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Journal of Nutritional Biochemistry 21 (2010) 1207–1213
Journal of Nutritional Biochemistry

Vitamin E decreases endogenous cholesterol synthesis and apo-AI-mediated cholesterol secretion in Caco-2 cells☆

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Received 27 July 2009; received in revised form 17 October 2009; accepted 20 October 2009

Abstract

Intestine is the gateway for newly absorbed tocopherols. This organ also plays a crucial role in cholesterol metabolism. Because tocopherols are known to impact cholesterol metabolism in the liver, we hypothesized that tocopherols could also modulate cholesterol metabolism in the intestine. This study aimed to verify this hypothesis and to unveil the mechanisms involved, using Caco-2 cells as a model of the human intestinal cell.

Both α- and γ-tocopherol significantly ($P<.05$) decreased endogenous cholesterol synthesis and apo-AI-mediated cholesterol secretion in Caco-2 cells. Tocopherols down-regulated ($P<.05$) up to half of the genes involved in the cholesterol synthesis pathway, together with CYP27A1, which is involved in oxysterol production. The activity of this enzyme, as well as the levels of intracellular oxysterols, was significantly diminished by tocopherols. Finally, tocopherols significantly reduced ABCA1 mRNA levels in Caco-2 cells.

We conclude that tocopherols impair the endogenous synthesis and apo-AI-mediated secretion of cholesterol in Caco-2 cells. This effect involves a down-regulation of genes involved in the cholesterol synthesis pathway, resulting in down-regulation of CYP27A1 which, in turn, diminishes oxysterol concentrations. The outcome is a decrease of LXR activity, resulting in down-regulation of ABCA1. These data reinforce the effect of α- and γ-tocopherol on cholesterol metabolism *via* gene expression regulation.

Keywords: Tocopherol; Intestine; Nutrition; Cholesterol; Vitamin; Microarrays

1. Introduction

The small intestine, together with the liver, is a key organ in the metabolism of cholesterol. Indeed, it has been established that this organ is capable of *de novo* cholesterol synthesis [1–6]. Even if the precise contribution of this organ appears species specific and hard to establish, it seems that the intestine contributes significantly to the circulating pool of cholesterol, in a range of 10% in rats to 35% in monkeys [1,2]. More recently, it has been demonstrated that the intestine is responsible for approximately 30% of fasting blood HDL cholesterol [7]. ATP-binding cassette A1 has been linked to HDL metabolism in the intestine [7]. The role of ATP-binding cassette A1 (ABCA1) is well known and has recently been reviewed in detail [8]. Briefly, this membrane transporter, mutated in Tangier disease [9], promotes cholesterol efflux to apo-AI, an apolipoprotein synthesized half in the liver and half in the intestine in humans [10].

Vitamin E is a major fat-soluble antioxidant. This compound is naturally present in eight different forms [(*R*,*R*,*R*)-α, -β, -γ, -δ-tocopherols and tocotrienols], but (*R*,*R*,*R*)-α-tocopherol and (*R*,*R*,*R*)-γ-tocopherol are the two forms mainly found in our diet [11]. Recently, the molecular mechanisms of intestinal uptake of vitamin E have been reevaluated. It has been shown that, contrary to what was previously assumed, vitamin E uptake is not passive and involves, at least in part, the scavenger receptor class B type I (SR-BI), a transporter involved in the uptake of cholesterol [12] at the apical side of the enterocyte [13], and the Niemann-Pick C1-Like1 (NPC1L1),

☆ This work was funded by INRA and INSERM.
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doi:10.1016/j.jnutbio.2009.10.008

which is critical for intestinal cholesterol absorption [14]. Furthermore, two studies have shown that an ABC transporter [15], unambiguously identified as ABCA1 [16], is involved in the basolateral efflux of a fraction of newly absorbed vitamin E in intestinal HDL. These findings confirm that SR-BI, NPC1L1 and ABCA1 are all involved in cholesterol and vitamin E trafficking across enterocytes, suggesting common pathways in the metabolism of these molecules putatively resulting in cross-talk between these two metabolisms. To support this cross-talk assumption, it has been reported that α-tocopherol, in human hepatocytes in culture (HepG2), repressed the cholesterol *de novo* biosynthesis *via* a molecular mechanism involving SREBP2 [17], similarly to the mechanism by which cholesterol regulates its own biosynthesis [18].

