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Although cellular uptake of vitamin E was initially described as a passive process, recent studies in the liver and brain have shown that SR-BI (scavenger receptor class B type I) is involved in this phenomenon. As SR-BI is expressed at high levels in the intestine, the present study addressed the involvement of SR-BI in vitamin E trafficking across enterocytes. Apical uptake and efflux of the main dietary forms of vitamin E were examined using Caco-2 TC-7 cell monolayers as a model of human intestinal epithelium. (*R,R,R*)-γ-tocopherol bioavailability was compared between wild-type mice and mice overexpressing SR-BI in the intestine. The effect of vitamin E on enterocyte SR-BI mRNA levels was measured by real-time quantitative reverse transcription-PCR. Concentration-dependent curves for vitamin E uptake were similar for (*R,R,R*)-α-, (*R,R,R*)-γ-, and DL-α-tocopherol. (*R,R,R*)-α-tocopherol transport was dependent on incubation temperature, with a 60% reduction in absorption at 4 °C compared with 37 °C ($p < 0.05$). Vitamin E flux in enterocytes was directed from the apical to the basal side, with a relative 10-fold reduction in the transfer process when measured in the opposite direction ($p < 0.05$). Co-incubation with cholesterol, γ-tocopherol, or lutein significantly impaired α-tocopherol absorption. Anti-human SR-BI antibodies and BLT1 (a chemical inhibitor of lipid transport via SR-BI) blocked up to 80% of vitamin E uptake and up to 30% of apical vitamin E efflux ($p < 0.05$), and similar results were obtained for (*R,R,R*)-γ-tocopherol. SR-BI mRNA levels were not significantly modified after a 24-h incubation of Caco-2 cells with vitamin E. Finally, (*R,R,R*)-γ-tocopherol bioavailability was 2.7-fold higher in mice overexpressing SR-BI than in wild-type mice ($p < 0.05$). The present data show for the first time that vitamin E intestinal absorption is, at least in part, mediated by SR-BI.

Vitamin E is a fat-soluble micronutrient that occurs in nature in eight different forms: (*R,R,R*)-α-, -β-, -γ-, and -δ-tocopherol and (*R,R,R*)-α-, -β-, -γ-, and -δ-tocotrienol. The human diet mainly provides (*R,R,R*)-α-tocopherol (RRR-α-T)[2] and (*R,R,R*)-γ-tocopherol (RRR-γ-T), whereas supplements generally supply vitamin E as DL-α-tocopherol (DL-α-T) or DL-α-tocopherol acetate. Although a recent study has described the fate of vitamin E in the human digestive tract (1), the major steps from micellar uptake to enterocyte trafficking and incorporation into chylomicrons are largely unknown (2). It has long been assumed that vitamin E, like other fat-soluble micronutrients, is absorbed by a passive process. However, recent data suggest that the absorption of fat-soluble micronutrients is more complex than previously thought. More precisely, several transporters, *i.e.* ABC transporters (3), Niemann-Pick C1-like 1 (NPC1L1) (4), cluster determinant 13 (CD13) (5), and scavenger receptor class B type 1 (SR-BI) (6), have been implicated in cholesterol trafficking in the enterocyte, and it has recently been shown that the absorption of the two carotenoids lutein and β-carotene is at least partly mediated by SR-BI (7, 8). Interestingly, recent data add support to the hypothesis of involvement of SR-BI in vitamin E transport in the enterocyte. First, SR-BI is expressed at substantial levels in the intestine (9). Second, SR-BI significantly contributes to the selective uptake of HDL-associated vitamin E in both porcine brain (10) and rat pneumocytes (11). Third, an SR-BI analog was reported to mediate cellular uptake of α-tocopherol in *Drosophila* (12). Fourth, vitamin E metabolism is abnormal in SR-BI-deficient mice (13). The objectives of this study were to assess whether intestinal absorption of vitamin E is protein-mediated and to determine whether SR-BI is involved in this phenomenon.

MATERIALS AND METHODS

Chemicals

RRR-α-T (≥99% pure), DL-α-T (≥98% pure), and RRR-γ-T (≥97% pure) were purchased from Fluka (Vaulx-en-Velin, France). Carotenoids (β-carotene, lycopene, and lutein) were generously provided by DSM Ltd. (the successor of Hoffmann-La Roche, Basel, Switzerland). Tocol, used as internal standard for HPLC analysis, was purchased from Lara Spiral (Couternon, France). 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. Sulfo-NHS-LC biotin was purchased from Uptima-Interchim (Montluçon, France). Streptavidin-agarose solution (manufactured by Oncogene Research Products), *n*-octyl-β-D-glucopyranoside (manufactured by Calbiochem) was purchased from VWR International SAS (Strasbourg, France). Mouse monoclonal IgG raised against the external domain (amino acids 104–294) of human SR-BI, also known as CLA-1, was purchased from BD Transduction Laboratories. BLT1 (blocks lipid transport 1, a chemical inhibitor of lipid transport mediated by SR-BI) was purchased from Chembridge (San Diego, CA). Dulbecco's modified Eagle's medium containing 4.5 g/liter glucose and trypsin-EDTA (500 and 200 mg/liter, respectively) was purchased from BioWhittaker (Fontenay-sous-Bois, France), fetal bovine serum (FBS) came from Biomedia (Issy-les-Moulineaux, France), and non-essential amino acids, penicillin/streptomycin, PBS, and PBS containing

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[2] The abbreviations used are: RRR-α-T, (*R,R,R*)-α-tocopherol; RRR-γ-T, (*R,R,R*)-γ-tocopherol; DL-α-T, DL-α-tocopherol; SR-BI, scavenger receptor class B type I; FBS, fetal bovine serum; Tg, transgenic (mice overexpressing SR-BI); PBS, phosphate-buffered saline; HPLC, high pressure liquid chromatography.

