretreated with either DMSO (control) or 10 μmol/L BLT1 or 400 μmol/L SSO for 1 h. The cells then received micelles (in the case of Caco-2) or enriched complete medium (in the case of HEK cells) supplemented with either DMSO (dimethyl sulfoxide alone as control) or scavenger receptor chemical inhibitors (10 μmol/L BLT1 or 400 μmol/L SSO in DMSO). Uptake was then measured as previously described.

All samples were stored at −80°C under nitrogen, with 0.5% pyrogallol as a preservative before lipid extraction and HPLC analysis. Aliquots of cell samples without pyrogallol and containing protease inhibitors were used to estimate the protein concentrations with the use of a bicinechonic acid kit (Pierce).

Retinol and carotenoid extraction and HPLC analysis

Retinol, carotenoids, and putative retinyl esters were extracted from 500 μL aqueous samples by using the following method. After adding ethanol containing 2 internal standards (retinyl acetate for retinol and putative retinyl esters and echinenone for carotenoids), the mixture was evaporated to dryness under nitrogen, and the dried extract was dissolved in 200 μL acetonitrile/dichloromethane (50:50, v:v). A volume of 160 μL was used for HPLC analysis. Retinol, retinyl esters, and carotenoids were separated by using a 250 × 4.6 mm reversed-phase C18, 5-μm Zorbax column (Interchim) equipped with a guard column. The mobile phase was 70% acetonitrile, 20% dichloromethane, and 10% methanol. The flow rate was 1.5 mL/min, and the column was kept at a constant temperature (35°C). The HPLC system was composed of a Dionex separation module (a P680 HPLC pump and an ASI-100 automated sample injector) and a Dionex UVD340 UV photodiode array detector (Dionex SA). Retinol, retinyl acetate, and retinyl palmitate were detected at 325 nm, whereas carotenoids were detected at 450 nm. All molecules were identified by comparing their retention times and spectra (190-500 nm) with pure standards. Quantification was performed by using Chromleon software (version 6.50, SP4 Build 1000; Dionex), comparing the peak area with standard reference curves.

Participants

The Supplémentation en Vitamines et Minéraux Antioxydants (SUVIMAX) study was designed to test the benefits of multivitamin-mineral supplementation on cancer and cardiovascular disease risk in France (28). Of a total of 6850 participants who agreed to participate in a postsupplementation follow-up, 4497 participants were randomly selected and genotyped for several SNPs in 14 candidate genes potentially involved in the metabolism of carotenoids and vitamin A. A subsample of 621 participants were then selected to obtain groups with contrasting haplotypes in whom plasma provitamin A carotenoids were measured. The characteristics of this subsample are shown in detail in a previous publication (29).

The Fish Oil Intervention and Genotype (FINGEN) study was a 4-center trial conducted at the Universities of Glasgow, Newcastle, Reading, and Southampton in the United Kingdom between June 2003 and September 2005. The study prospectively recruited 312 volunteers according to apolipoprotein E (APOE) genotype, sex, and age as described previously (30,31). The primary objective of this randomized controlled trial was to examine the impact of APOE genotype, age, and sex on the responsiveness of an array of established and putative cardiovascular disease biomarkers to physiologic fish oil supplementation. For the purpose of the current publication, baseline plasma provitamin A carotenoid concentrations were used.

The HELENA (Healthy Lifestyle in Europe by Nutrition in Adolescence) cross-sectional study has been described in detail elsewhere (32). Briefly, a total of 3865 adolescents were recruited between 2006 and 2007. Data were collected in a total of 10 centers in 9 European countries. Participants were randomly selected from schools by using a proportional cluster sampling methodology and taking age into account. One-third of the classes were randomly selected for plasma collection (n = 1155). BMI and plasma β-carotene measurements were available for 993 adolescents. Data were collected on a detailed case report form, in accordance with standardized procedures. Only plasma β-carotene concentrations are available from this study population because plasma α-carotene and β-cryptoxanthin were not analyzed.

All of the above-mentioned human studies were approved by local research ethics committees.

Choice of candidate SNPs in CD36 and SCARB1 and genotyping methods

In the SUVIMAX and FINGEN samples, SNPs were selected through an analysis of previous studies describing associations between these SNPs and digestion, transport, or lipid metabolism (33). In the HELENA study, 10 tag SNPs were selected to cover the whole genetic variability of CD36 (29), but only the rs5888 SNP was genotyped in scavenger receptor class B type I (SCARB1). Methods of genotyping have been described in detail in previous articles (29,33). Genotyped SNPs are presented in Supplemental Table 1.