Thus, we evaluated the ability of both α- and γ-tocopherols (the two main vitamers present in diet) to modulate cholesterol synthesis and cholesterol efflux in HDL, two major events of cholesterol metabolism that occur in the intestine. To this goal, we used Caco-2 monolayers, a model of human intestinal cells. We showed that both α- and γ-tocopherol diminished endogenous cholesterol synthesis as well as apolipoprotein-AI-(apo-AI)-mediated cholesterol efflux. These effects were the consequence of a tocopherol-mediated down-regulation of several genes implicated in endogenous cholesterol synthesis, as well as the down-regulation of ABCA1 *via* down-regulation of CYP27A1, which affects oxysterol synthesis and LXR activity.

2. Material and methods

2.1. Chemicals

Chemicals were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). 27(*R*)-Hydroxycholesterol [commercial name: 26(*R*)-hydroxycholesterol] came from Steraloids (Newport, RI, USA).

2.2. Preparation of tocopherol-rich micelles

For the delivery of tocopherol to cells, mixed micelles, which have similar lipid composition to those found *in vivo* [19], were prepared as previously described [13,20]. The concentration of tocopherols used in this study corresponds to the physiological concentration of tocopherols present in intestinal lumen [21].

2.3. Cell culture

Caco-2 clone TC-7 cells [22,23] were a gift from Dr. M. Rousset (U178 INSERM, Villejuif, France). Cells were cultured in the presence of DMEM supplemented with 20% heat-inactivated FBS, 1% nonessential amino acids and 1% antibiotics (complete medium). For each experiment, cells were seeded and grown on six-well plates to obtain confluent, differentiated cell monolayers. Twelve hours prior to each experiment, the medium was replaced with complete, serum-free medium. Differentiated cell monolayers were incubated with either 90 μM RRR-α-tocopherol-rich, 90 μM RRR-γ-tocopherol-rich micelles or tocopherol-free micelles (controls) for 24 h before experiments.

2.4. Cholesterol efflux experiment

Cholesterol efflux was assayed according to an established method [24]. Briefly, Caco-2 cells were differentiated for 14 days, then cells were cultured for 24 h in medium containing [^{3}H]cholesterol (3 μCi/ml; PerkinElmer SAS, Courtaboeuf, France) together with cholesterol (50 μM). Tocopherol-rich mixed micelles were incubated for an additional 24 h. Media were removed and replaced for 4 h by serum-free medium containing either 0.1% BSA for the control or 15 μg/ml apo A-I (VWR International, Strasbourg, France). Media were taken out, and cells were washed with PBS and lysed by 0.5N NaOH. Radioactivity in the medium and cell lysate was measured by scintillation counting. Cholesterol efflux was calculated by dividing radioactivity of the medium by total radioactivity in cell lysate plus medium. Cholesterol efflux data in the presence of apo A-I were standardized by efflux in the presence of BSA. Assays were performed in triplicate.

2.5. De novo cholesterol synthesis

De novo cholesterol synthesis was assayed according to an established method [17]. Caco-2 cells were differentiated for 14 days and then incubated for 24 h with mixed micelles enriched or not with tocopherols. Cells were pulsed with 2 μCi of [1-^{14}C] acetic acid (1.702 GBp/mmol, 46.0 mCi/mmol; PerkinElmer SAS) for 6 h, then washed with PBS and scrapped in 1 ml PBS. Lipids were extracted from the cell lysate by incubation with 3 ml of 2:1 methanol/chloroform. The chloroform phase obtained after centrifugation (500×*g*, 10 min, 4°C) was evaporated to dryness under nitrogen, and the dried extract was dissolved in 60 μl of 2:1 chloroform/methanol. Samples were resolved by thin layer chromatography developed with 60:40:1 hexane/diethylether/acetic acid. Lipids were visualized with 0.1% dichlorofluorescein, and radioactive incorporation into cholesterol was quantified by scintillation counting.

2.6. Hybridization arrays and microarray data analysis

RNA quality control was performed on an Agilent 2100 Bioanalyzer (Agilent, Massy, France) with 6000 Nano Chips, according to the manufacturer's instructions. RNA from three independent experiments were pooled per treated group and hybridized to Agilent Whole Human Genome (4×44K). All labeling, hybridization, washing and scanning were performed as described in the manufacturer's protocol. Arrays were scanned with an Agilent Scanner. Data were extracted with Agilent Feature Extraction v. 9.5.3 and analyzed with Agilent GeneSpring GX v. 7.3.1. Pathway analyses were performed with Metacore (http://www.genego.com/metacore.php) and GenMAPP (http://www.genmapp.org/) software.