0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PBS-CM) were purchased from Invitrogen. The protease inhibitor mixture was a gift from F. Tosini (Avantage Nutrition, Marseille, France).

Preparation of Tocopherol-rich Micelles

For delivery of tocopherol to cells, mixed micelles, which had a lipid composition similar to those found *in vivo* (14), were prepared as described previously (7) to obtain the following final concentrations: 0.04 mM phosphatidylcholine, 0.16 mM lysophosphatidylcholine, 0.3 mM monoolein, 0.1 mM free cholesterol, 0.5 mM oleic acid, 5–90 μM tocopherol (1), and 5 mM taurocholate. The concentration of tocopherol in the micellar solutions was checked before each experiment.

Preparation of Tocopherol-rich Emulsions

For delivery of tocopherol to mice, emulsions were prepared as follows. An appropriate volume of tocopherol stock solution was transferred to Eppendorf tubes to obtain a final amount of 5 mg in each tube. Stock solution solvent was carefully evaporated under nitrogen. Dried residue was solubilized in 100 μl of Isio-4 vegetable oil (Lesieur, Asnières-sur-Seine, France), and 200 μl of physiological serum were added. The mixture was vigorously mixed in ice-cold water in a sonication bath (Branson 3510) for 15 min and used for force-feeding within 10 min.

Cell Culture

Caco-2 clone TC-7 cells (15, 16) were a gift from Dr. M. Rousset (INSERM U178, Villejuif, France). Cells were cultured in the presence of Dulbecco's modified Eagle's medium supplemented with 20% heat-inactivated FBS, 1% non-essential amino acid, and 1% antibiotics (complete medium) as described previously (7).

For each experiment, cells were seeded and grown on transwells as described previously (7) to obtain confluent differentiated cell monolayers. Twelve hours prior to each experiment, the medium used in apical and basolateral chambers was a serum-free complete medium.

During preliminary tests, the integrity of the cell monolayers was checked by measuring transepithelial electrical resistance before and after the experiment using a Millicell-ERS voltohmmeter fitted with a chopstick electrode (Millipore, Saint-Quentin-en-Yvelines, France).

Identification of Transport Characteristics by Uptake and Efflux Measurement

At the beginning of each experiment, cell monolayers were washed twice with 0.5 ml of PBS. For uptake experiments, the apical or basolateral side of the cell monolayers received the tocopherol-rich micelles, whereas the other side received the serum-free complete medium. Cells were incubated for 15 min at either 37 or 4 °C depending on the experiment. Incubation time was chosen after a preliminary experiment measuring maximal absorption rate to obtain sufficient amounts of absorbed tocopherol for accurate measurements. At the end of each experiment, media from each side of the cell monolayer were harvested. Cells were washed twice in 0.5 ml of ice-cold PBS containing 10 mM taurocholate, to eliminate adsorbed tocopherol, and then scraped and collected in 2 ml PBS. Absorbed tocopherol was estimated as tocopherol found in scraped cells plus tocopherol found on the opposite side of the cell monolayer (the basolateral side when micellar tocopherol was added to the apical side, and vice versa).

For apical efflux experiments, the cells first received the tocopherol-rich micelles at the apical side for 60 min. They were then washed three times with PBS and received apical medium containing vitamin E acceptors, *i.e.* vitamin E-free mixed micelles. Aliquots of apical medium were taken at different times and replaced by the same volume of new medium.

All of the samples were stored at −80 °C under nitrogen with 0.5% pyrogallol as a preservative before tocopherol extraction and HPLC analysis. Aliquots of cell samples without pyrogallol were used to assess protein concentrations using a bicinchoninic acid kit (Pierce).

Competition Studies

With Cholesterol—RRR-α-T and RRR-γ-T uptake was measured after incubation of vitamin E-rich mixed micelles (70.4 μM) containing either no cholesterol, 0.1 mM cholesterol (control), or 0.2 mM cholesterol.

With Other Microconstituents—RRR-α-T and RRR-γ-T uptake was measured after incubation of vitamin E-rich mixed micelles (final concentration, 40 μM) containing either no other microconstituent, RRR-γ-T (final concentration of 40 μM when RRR-α-T uptake was measured), RRR-α-T (final concentration of 40 μM when RRR-γ-T uptake was measured), or carotenoids (final concentration: 6.0 μM lutein, 2.8 μM β-carotene, or 0.4 μM lycopene).