Statistical analysis

Cell studies. Results are expressed as means ± SD. Differences between the uptake efficiency in the different cell groups (control, HEK cells that overexpressed a scavenger receptor, cells + a chemical inhibitor of either SR-BI or CD36) were analyzed by using the Mann-Whitney test as a post hoc test when the Kruskal-Wallis test showed significant differences (P < 0.05) between groups. P < 0.05 was considered significant.

Candidate gene association studies in the 3 studies. Associations between SNPs and plasma concentrations of provitamin A carotenoids in the 3 independent samples were first assessed by using the general linear model on log-transformed data. In a first step, interfering covariables (adjustment factors) were identified. Any covariable that was significant at P < 0.05 (namely age, sex, and BMI) was included as an adjustment factor for testing the genotype effect. The effects of the genotypes on the dependent variables (i.e., plasma provitamin A carotenoids) were tested systematically in the 3 populations and additionally in the SUVIMAX and FINGEN studies, for all participants and for men and women separately. The dominant model specifically tests the association of having at least 1 minor allele versus not having a minor allele. The recessive model specifically tests the association of having the minor allele as both alleles versus having at least 1 major allele. Under the additive model, testing is designed specifically to reveal associations that depend additively upon the minor allele—that is, where having 2 minor alleles rather than having no minor alleles is twice as likely to affect the outcome in a certain direction as is having just 1 minor allele rather than no minor alleles.

In a second approach, we performed a haplotype-based association analysis of data with Thesias software, which is based on the Stochastic-EM algorithm. This permits haplotypes to be inferred from genotypic data and to test their associations with phenotypes of interest (34). Analyses were performed after adjustment for sex, age, and BMI.

To correct for multiple comparisons, Bonferroni corrections to account for the number of comparisons that were performed in each data
set were performed. The $\alpha$ values to reach significance were lowered as follows: $P < 0.0083$ for the SUVIMAX study (6 tested SNPs), $P < 0.0063$ for the FINGEN study (8 tested SNPs), and $P < 0.0045$ for the HELENA study (11 tested SNPs). Similar corrections were applied to the haplotype-based association analysis.

Results

Retinol and provitamin A carotenoid absorption efficiency in Caco-2 TC-7 cells

After 1 h incubation, retinol was more efficiently absorbed than provitamin A carotenoids (up to 30.3 ± 0.2% absorbed) for the cells for retinol vs. 2.2 ± 0.3% for $\beta$-cryptoxanthin, 2.4 ± 0.3% for $\alpha$-carotene, and 4.2 ± 0.3% for $\beta$-carotene. The addition of BLT1 [a specific chemical inhibitor of SR-BI (19)] significantly decreased uptake of all provitamin A carotenoids by ~50 to 70% ($P < 0.05$) but had no effect on retinol uptake (Fig. 1).

Effect of SCARB1 and CD36 transfection on provitamin A carotenoid and retinol uptake in HEK cells

Transfection with a human SCARB1 expression vector led to a significant 1- to 2-fold increase in provitamin A carotenoid uptake when compared with control (cells transfected with an empty plasmid). Furthermore, this increase was completely suppressed by addition of BLT1 (Fig. 2A). Conversely, HEK cell transfection with human SCARB1 did not modify retinol uptake, and BLT1 had no effect on retinol uptake by SCARB1 transfected cells. HEK cell transfection with a human CD36 expression vector also significantly increased provitamin A carotenoid uptake from ~40 to 100% (Fig. 2B). This increase was suppressed by the addition of SSO (the chemical inhibitor of CD36). With regard to SR-BI, the transfection of HEK cells with CD36 and the addition of SSO had no effect on retinol uptake.