2.7. RNA Isolation and qPCR

Experiments were performed as previously described [20]. The sequences of primers used for this study are reported in Supplementary Table 1.

2.8. Oxysterol quantification

Oxysterols were analyzed as described elsewhere [25]. Briefly, 1 ml cell homogenate sample was saponified with a mixture of NaOH/ethanol (1:9) for 1 h at 55°C in a shaking water bath after adding 3-acetate-19-hydroxycholesterol (0.3 μg) as an internal standard. Oxysterols were extracted by adding 2 ml of hexane and evaporating the solvent under nitrogen gas. The solute was dissolved by 75 mM potassium phosphate buffer (pH 7.4) containing EDTA (1 mM), DTT (0.5 mM) and $MgCl_2$ (5 mM) with 45% (w/v) HPβCD. The solute was incubated in 200 μl potassium phosphate buffer (pH 7.4) with 3 IU of cholesterol oxidase (cellulomonas species) at 37°C for 30 min. The reaction was terminated by adding a mixture of methanol–chloroform (2:1 v/v, 2 ml). Sterols were extracted with chloroform (2 ml) and water (1.5 ml). The combined extracts were evaporated to dryness under nitrogen and redissolved in 250 μl of acetonitrile. Oxysterols (ketone derivatives) were separated by HPLC (Waters Symmetry, C18, 4.6×250 mm, 5 μm particle size). The mobile phase was acetonitrile/ethanol (98:2). Flow rate was kept at 1 ml/min and absorbency was monitored at 240 nm. Oxysterol peaks were identified by their retention times compared with those of known standards and quantified by comparison with samples containing known quantities of each of the analyzed oxysterols, namely, 25-hydroxycholesterol and 27-hydroxycholesterol.

2.9. CYP27A1 Activity

Sterol 27-hydroxylase (CYP27A1) in the Caco-2 cell homogenate was assayed with a radioisotopic method that used [4-^{14}C]cholesterol solubilized in hydroxypropyl-β-cyclodextrin [26].

2.10. Statistical analysis

Results are expressed as means±S.E. For mRNA expression levels, differences were tested using the Student's *t* test. For other experiments, differences were tested by ANOVA. The Tukey–Kramer test was used as a *post hoc* test when ANOVA showed significant differences between groups. *P* values <.05 were considered significant. All statistical analyses were performed using Stat View software 5.0 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Tocopherols reduce apo-AI-mediated efflux of cholesterol, as well as endogenous cholesterol synthesis

The impact of tocopherols (both α- and γ-tocopherol) on apo-AI-mediated efflux of cholesterol at the basolateral side of enterocytes was studied by using Caco-2 cell monolayers cultivated on filters. A significant ($P<.05$) decrease of around 20% of cholesterol efflux was observed when mixed micelles containing dietary concentrations of either α- or γ-tocopherol (between 100 and 600 μM in intestinal lumen [21]) were added to the apical side of the cells for 24 h (Fig. 1A). No impact on cholesterol uptake was observed under tocopherol effect (data not shown). *De novo*