Involvement of Proteins in Tocopherol Transport

Test for Localization of SR-BI in Apical or Basolateral Membranes of Caco-2 TC-7 Cells—Biotinylation of cell monolayers and dot blotting were performed as described previously (7). Blots were then incubated with mouse anti-human SR-BI IgG at 1/1000 dilution. Anti-mouse IgG were used as secondary antibodies at a 1/5000 dilution for visualization.

Tocopherol Apical Transport Inhibition by Anti-human SR-BI Antibody—For uptake experiments, cell monolayers were incubated for 2 min with 3.75 μg/ml anti-human SR-BI monoclonal antibody raised against the external domain before tocopherol-rich micelles were added for 60 min. Previous experiments have shown that this antibody concentration allows a maximal inhibition of absorption (7). Anti-human SR-BI antibody raised against the C-terminal domain, which is located at the internal side of the apical membrane, was used as a control at 3.75 μg/ml (17).

For efflux experiments, tocopherol-enriched cells (see previous section on uptake and efflux measurements) received apical medium containing 3.75 μg/ml anti-human SR-BI antibody and vitamin E-free mixed micelles (as acceptors) for up to 24 h.

Tocopherol Apical Transport Inhibition by BLT1—BLT1 cytotoxicity had been controlled previously using a CellTiter 96 Aqueous One Solution assay (Promega, Madison, WI) (7). These control experiments showed that BLT1 was not toxic up to 10 μM. The effect of BLT1 on tocopherol uptake was assessed as follows. Cell monolayers were pretreated with either Me_2SO (control) or BLT1 at 10 μM for 1 h. The cells then received tocopherol-rich mixed micelles with 10 μM BLT1 for 60 min, and uptake was measured as described above.

For efflux experiments, tocopherol-enriched cells received apical medium supplemented with either Me_2SO or BLT1 at 10 μM and vitamin E-free mixed micelles (as acceptors) for up to 6 h. Efflux was measured as described above.

Tocopherol Bioavailability in Mice Overexpressing SR-BI in the Intestine

Transgenic (Tg) mice were created as follows. The SR-BI cDNA was obtained by digestion of pRC/CMV vector by restriction enzymes HindIII and XbaI. The apolipoprotein C III enhancer (−500/−890 bp)-apolipoprotein A IV promoter (−700 bp) was excised from pUCSH-CAT plasmids using restriction enzymes XbaI and HindIII. The construct containing the apolipoprotein C III enhancer-apolipoprotein

A IV promoter to the intestinal specificity and the 1.8-kilobase cDNA fragment of murine SR-BI was cloned into the XbaI site of the pc DNA 1.1 vector. A linearized fragment of the construct, obtained by digestion of the vector by restriction enzymes SalI and AvrII, was used to generate transgenic mice by standard procedures on a B6D2 background.

Detailed information on the characteristics of SR-BI Tg mice are provided in a study by Bietrix *et al.* (18). RNA analysis showed that SR-BI Tg mice had a 25-fold increase of SR-BI in the intestine as compared with wild-type mice. Immunocytochemistry confirmed the intestinal overexpression of SR-BI in Tg mice and showed that the protein was located at the same sites as found in wild-type mice.

The mice were housed in a temperature-, humidity- and light-controlled room. They were given a standard chow diet with water *ad libitum*. They were fasted overnight before each experiment. On the day of the experiment, a first blood sample was obtained at fasting (zero base-line sample) by cutting the extremity of the tails. The mice were then force-fed with a (*R,R,R*)-γ-tocopherol-enriched emulsion. Additional blood samples were taken at 1, 2, 3, 4, 7, 10, and 24 h after the force-feeding. Plasma samples were stored at −80 °C until vitamin E analysis was done by HPLC.

Tocopherol Extraction

Tocopherol was extracted from 500 μl of aqueous samples using the following method. Distilled water was added to sample volumes below 500 μl to reach a final volume of 500 μl. Tocol, which was used as the internal standard, was added to the samples in 500 μl of ethanol. The mixture was extracted once with one volume of hexane. The hexane phase obtained after centrifugation (500 × *g*, 10 min, 4 °C) was evaporated to dryness under nitrogen, and the dried extract was dissolved in 100 μl of methanol. A volume of 5–20 μl was used for HPLC analysis.

Tocopherol Analysis

RRR-α-, RRR-γ-, DL-α-T, and tocol were separated using a 250 × 4.6-nm RP C_{18}, 5-μm Zorbax column (Interchim, Montluçon, France) and a guard column. The mobile phase was 100% methanol. Flow rate was 1.5 ml/min, and the column was kept at a constant temperature (30 °C). The HPLC system comprised a Dionex separation module (P680 HPLC pump and ASI-100 automated sample injector, Dionex, Aix-en-Provence, France) and a Jasco fluorimetric detector (Jasco, Nantes, France). Tocopherols were detected at 325 nm after light emission at 292 nm and were identified by retention time compared with pure (>95%) standards. Quantification was performed using Chromeleon software (version 6.50, SP4 Build 1000) comparing the peak area with standard reference curves. All solvents used were HPLC grade from sodium dodecyl sulfate (Peypin, France).