Associations between SNPs and haplotypes in SCARB1 and CD36 and plasma concentrations of provitamin A carotenoids in the 3 studies

SCARB1 gene variants and plasma provitamin A carotenoid concentrations. In the SUVIMAX sample, significant Bonferroni-corrected associations between the rs61932577 SNP in SCARB1 and plasma provitamin A carotenoids were observed (Table 1). More precisely, participants bearing the TT genotype had higher provitamin A carotenoid concentrations than did participants with the C allele (recessive and additive models). Note that similar findings were observed in men and in women suggesting no sex effect on this association. There were no significant associations between the 2 other SCARB1 SNPs genotyped in the SUVIMAX study (i.e., rs5888 and rs4238001) and plasma provitamin A carotenoid concentrations (data not shown). This was confirmed in the HELENA and FINGEN studies where no significant association between the genotyped SCARB1 SNPs and plasma provitamin A carotenoid concentrations was observed (data not shown). Finally, haplotype analysis suggested that SUVIMAX participants bearing the CCT haplotype (rs5888, rs4238001, rs61932577) had lower (~18%; data not shown) plasma $\beta$-cryptoxanthin than did participants bearing the TCC haplotype (Table 2).

CD36 genetic variants and plasma provitamin A carotenoid concentrations. As shown in Table 3, plasma $\beta$-cryptoxanthin concentrations in women from the SUVIMAX study were significantly associated (even after Bonferroni correction) with rs1984112 and with rs1761667 (recessive effects). Women who were homozygous for the G minor allele at either rs1984112 or rs1761667 had ~25% less plasma $\beta$-cryptoxanthin than did women who bore an A allele at either of these loci. Conversely, there was no association between these SNPs and plasma $\beta$-cryptoxanthin concentration in men. There were also significant Bonferroni-corrected associations between plasma $\beta$-cryptoxanthin and rs7755 that differed between sexes. Women homozygous for the A minor allele at this locus had ~23% less $\beta$-cryptoxanthin than did women carrying the G allele. Conversely, men carrying the AA genotype in rs7755 had ~21% more $\beta$-cryptoxanthin than did men who bore a G allele.

In the FINGEN study, plasma concentrations of the provitamin A carotenoid $\alpha$-carotene were associated with 3 CD36 SNPs (Table 4) (2 of these associations remained significant after Bonferroni correction). Haplotype analysis showed that, in the FINGEN study (Table 5), 1 haplotype constituting 5 SNPs (rs1984112, rs1761667, rs1527479, rs1527483, rs13230419), GACC with a frequency of ~29% in the sample, achieved nominal association with higher (+12%; data not shown) plasma $\alpha$-carotene ($P = 0.035$) and higher (+16%; data not shown) $\beta$-cryptoxanthin concentrations in comparison with the most frequent haplotype (AAGCT; frequency of 47%). A nominal association between the rs1984112 SNP and plasma $\beta$-cryptoxanthin concentration was also observed in the SUVIMAX sample when the haplotypic background was G at rs1761667 and A at rs7755 (Supplemental Table 2). Finally, no significant association was detected (in individual SNP analysis or haplotype analysis) between the CD36 SNPs and plasma $\beta$-carotene concentrations in any of the 3 populations.

Discussion

The scavenger receptor family is a multiligand, multifunctional receptor system including class A, class B, mucin-like, and endothelial receptors. SR-BI is a class B receptor found in numerous tissues including the intestine. It is involved in the cellular uptake of a wide range of lipid molecules, including cholesterol (12,35), vitamin E (36), vitamin D (21), and the non-

FIGURE 1 Effect of BLT1 on provitamin A carotenoids and retinol uptake by differentiated Caco-2 TC-7 cell monolayers. Cells were incubated with either $\beta$-carotene, $\beta$-cryptoxanthin, $\alpha$-carotene–enriched mixed micelles or retinol-enriched mixed micelles with or without BLT1 at 10 $\mu$mol/L. Values are mean ± SEM of 3 assays. Data are from 1 experiment representative of at least 2 independent experiments. *Different from DMSO control, $P < 0.05$ (Mann-Whitney $U$ test). BLT1, blocks lipid transport 1.
FIGURE 2 Effect of transfection with human SCARBl and CD36, and chemical inhibitors of SR-BI and CD36, on uptake of retinol and provitamin A carotenoids by 293-T HEK cells. Values are mean ± SEM of 3 assays. Data are from 1 experiment representative of at least 2 independent experiments. Labeled means without a common letter differ, \( P < 0.05 \). Insets: Western blots of protein expression in HEK cells: 1, size marker; 2, control cells; 3, HEK cells overexpressing SR-BI (A) or CD36 (B). (A) Effect of SCARBl overexpression and BLT1. Cells were transfected with either an empty pcDNA3.1 plasmid (control) or with a pcDNA3.1 plasmid containing human SCARBl. (B) Effect of CD36 overexpression and SSO. Cells were transfected with either an empty pIRES plasmid (control) or with a pIRES plasmid containing human CD36. SEM of 3 experiments. Labeled means representative of at least 2 independent experiments. Data are from 1 experiment analyzed by 1-way ANOVA, followed by Bonferroni post hoc analysis. *Nominal association before Bonferroni correction (i.e., \( P < 0.05 \)).