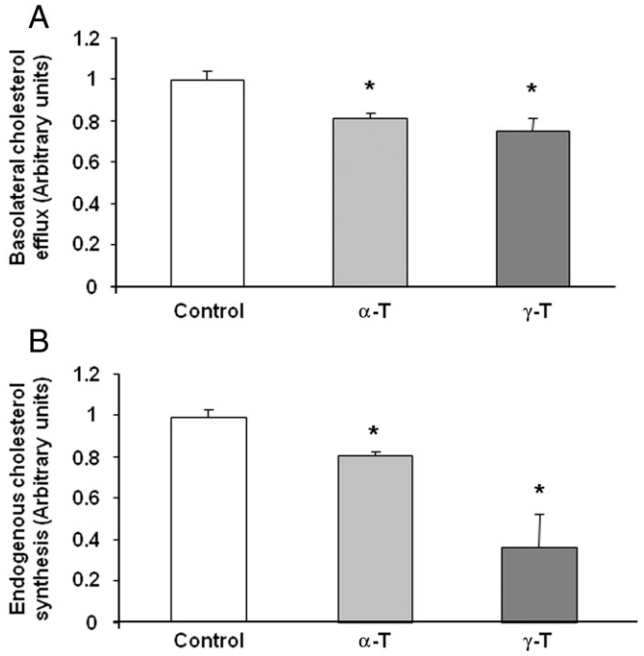

Fig. 1. α- and γ-Tocopherol-induced apo-AI-mediated basolateral cholesterol efflux reduces endogenous cholesterol synthesis in Caco-2. (A) Differentiated Caco-2 monolayers were cultured on a filter for 24 h in apical medium containing [^{3}H] cholesterol together with nonlabeled cholesterol (50 μM). Cells were then incubated (apical side) for an additional 24 h with tocopherol-rich mixed micelles [90 μM α-tocopherol-rich micelles (α-T) or 90 μM γ-tocopherol-rich micelles (γ-T)] or tocopherol-free micelles (control). After washing, cells were lysed, then radioactivity in the basolateral medium and cell lysate was measured by scintillation counting. Data represent means±S.E. of three assays. An asterisk indicates a significant difference (P<.05) when compared to the control. (B) Differentiated Caco-2 monolayers incubated for 24 h with either 90 μM α-tocopherol-rich micelles (α-T), 90 μM γ-tocopherol-rich micelles (γ-T) or tocopherol-free micelles (control). Cells were then pulsed with [1-^{14}C]acetic acid. Lipids were extracted from the cell lysate and resolved by thin layer chromatography. Radioactive incorporation into cholesterol was quantified by scintillation counting. Data represent means±S.E. An asterisk indicates a significant difference (P<.05) when compared to the control.

cholesterol synthesis experiments were undertaken. These experiments clearly showed that both tocopherols significantly (P<.05) decreased *de novo* cholesterol synthesis in Caco-2 cells: 16% and 63% for α- and γ-tocopherol, respectively (Fig. 1B).

3.2. Tocopherols diminish transcription of a significant portion of genes involved in the cholesterol synthesis pathway

Microarray experiments were performed to elucidate the inhibition of *de novo* cholesterol synthesis by tocopherols observed above. Pathway analysis, performed with Metacore and GenMapp software, revealed that a large proportion of genes involved in the *de novo* cholesterol synthesis pathway were strongly and significantly (P<.05 in Metacore analysis) inhibited by tocopherols (Supplementary Table 2). More precisely, α-tocopherol down-regulated approximately one-third of the genes; γ-tocopherol affected more than half of the genes. All genes regulated by α-tocopherol and γ-tocopherol are reported in Tables 1 and 2, respectively. Of note, because the HMG-CoA-reductase (HMGCR) log ratio value was lost, probably during the filtering step of microarray data analysis, qPCR analysis was performed to estimate the mRNA level of this essential enzyme in the cholesterol synthesis pathway. In agreement with data related to other genes of the

Table 1
Regulation of genes involved in cholesterol synthesis pathway by α-tocopherol

Gene	Accession number	Log ratio (microarray)	Fold change (qPCR)
HMGCR	NM_000859	–	0.90
MVK	NM_000431	0.475	0.73
FDPS	NM_002004	0.447	–
FDFT1	NM_004462	0.494	–
SQLE	NM_003129	0.500	0.74
LSS	NM_002340	0.386	–
EBP	NM_006579	0.397	–
SC4MOL	NM_006745	0.500	–
TM7SF2	NM_003273	0.419	–

–: Not determined.

Table 2
Regulation of genes involved in cholesterol synthesis pathway by γ-tocopherol

Gene	Accession number	Log ratio (microarray)	Fold change (qPCR)
HMGCS1	NM_002130	0.489	–
HMGCR	NM_000859	–	0.71
MVK	NM_000431	0.419	0.37
MVD	NM_002461	0.478	–
FDPS	NM_002004	0.350	–
IDI1	NM_004508	0.410	–
FDFT1	NM_004462	0.418	–
SQLE	NM_003129	0.426	0.43
LSS	NM_002340	0.358	–
CYP51A1	NM_000786	0.477	–
EBP	NM_006579	0.286	–
SC4MOL	NM_006745	0.368	–
TM7SF2	NM_003273	0.411	–

–: Not determined.