Effect of Vitamin E on SR-BI mRNA Levels

Differentiated cell monolayers cultivated on filters were incubated with either 80 μM RRR-α-T-rich micelles, 80 μM RRR-γ-T-rich micelles, or tocopherol-free micelles (controls) for 24 h. Total RNA was isolated from the cells using RNA II nucleospin™ kits (Macherey-Nagel, Düren, Germany). The cDNA was prepared by reverse transcription of 1 μg of total RNA using random hexamers as primers. An equivalent volume of 5 μl of cDNA solution was used for quantification of specific cDNA by real-time quantitative reverse transcription-PCR. The sequences of the primers for 18 S RNA, used as an internal standard for sample normalization, have been published previously (19). The sequences of the forward and reverse human SR-BI primers were 5′-CGGCTCGGAGAGCGACTAC-3′ and 5′-GGGCTTATTCTCCATCATCACC-3′, respectively.

Real-time quantitative reverse transcription-PCR reactions were performed in duplicate on an ABI PRISM™ 7700 sequence detection system (PE Applied Biosystems) using SYBR Green kits (Eurogentec, Angers, France) in line with the manufacturer's instructions. A melt curve peak analysis was performed at the end of all real-time PCR runs to ensure that only the correct product was amplified. The relative levels of SR-BI mRNA were calculated as recommended by the manufacturer using the comparative $\Delta\Delta C_t$ method (User Bulletin No. 2, ABI PRISM 7700 sequence detection system, PE Applied Biosystems) and then analyzed as described by Livak and Schmittgen (20).

Statistical Analysis

Results are expressed as means ± S.D. The Differences between the two groups of unpaired data were tested using the nonparametric Mann-Whitney *U* test. Values of $p < 0.05$ were considered significant. All statistical analyses were performed using Statview software, version 5.0 (SAS Institute, Cary, NC). Relationships between the two variables were examined by regression analysis on KaleidaGraph version 3.6 software (Synergy software, Reading, PA).

RESULTS

Incorporation of Tocopherol in Mixed Micelles

Increasing amounts of RRR-α-T, RRR-γ-T, or DL-α-T, corresponding to theoretical final concentrations ranging from 2.5 to 90 μM, were added to the lipid mixture during micelle preparation, and the concentrations of tocopherol recovered in the mixed micelles was measured. The incorporation of all three tocopherols increased linearly (data not shown).

Effect of Incubation Time on Tocopherol Uptake

RRR-α-T and RRR-γ-T absorbed by the differentiated monolayers increased curvilinearly for up to 60 min of incubation for both a high (40 μM) and a low (2.5 μM) concentration (data not shown). At incubation times less than 15 min and at the lower concentration, the amounts of tocopherol absorbed were insufficient for accurate measurement. Thus, to remain as close as possible to the initial absorption rate and to obtain adequate tocopherol absorption, we decided to work at 15 min of incubation. Competition studies were performed at 30 min, which represents approximately the time during which the bowel content remains in contact with duodenal cells during digestion (21). Finally, some absorption experiments were carried out at 60 min to increase the differences between groups.

Effect of Micellar Tocopherol Concentration on Tocopherol Absorption

Absorption of RRR-α-T, RRR-γ-T, and DL-α-T was not linear (Fig. 1). The best fits were the hyperbolic curves $y = ax/(x + b)$, $R^2 > 0.99$. Apparent Q_{max} and *K* values were calculated (Table 1). Q_{max} represents maximal amount of tocopherol absorbed, and *K* represents the concentration of micellar tocopherol required to reach $Q_{max}/2$. Note that these values likely depend on micelle composition. There was no significant difference between vitamin E species in terms of affinity for a potential transporter (*K* values).

Effect of Temperature and Transport Direction

Table 2 shows that there was (i) a significant decrease in RRR-α-T and RRR-γ-T absorption at 4 °C compared with that at 37 °C and (ii) a drastic fall (up to 93.7%) in RRR-α-T and RRR-γ-T absorption when vitamin

E-rich micelles were supplied at the basolateral side compared with the apical side.

Effect of the Amount of Cholesterol Incorporated in Micelles with Vitamin E on Vitamin E Absorption

Cholesterol is naturally present in micelles during digestion but in concentrations that increase or decrease depending on diet and biliary secretion. RRR-α-T was significantly affected by micellar cholesterol concentration. More precisely, absorption decreased when micellar cholesterol concentration increased (Table 3). Similar results were obtained for RRR-γ-T (data not shown).