provitamin A carotenoids lutein (11) and lycopene (14). Its involvement in the cellular uptake of these carotenoids has raised questions as to its role in the cellular uptake of vitamin A, either as retinol or as provitamin A carotenoids, as well as to the involvement of other scavenger class B receptors, such as CD36/SCARB1. It has been shown that CD36 is involved both in \( \beta \)-carotene uptake by mouse brush border membrane vesicles (12) and in cellular uptake of lutein and lycopene by adipocytes (16).

Our first objective was to investigate for the first time to our knowledge the involvement of SR-BI and CD36 in cellular uptake of \( \alpha \)-carotene, as well as the involvement of CD36 in \( \beta \)-cryptoxanthin and retinol uptake. Our second objective was to assess whether genetic variants in the genes that encode these scavenger receptors may affect plasma concentrations of provitamin A carotenoids. To achieve these objectives we used 2 different approaches: 1) uptake studies in a human intestinal cell line (Caco-2) and in HEK cells that can be easily transfected and 2) association studies between genetic variants in the genes that encode these transporters and plasma concentrations of provitamin A carotenoids in 3 independent populations.

Our initial objective was to work with a human intestinal cell line and either to modulate the expression of the scavenger receptors (siRNA or overexpression by transfection) or to inhibit the scavenger receptors by using specific chemical inhibitors: BLT1 for SR-BI and SSO for CD36. Nevertheless, it was not possible to fully inhibit SR-BI expression in Caco-2 cells with the siRNA methodology (13), and the fact that these cells do not express CD36 (35) did not allow us to study the inhibitory effect of the specific chemical inhibitor of CD36, i.e., SSO, on vitamin A uptake. We thus used HEK cells transfected with either human SCARBl or CD36 to confirm and extend the data obtained in Caco-2 cells.

Our first result showed, in both human intestinal cells and in SCARBl transfected HEK cells, that the chemical inhibitor of SR-BI, i.e., BLT1, impairs provitamin A carotenoid uptake. This result is in agreement with the 2 studies that suggested the

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\( ^{1} \text{Values are mean ± SD. } \)P values were calculated by using a general linear model and were adjusted for age and BMI. *Nominal association before Bonferroni correction (i.e., 0.0083 < \( P < 0.05 \)). SCARBl, scavenger receptor class B type I; SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants.
involvement of SR-BI in cellular uptake of β-carotene (12,13) and β-cryptoxanthin (13). Furthermore, it shows for the first time that SR-BI is also involved in the uptake of the third main provitamin A carotenoid of the human diet, α-carotene. The lack of effect of BLT1 on retinol uptake, both in Caco-2 and in HEK cells, confirms the result obtained by During and Harrison (7) using siRNA and shows for the first time that this scavenger receptor is also involved in the cellular uptake of α-carotene by comparison to the most frequent haplotype (P values calculated by Student’s t test).

The second objective of this study was to evaluate whether genetic variants in SCARB1 and CD36 are related to plasma vitamin A concentrations in humans. To diminish the risk of associations that occur only in a particular genetic background, we assessed these associations in 3 independent populations. We focused on provitamin A carotenoids because cell experiments showed that cellular uptake of retinol is not mediated by these scavenger receptors and because our sample size was underpowered to detect SNP effects on circulating retinol concentrations. Indeed, a previous genomewide association study indicated that a sample size of n = 1242 gives a power of only 13% to detect a SNP explaining 1.5% of the variation in serum retinol concentrations.