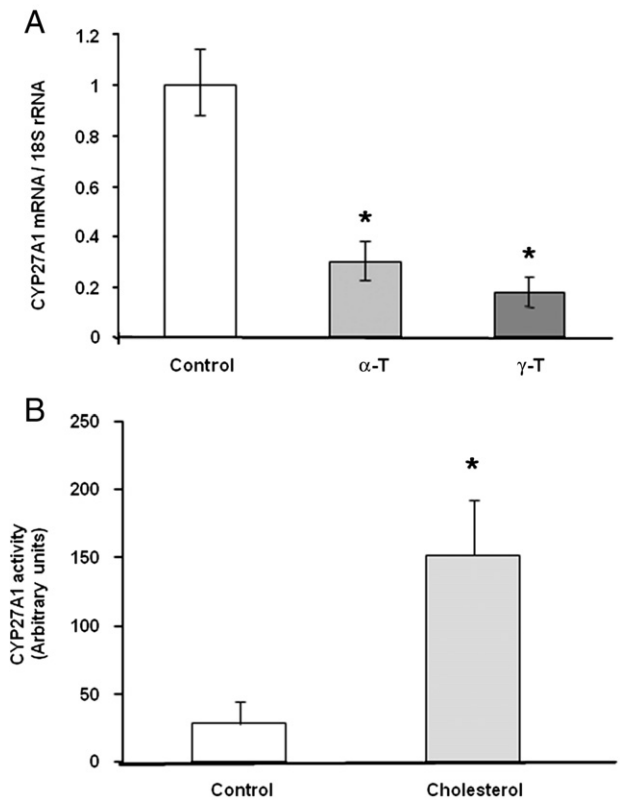

Fig. 2. CYP27A1 expression and activity are modulated by tocopherols and cholesterol, respectively. (A) Differentiated Caco-2 monolayers received either 90 μM α-tocopherol-rich micelles (α-T), 90 μM γ-tocopherol-rich micelles (γ-T) or tocopherol-free micelles (control) for 24 h. Cells were then scraped, mRNA was extracted and CYP27A1 mRNA levels were analyzed by qPCR. (B) Differentiated Caco-2 monolayers were incubated for 24 h with cholesterol (10 mg/ml). Cells were scrapped and CYP27A1 activity was measured using a radioisotopic method. Data represent means±S.E. An asterisk indicates a significant difference (P<.05) when compared to the control.

Table 3
Oxysterol quantification following tocopherol treatment

Treatment	25-Hydroxycholesterol	27-Hydroxycholesterol
Control	10.5±0.4	4.2±0.4
α-Tocopherol	7.4±0.2 *	nd
γ-Tocopherol	5.3±0.3 *	nd

Quantification of oxysterols (25-hydroxycholesterol and 27-hydroxycholesterol) was performed using HPLC as described in Material and Methods. Values are expressed in micrograms per milligrams of protein. nd: Not detected (below the limit of quantification).
* $P<.05$.

pathway, HMGCR mRNA levels were significantly decreased by tocopherol treatment (Tables 1 and 2). To validate the microarray data, qPCR was used on two genes significantly affected by tocopherol treatment in microarray experiments [mevalonate kinase (MVK) and squalene epoxidase (SQLE)]. This validation was successful (Tables 1 and 2), strongly supporting our microarray results.

3.3. Tocopherols decrease intracellular oxysterol concentrations via a CYP27A1 down-regulation that is related to a decrease in cholesterol synthesis

Microarray experiments showed an interesting decrease in CYP27A1 (the enzyme involved in the intestinal production of oxysterols; other enzymes including CYP11A1 that mediate the synthesis of 22-hydroxycholesterol are not modified; data not shown) mRNA when Caco-2 cells were incubated with tocopherols (data not shown). This result was confirmed by qPCR, where the mRNA level of this enzyme was strongly inhibited (around 70%) (Fig. 2A), and by quantification of CYP27A1 activity, which was inhibited in the presence of α-tocopherol (data not shown). The consequence of this regulation in terms of oxysterol concentration, mainly 25-hydroxycholesterol and 27-hydroxycholesterol [27,28], within cells was evaluated by HPLC. We observed, as reported in Table 3, a decrease in the quantity of 25-hydroxycholesterol when Caco-2 cells were incubated with tocopherols, while 27-hydrocholesterol was no longer detectable after tocopherol treatment.

To find a putative link between the modulation of CYP27A1 expression and activity and the cholesterol content of the cells, Caco-2 cells were incubated in the presence of cholesterol and CYP27A1 activity was determined. Interestingly, we found that the activity of CYP27A1 was significantly ($P<.05$) induced (a factor 5.4) by cholesterol (Fig. 2B). Thus, in Caco-2 cells treated with tocopherols, where cholesterol synthesis is decreased, it was highly consistent to find a decrease in CYP27A1 expression and activity.