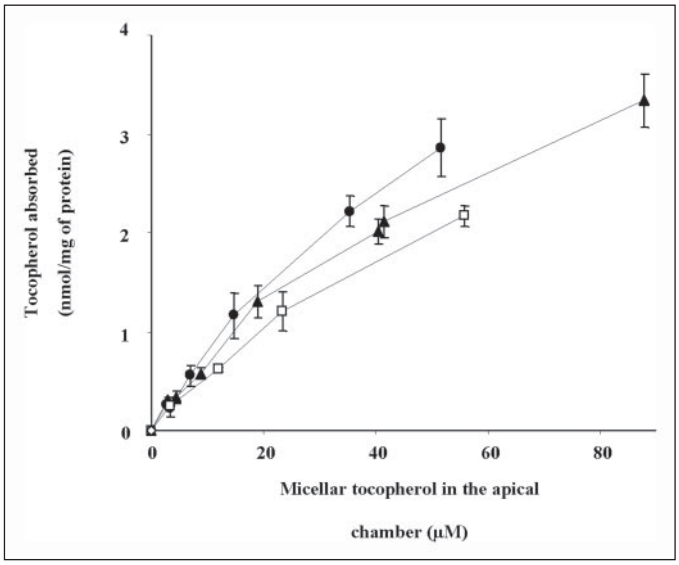

FIGURE 1. **Effect of micellar tocopherol concentration on tocopherol absorption by differentiated Caco-2 TC-7 cell monolayers at 37 °C.** The apical side received FBS-free medium containing either RRR-α-T (●)-, RRR-γ-T (▲)-, or DL-α-T (□)-rich mixed micelles, and the basolateral side received FBS-free medium. Incubation time was 15 min. Data are means ± S.D. of three assays. Tocopherol absorbed = tocopherol recovered in the scraped cells + tocopherol recovered in the basolateral medium.

TABLE 1

Parameters of tocopherol absorption as a function of micellar tocopherol concentrations in differentiated Caco-2 TC-7 cell monolayers

Tocopherol uptake was measured at 37 °C after apical micellar tocopherol delivery in increasing concentrations. Incubation time was 15 min. Best fitting curves were hyperbolic curves $y = ax/(b + x)$. Q_{max} represents the maximal amount of tocopherol absorbed, and K represents the concentration of micellar tocopherol required to reach $Q_{max}/2$. Data are means ± S.D. of three assays.

Vitamin E species	Apparent Q_{max}	Apparent K
	nmol of tocopherol absorbed/mg of protein	*μM*
RRR-α-T	7.5 ± 0.6	84.3 ± 9.9
RRR-γ-T	6.5 ± 0.5	83.9 ± 11.5
DL-α-T	5.6 ± 0.7	88.3 ± 15.2

Effect of Co-incubation with Other Fat-soluble Microconstituents on Vitamin E Absorption

As shown in Table 4, co-incubation of micellar RRR-α-T with micellar RRR-γ-T significantly decreased the absorption of micellar RRR-α-T. Co-incubation with lutein also significantly decreased RRR-α-T absorption. Conversely there was no significant effect of β-carotene and lycopene on RRR-α-T absorption, but it should be noted that the micellar concentrations of these carotenoids were necessarily lower than for lutein because of their naturally lower solubility in micelles. Similar results were obtained with RRR-γ-T (data not shown).

Involvement of SR-BI in Vitamin E Transport across the Apical Membrane of the Enterocyte

The biotinylation experiment that we performed demonstrated that SR-BI is located mainly at the apical membrane of Caco-2 TC7 cells, according to our previous work (7).

Uptake Experiment—Fig. 2 shows that the addition of anti-human SR-BI antibody raised against the external domain significantly decreased RRR-α-T and RRR-γ-T apical absorption (by about 20%). Conversely, and as expected, the control antibody, *i.e.* the anti-human SR-BI antibody raised against an internal domain, did not impair vitamin E absorption (data not shown). The specific chemical inhibitor of SR-BI (BLT1) also significantly decreased RRR-α-T and RRR-γ-T absorption (by up to 80%).

Apical Efflux Experiment—The apical effluxes of both RRR-α-T and RRR-γ-T (data not shown) increased dramatically (~300% after 24 h) when a putative physiological acceptor of vitamin E, in this case vitamin E-free mixed micelles, was added to the apical medium (Fig. 3). Adding either anti-human SR-BI antibody raised against the external domain or BLT1 at the apical side of the cells in the presence of vitamin E-free mixed micelles significantly reduced the apical efflux of RRR-α-T (Fig. 4) and RRR-γ-T (data not shown, but similar for this vitamin).

Regulation of Enterocyte SR-BI mRNA Levels by Vitamin E

SR-BI mRNA levels were not significantly affected by incubation of differentiated cell monolayers with either RRR-α-T or RRR-γ-T (data not shown).

RRR-γ-T Bioavailability in Mice Overexpressing SR-BI in the Intestine

Plasma RRR-γ-T response (expressed as area under the postprandial 0–24-h curves) after force-feeding with RRR-γ-T-enriched emulsion was significantly higher in SR-BI Tg mice than in control mice (106.9 ± 21.0 μmol·h/liter *versus* 54.9 ± 22.5 μmol·h/liter) ($p = 0.0062$; Fig. 5*A*). Plasma RRR-γ-T response remained significantly higher in SR-BI Tg mice than in wild-type mice after lipid adjustment of the vitamin E response (35.7 ± 8.4 μmol·h/g of lipid *versus* 17.0 ± 6.9 μmol·h/g of lipid, $p = 0.0062$, Fig. 5*B*).

TABLE 2

Effect of temperature and transport direction on RRR-α-tocopherol and RRR-γ-tocopherol absorption by differentiated Caco-2 TC-7 cell monolayers

Tocopherol absorption (pmol/mg of protein) was measured at 37 °C after apical micellar tocopherol delivery (control = 100%). Tocopherol uptake at 4 °C and tocopherol uptake following addition of micellar tocopherol in the basolateral chamber were compared with absorptions measured with vitamin E-rich micelles at the same micellar concentration at 37 °C and in the apical to basolateral direction. Incubation time was 15 min. Data are means ± S.D. of three assays. Each value was significantly different from the control.