The first important findings of these association studies are the significant associations between plasma α-carotene and β-cryptoxanthin concentrations and SNPs, or haplotypes, in CD36. Indeed, this suggests that CD36 is involved in the plasma concentration of provitamin A carotenoids. Nevertheless, the fact that no significant association was observed between plasma β-carotene and CD36 genetic variants in any of the 3 populations was surprising. We hypothesized that this was due to a confounding effect of β-carotene-15,15-monoxygenase (BCMO1) genetic variants on β-carotene cleavage (18,38). To verify this hypothesis we evaluated associations between 2 SNPs in BCMO1 [rs7501331 and rs12934922 (33)] and plasma provitamin A carotenoid concentrations in both the SUVIMAX and FINGEN samples (SNPs in BCMO1 were not genotyped in the HELENA study). Results of these analyses (Supplemental Table 3) clearly showed that these 2 SNPs, as well as one of their haplotypes (data not shown), were significantly associated with blood concentrations of β- and α-carotene, but not with that of β-cryptoxanthin. This result supports the hypothesis that the effects of the genetic variants in BCMO1 masked the effect of the CD36 SNPs with regard to blood β-carotene in the 3 studies.

### TABLE 3  Plasma β-cryptoxanthin concentrations according to genotypes of CD36 in the SUVIMAX clinical study

<table>
<thead>
<tr>
<th>SNP/participant groups</th>
<th>Genotype</th>
<th>P value</th>
<th>n</th>
<th>μmol/L</th>
<th>Dominant model</th>
<th>Recessive model</th>
<th>Additive model</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1984112</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All participants</td>
<td></td>
<td></td>
<td>312</td>
<td>189</td>
<td>119</td>
<td>0.35 ± 0.31</td>
<td>0.38 ± 0.25</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td>176</td>
<td>102</td>
<td>63</td>
<td>0.40 ± 0.31</td>
<td>0.39 ± 0.25</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td>138</td>
<td>87</td>
<td>56</td>
<td>0.28 ± 0.31</td>
<td>0.26 ± 0.24</td>
</tr>
<tr>
<td>rs1761667</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All participants</td>
<td></td>
<td></td>
<td>249</td>
<td>192</td>
<td>179</td>
<td>0.33 ± 0.31</td>
<td>0.34 ± 0.26</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td>136</td>
<td>105</td>
<td>99</td>
<td>0.39 ± 0.30</td>
<td>0.40 ± 0.29</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td>113</td>
<td>87</td>
<td>80</td>
<td>0.27 ± 0.31</td>
<td>0.26 ± 0.21</td>
</tr>
<tr>
<td>rs7755</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All participants</td>
<td></td>
<td></td>
<td>216</td>
<td>250</td>
<td>151</td>
<td>0.35 ± 0.30</td>
<td>0.32 ± 0.29</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td>125</td>
<td>132</td>
<td>82</td>
<td>0.39 ± 0.28</td>
<td>0.39 ± 0.30</td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td>91</td>
<td>118</td>
<td>69</td>
<td>0.29 ± 0.32</td>
<td>0.24 ± 0.25</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD. P values were calculated by using a general linear model and were adjusted for age and BMI. *Nominal association before Bonferroni correction (i.e., 0.0083 < P < 0.05). Models are explained in the statistic paragraph of the Participants and Methods section. CD36, cluster determinant 36; SUVIMAX, Suppl´ementation en Vitamines et Min´eraux AntioXydants.
The mechanism by which CD36 affects plasma provitamin A carotenoid concentrations is not known. CD36 recognizes a broad variety of lipid ligands, including fatty acids, oxidized LDL, apoptotic cells, and β-carotene (12). Thus, recognition of the fat-soluble provitamin A carotenoids by CD36 is plausible. Because CD36 is expressed in the human enterocyte, although not in the Caco-2 cell line (11), our result in CD36 transfected HEK cells suggests that CD36 may facilitate the transfer of provitamin A carotenoids from mixed micelles (39) across the apical membrane of the enterocyte. However, because CD36 has been shown to be involved in active transport of provitamin A carotenoids into the adipose tissue (16), it is unclear at this time whether the observed effect of genetic variants in CD36 on fasting plasma carotenoid concentrations is caused at the site of the intestine or in other tissues involved in provitamin A uptake. The potential functional importance of SNPs in CD36 has been shown by a recent study of monocyte and/or platelet CD36 protein expression in African Americans (40). In this work, the A allele of rs1761667 was associated with decreased CD36 expression in monocytes. These functional data allow us to hypothesize that the higher plasma β-cryptoxanthin concentrations observed in participant carriers of the A allele at rs1761667 (Table 4) might be related to a lower CD36 expression in some tissues, and therefore to a lower uptake of this carotenoid by these tissues, which may lead to an accumulation of this carotenoid in the plasma compartment. If confirmed, this mechanism might help explain why high plasma concentrations of carotenoids could result not only from high intakes and/or high bioavailability of carotenoids but also from an impaired uptake of these compounds by tissues. In other words, a high plasma concentration of provitamin A carotenoids would not always mean that tissue status for these carotenoids is also high.