3.4. ABCA1 is down-regulated by vitamin E via CYP27A1, oxysterols and LXR in Caco-2 cells

Because the apo-AI-dependent efflux of cholesterol is mediated by ABCA1 [29,30], we hypothesized that the inhibition of cholesterol efflux by tocopherols was due to an effect of these molecules on ABCA1 expression. To verify this hypothesis, Caco-2 cells were incubated with α- or γ-tocopherol as shown in Fig. 3A, both vitamers significantly ($P<.05$) reduced ABCA1 mRNA levels (Fig. 3A).

α- and γ-Tocopherol are well-known antioxidants. As such, we investigated whether the down-regulation of ABCA1 was due to the

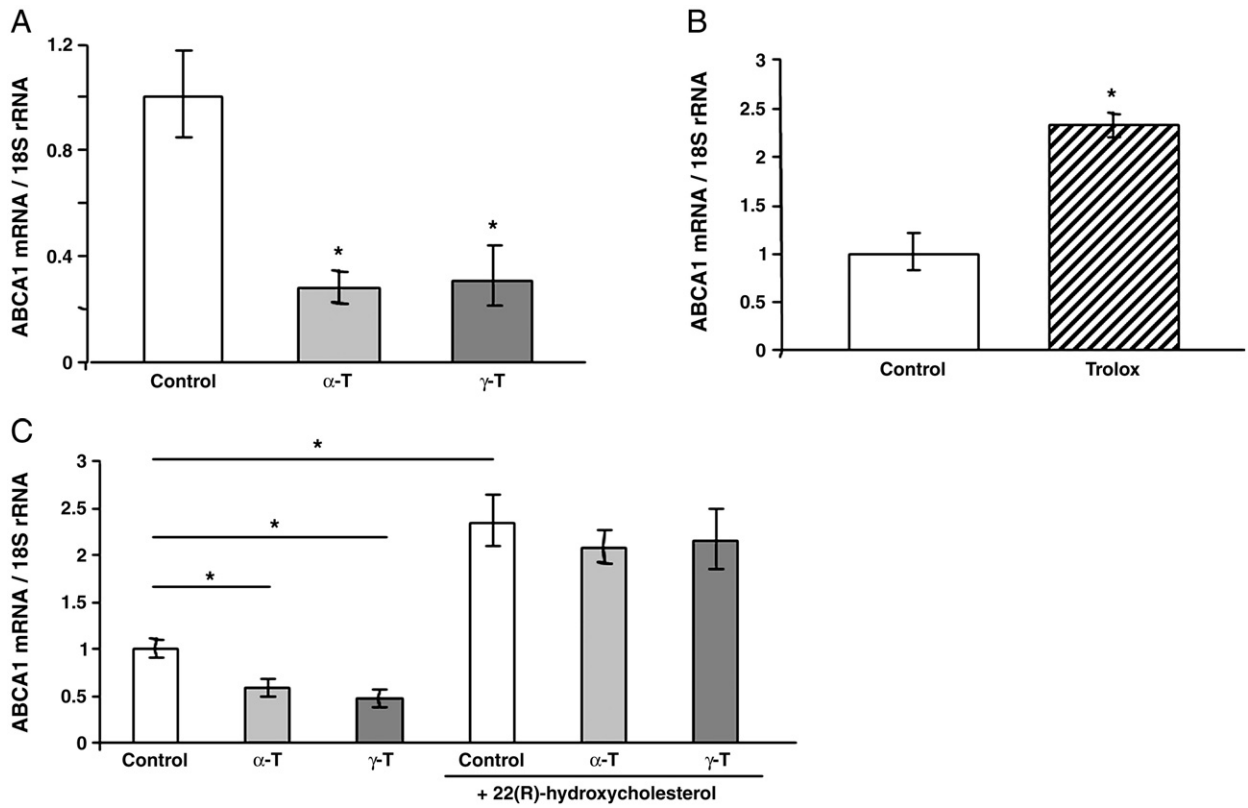

Fig. 3. ABCA1 mRNA levels are decreased by tocopherols. (A) Differentiated Caco-2 monolayers received either 90 μM α-tocopherol-rich micelles (α-T), 90 μM γ-tocopherol-rich micelles (γ-T) or tocopherol-free micelles (control) for 24 h. (B) Differentiated Caco-2 monolayers received either 90 μM trolox together with tocopherol-free micelles (trolox) or tocopherol-free micelles (control) for 24 h. (C) Differentiated Caco-2 monolayers received either 90 μM α-tocopherol-rich micelles (α-T), 90 μM γ-tocopherol-rich micelles (γ-T) or tocopherol-free micelles (control) for 24 h in the presence or absence of 22(*R*)-hydroxycholesterol (25 μM). In all experiments, ABCA1 mRNA levels were analyzed by qPCR. Data represent means±S.E. An asterisk indicates a significant difference ($P<.05$) when compared to the control.