(*R,R,R*)-α-Tocopherol absorption			(*R,R,R*)-γ-Tocopherol absorption		
RRR-α-T micellar concentration	4 °C	Basolateral to apical	RRR-γ-T micellar concentration	4 °C	Basolateral to apical
μM	*% of control*	*% of control*	*μM*	*% of control*	*% of control*
15	29.5 ± 6.9	6.3 ± 0.0	19	48.6 ± 5.7	9.3 ± 0.5
31	46.4 ± 9.6	6.6 ± 2.5	41	46.5 ± 4.9	6.6 ± 1.6
52	42.0 ± 8.0	6.5 ± 0.9	88	50.0 ± 18.2	8.7 ± 0.1

TABLE 3

Effect of micellar cholesterol concentration on micellar (*R,R,R*)-α-tocopherol absorption by differentiated Caco-2 TC-7 cell monolayers

The apical side received FBS-free medium containing vitamin-E-rich mixed micelles (70.4 μM) with either no cholesterol, 0.1 mM cholesterol (control), or 0.2 mM cholesterol. The basolateral side received FBS-free medium. Incubation time was 30 min. Data are means ± S.D. of three assays.

Micellar cholesterol concentration	RRR-α-T absorption
mM	*% of control*
0.0	140.3 ± 13.1[a]
0.1	100.0 ± 12.6
0.2	93.3 ± 10.7

[a] Significantly different from the control.

TABLE 4

Effect of (*R,R,R*)-γ-tocopherol and carotenoids on (*R,R,R*)-α-tocopherol absorption by differentiated Caco-2 TC-7 cell monolayers

The apical side received FBS-free medium containing RRR-α-T-rich mixed micelles (40 μM) plus mixed micelles containing either no other microconstituent, RRR-γ-T, or carotenoids. The basolateral side received FBS-free medium. Incubation time was 30 min. Data are means ± S.D. of three assays.

Experimental conditions	RRR-α-T absorption
	% of control
40 μM RRR-α-T + 40 μM RRR-γ-T	59.5 ± 11.9[a]
40 μM RRR-α-T + 6.0 μM lutein	78.9 ± 6.7[a]
40 μM RRR-α-T + 2.8 μM β-carotene	80.6 ± 10.1
40 μM RRR-α-T + 0.4 μM lycopene	97.0 ± 0.7

[a] Significantly different from the control (RRR-α-T-rich micelles plus microconstituent-free mixed micelles).

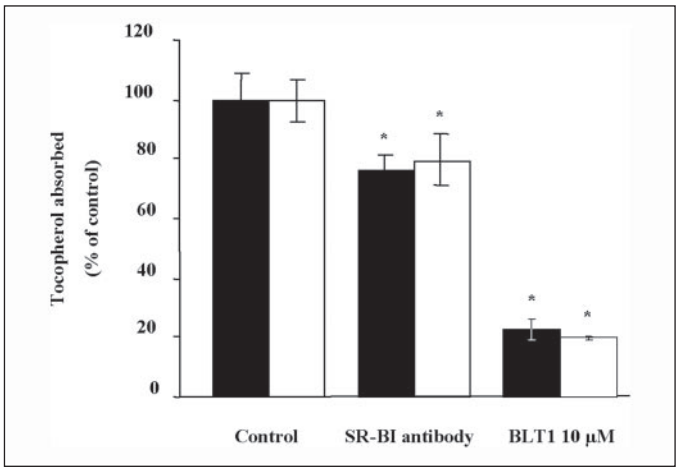

FIGURE 2. **Effect of anti-human SR-BI antibody and BLT1 on RRR-α-T and RRR-γ-T absorption by differentiated Caco-2 TC-7 monolayers.** The apical sides of the cell monolayers were preincubated with either the anti-human SR-BI antibody raised against the external domain at 3.75 μg/ml or BLT1 at 10 μM, and they then received FBS-free medium containing either RRR-α-T (■)- or RRR-γ-T (□)-enriched mixed micelles at 40 μM. The basolateral sides received FBS-free medium. Incubation time was 60 min. Data are means ± S.D. of three assays. *, significant difference from the control (assay performed with control antibody and without BLT1).

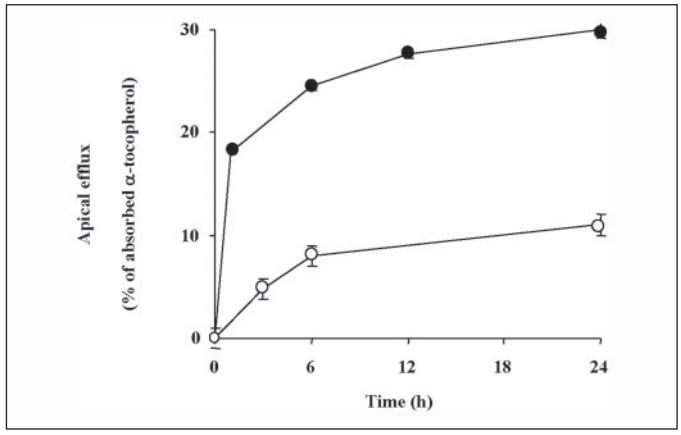

FIGURE 3. **Effect of a physiological acceptor of vitamin E on RRR-α-T apical efflux by differentiated Caco-2 TC-7 cell monolayers.** Cells were enriched with RRR-α-T (see "Materials and Methods"), and the apical side then received either FBS-free medium (○) or FBS-free medium containing tocopherol-free mixed micelles as physiologiocal acceptor (●). The basolateral side received FBS-free medium. Data are means ± S.D. of three assays.