The second important finding of these association studies was the significant associations observed between the rs61932577 SNP in SCARB1 and plasma provitamin A carotenoid concentrations. This confirms, in a new population, findings from a previous study showing an association between this SNP and plasma α- and β-carotene concentrations (41). This association can be explained by an effect of the genetic variants on the ability of SR-BI to either absorb provitamin A carotenoids at the intestinal level and/or to bind HDL, which transports a fraction of carotenoids in the plasma (42).

In summary, our results show that CD36 and SR-BI are involved in cellular uptake of the 3 main dietary provitamin A carotenoids but not in that of retinol. Genetic variants in both

### Table 4

<table>
<thead>
<tr>
<th>Genotype</th>
<th>rs1984112</th>
<th>rs1761667</th>
<th>rs1527479</th>
<th>rs1527483</th>
<th>rs13230419</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNP/participant groups</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>AA</td>
<td>AG</td>
</tr>
<tr>
<td>All participants</td>
<td>92</td>
<td>106</td>
<td>33</td>
<td>0.22 ± 0.13</td>
<td>0.26 ± 0.17</td>
</tr>
<tr>
<td>Men</td>
<td>47</td>
<td>42</td>
<td>21</td>
<td>0.21 ± 0.13</td>
<td>0.20 ± 0.09</td>
</tr>
<tr>
<td>Women</td>
<td>45</td>
<td>64</td>
<td>12</td>
<td>0.22 ± 0.13</td>
<td>0.29 ± 0.19</td>
</tr>
<tr>
<td>All participants</td>
<td>63</td>
<td>123</td>
<td>48</td>
<td>0.21 ± 0.13</td>
<td>0.26 ± 0.17</td>
</tr>
<tr>
<td>Men</td>
<td>35</td>
<td>55</td>
<td>22</td>
<td>0.20 ± 0.13</td>
<td>0.22 ± 0.11</td>
</tr>
<tr>
<td>Women</td>
<td>31</td>
<td>67</td>
<td>24</td>
<td>0.22 ± 0.12</td>
<td>0.29 ± 0.20</td>
</tr>
</tbody>
</table>

1 Values are mean ± SD. P values were calculated by using a general linear model and were adjusted for age, BMI, and smoking habits. *Nominal association before Bonferroni correction (i.e., 0.00625 < P < 0.05). Models are explained in the statistic paragraph of the Participants and Methods section. CD36, cluster determinant 36; FINGEN, The Fish Oil Intervention and Genotype study.

### Table 5

<table>
<thead>
<tr>
<th>rs1984112</th>
<th>rs1761667</th>
<th>rs1527479</th>
<th>rs1527483</th>
<th>rs13230419</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haplotype frequency</td>
<td>0.465</td>
<td>0.39</td>
<td>0.041</td>
<td>0.10</td>
</tr>
</tbody>
</table>

1 The associations between CD36 SNPs and plasma provitamin A carotenoids were assessed in 207 participants (59% men). *Nominal association before Bonferroni correction (i.e., 0.01 < P < 0.05). CD36, cluster determinant 36; FINGEN, The Fish Oil Intervention and Genotype study.

2 Haplotype frequencies were estimated by using Thesias software (34). The most frequent haplotype (AAGCT) is the reference haplotype. Haplotypes with a frequency <2.5% are not shown.

3 Haplotype effect on plasma provitamin A carotenoids by comparison to the most frequent haplotype (P values calculated by Student’s t test).
genes are associated with plasma concentrations of provitamin A carotenoids. All of these data corroborate the importance of these receptors in regulating plasma concentrations of provitamin A carotenoids. The presented data therefore add to our understanding of the molecular regulation of provitamin A uptake and add information to explore the observed interindividual variations in plasma carotenoid concentrations.

Acknowledgments

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SCARB1 and CD36 and blood provitamin A carotenoids

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