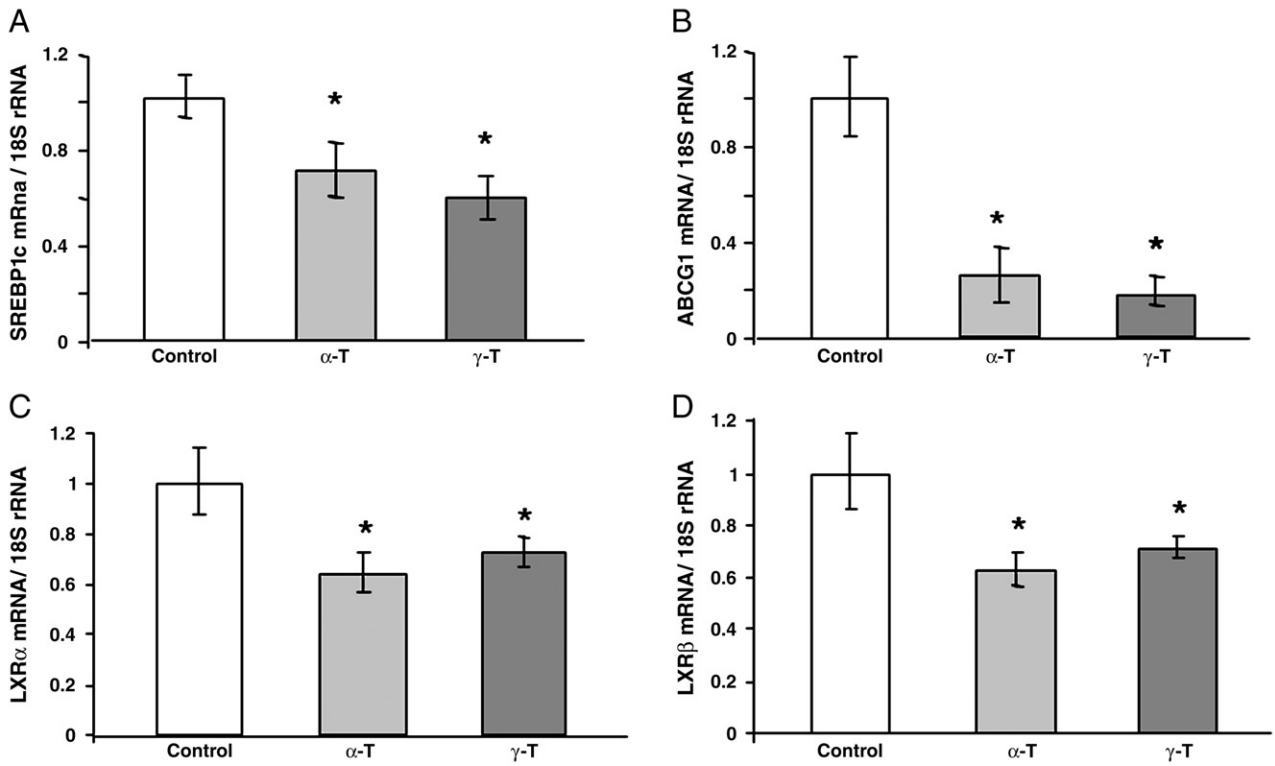

Fig. 4. LXR target gene mRNA as well as LXRα and LXRβ mRNA levels is repressed by α-tocopherol and γ-tocopherol. Differentiated Caco-2 monolayers received either 90 μM α-tocopherol-rich micelles (α-T), 90 μM γ-tocopherol-rich micelles (γ-T) or tocopherol-free micelles (control) for 24 h. Cells were then scraped, mRNA was extracted and mRNA levels of SREBP1C (A), ABCG1 (B), LXRα (C) and LXRβ (D) were analyzed by qPCR. Data represent means±S.E. An asterisk indicates a significant difference ($P<.05$) when compared to the control.

antioxidant property of the tocopherols. Caco-2 cells were incubated with trolox, a commonly used antioxidant. Interestingly, this antioxidant did not diminish ABCA1 expression; on the contrary, it significantly increased it by 230% (Fig. 3B).