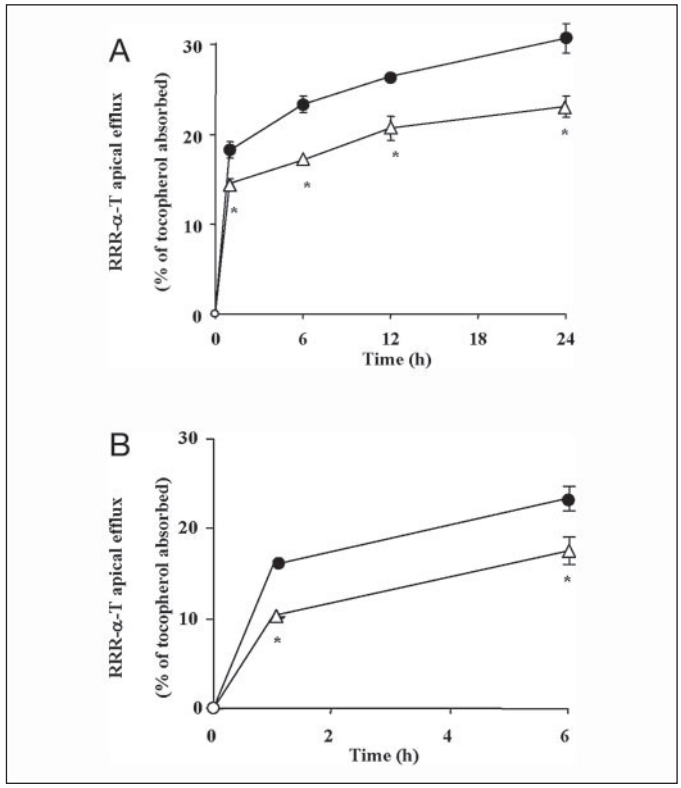

FIGURE 4. **Effect of anti-human SR-BI antibody and BLT1 on RRR-α-T apical efflux by differentiated Caco-2 TC-7 cell monolayers.** Cells were enriched in RRR-α-T, and the apical side then received either FBS-free medium containing tocopherol-free mixed micelles (●) or the same mixture plus anti-human SR-BI antibody (△, *A*) or plus BLT1 (△, *B*). The basolateral side received FBS-free medium. Aliquots of apical medium were taken at different times and replaced by the same volume of new medium. Data are means ± S.D. of three assays. *, significant difference from the control (assay performed without antibody or inhibitor (●).

DISCUSSION

To study in detail the mechanisms involved in vitamin E transport across the enterocyte, we used the frequently employed Caco-2 TC-7 cell model, which gives reproducible figures that correlate closely with human *in vivo* data (22). Our first result confirms that RRR-α-T, RRR-γ-T, and DL-α-T show similar absorption patterns. This is in full agreement with previous studies in which there was no intestinal discrimination between α-tocopherol stereoisomers (23) or between α- and γ-T forms (24). Taken together, the saturable uptake of vitamin E, the temperature dependence, and the direction dependence strongly argue in favor of a protein-mediated uptake (25). However, the possibility cannot be excluded that a fraction of vitamin E was absorbed by passive diffusion because of the 30–50% absorption observed at 4 °C. After having demonstrated that a least a fraction of vitamin E was absorbed through a receptor-mediated process, the question that arises is "what transporter(s) is involved?" The best candidate is SR-BI, as its involvement in vitamin E trafficking has been demonstrated in several tissues (10, 12, 13, 26). The finding that RRR-α-T and RRR-γ-T uptake in Caco-2 cells was inhibited by both anti-human SR-BI antibody and BLT1, which is a

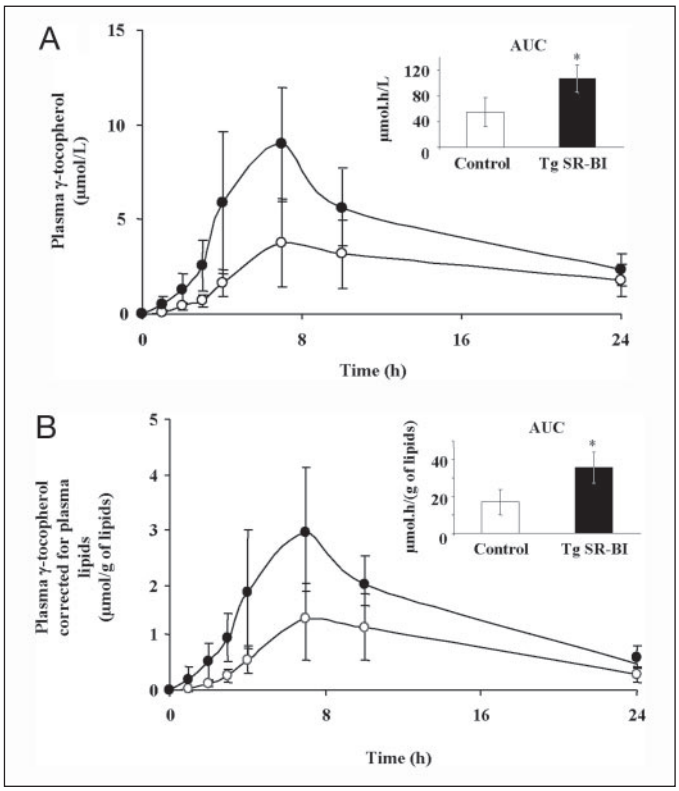

FIGURE 5. **Plasma γ-tocopherol response in wild-type mice and in mice overexpressing SR-BI in the intestine.** Mice were force-fed with an RRR-γ-T-enriched emulsion. Data are means ± S.D., $n = 6$ for control mice (○) and $n = 5$ for SR-BI Tg mice (●). *A*, plasma RRR-γ-T. *B*, plasma RRR-γ-T corrected for plasma lipids (cholesterol plus phospholipids plus triglycerides). *AUC*, area under the curve.

specific chemical inhibitor of SR-BI (27, 28), is taken as initial evidence that SR-BI is involved in tocopherol uptake. The difference in inhibition efficiency between the antibody and the chemical inhibitor can be explained by the fact that the antibody was monoclonal, and consequently its high site specificity may lead to only a partial inhibition of SR-BI. The fact that we did not manage to fully inhibit tocopherol absorption suggests that other means of transport (passive diffusion or via other transporters) are involved. To further examine the involvement of SR-BI in tocopherol absorption, we compared tocopherol bioavailability between wild-type mice and mice overexpressing SR-BI in the intestine. To do this, we used RRR-γ-T instead of RRR-α-T, for three reasons. First, as shown in this study in Caco-2 cells and as reported previously in a clinical study by Traber *et al.* (29), there is no intestinal discrimination between RRR-γ-T and RRR-α-T, making it possible to choose between either of these isomers. Second, mice bred in-house had RRR-γ-T plasma concentrations close to zero, making RRR-γ-T equivalent to a tracer to measure newly absorbed tocopherol. Finally, because hepatic α-TTP discriminates between tocopherol isomers and resecretes more α-T than γ-T in very low density lipoproteins, using γ-tocopherol generates less contamination of plasma tocopherol by tocopherol from hepatic origin and thus provides a better evaluation of tocopherol secreted by the intestine, *i.e.* newly absorbed tocopherol. The results obtained in mice provide further evidence that SR-BI is involved in tocopherol absorption. Having obtained evidence that SR-B1 is involved in the apical uptake of tocopherols, and given that this transporter is involved in both lipid uptake and efflux (30), the obvious question was "is this transporter involved in the apical efflux of tocopherol as well?" The fact that both anti-human SR-BI antibody and BLT1 inhibited tocopherol efflux from enterocytes to the apical side suggests that SR-BI also plays a role in intestinal tocopherol reverse transport.

Given that the main dietary sources of vitamin E are (*R,R,R*)-α- and (*R,R,R*)-γ-tocopherol and that both vitamers are transported through the SR-BI, we raised the question of whether there was competition between these two forms of vitamin E. This is an important issue, as γ-tocopherol has different biological properties than α-tocopherol, and it has been suggested that a high consumption of vitamin E supplements, which contain mostly α-tocopherol, may impair γ-tocopherol bioavailability, which would explain the disappointing results of large scale clinical trials designed to evaluate the effects of vitamin E on cardiovascular diseases (31). The significant inhibition of RRR-γ-T absorption when 40 μM RRR-α-T was added (and vice versa), together with the finding that α-tocopherol concentrations in the human gut range between 100–600 μM following the intake of a 440 mg vitamin E supplement (1), shows that competition between these forms of vitamin E likely occurs *in vivo*. Because SR-BI is also involved in the intestinal uptake of lutein (7) (a carotenoid that, like tocopherol, is an isoprenoid), it is possible that carotenoids may impair tocopherol absorption. We therefore performed competition studies between RRR-α-T or RRR-γ-T and carotenoids. These experiments showed that lutein, but not β-carotene and lycopene, impaired tocopherol absorption. This difference can be explained by the necessarily lower concentration of lycopene and β-carotene able to have been incorporated into micelles (lutein is more soluble in micelles than the other two carotenoids). These results suggest that cholesterol, carotenoids, and vitamin E may compete for transport by SR-BI. This is consistent with *in vivo* studies that have shown that vitamin E decreases canthaxanthin absorption in the rat (32) and that a diet supplementation with 400 IU α-T/day reduces serum concentrations of γ- and δ-T in humans (33). Finally, although the physiopathological consequences of our findings remain unknown, we suggest that inter-individual variations in SR-BI expression or efficiency may explain the highly significant inter-individual variability in tocopherol absorption (34).

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