Because intracellular oxysterol concentrations were reduced in tocopherol-treated Caco-2 cells (Table 3), and because ABCA1 is a well-known target gene of the oxysterol-activated LXR nuclear receptors [31], we decided to study the putative involvement of LXRs in this regulation. We co-incubated Caco-2 with tocopherols and 22(*R*)-hydroxycholesterol, a LXR agonist [32]. This co-incubation led to an attenuation of the tocopherol-induced decrease of ABCA1 mRNA levels (Fig. 3C). Note that the positive control, 22(*R*)-hydroxycholesterol alone, demonstrated an induction of ABCA1, as expected (Fig. 3C). These findings, together with the observed decrease of oxysterol concentrations, suggest that under tocopherol treatment, LXRs are deactivated by a lack of ligand, leading to a down-regulation of LXR target genes, including ABCA1. In agreement with this hypothesis, tocopherols significantly down-regulated two other LXR target genes: SREBP1c and ABCG1 (Fig. 4A and B). Finally, LXRα and LXRβ mRNA levels were also down-regulated by tocopherols, which may also participate in the down-regulation of ABCA1 (Fig. 4C and D).

4. Discussion

The data obtained herein show for the first time that tocopherols (both α- and γ-tocopherols) have an impact on intestinal cholesterol metabolism. Indeed, the inhibitory effect of tocopherols on the *de novo* synthesis of cholesterol within human intestinal cells, which are the gateway for newly absorbed tocopherols, has never been reported. However, this effect is in full agreement with recent results reported in HepG2 cells [17] and in adrenal glands [33]. The molecular mechanism behind this effect has not been elucidated, but Valastyan et al. [17] have suggested that SREBP2 is probably involved. Indeed, they observed an attenuation of the proteolytic cleavage of SREBP2 by α-tocopherol in HepG2 and CHO cells, which can lead to a less active concentration of SREBP2 in the nucleus. However, no data were reported for γ-tocopherol. Interestingly, we report here that both α- and γ-tocopherol could share common nonantioxidant properties, based on an effect on SREBP activation. Indeed, because all the genes down-regulated by tocopherols in our study are already known as SREBP2 target genes [18], we suggest that tocopherols interfere with

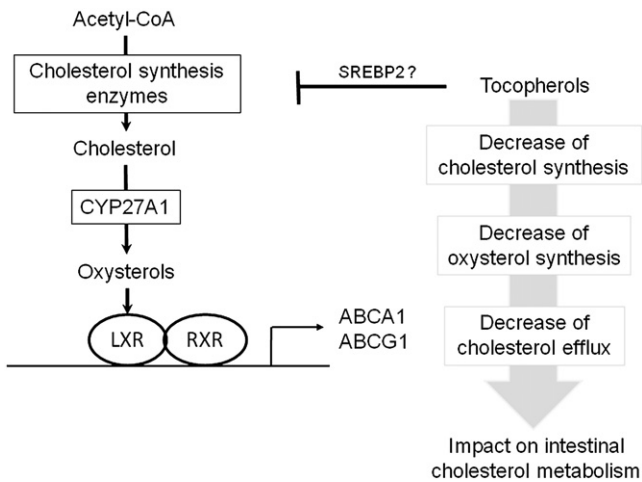

Fig. 5. Scheme of the proposed regulatory mechanism. The inhibitory effect of tocopherols on *de novo* cholesterol synthesis within the enterocytes is probably due to a SREBP2-mediated down-regulation of several genes implicated in the cholesterol synthesis pathway. The inhibitory effect of tocopherols on the apo-AI-mediated cholesterol is likely explained by the down-regulation of CYP27A1, which decreases intracellular oxysterols and, in turn, down-regulates several LXR target genes, including ABCA1.

the proteolytic cleavage of SREBP2 and consequently also down-regulate SREBP2-dependent genes in enterocytes. This hypothesis requires further experimentation to be definitively validated.

The observed inhibition of endogenous cholesterol synthesis by tocopherols could, by itself, explain the inhibition of apo-AI-stimulated cholesterol secretion. Indeed, the tocopherol-induced decrease of cholesterol synthesis results in a down-regulation of CYP27A1. This mechanism involves a cascade of events, starting with an attenuation of oxysterols, the products of CYP27A1 activity [27,28,34]. A decrease in oxysterols leads to a reduction of LXR transactivation, which in turn leads to a decrease in ABCA1 expression, a gene under the control of LXR [31]. This would eventually lead to a decrease in ABCA1-mediated efflux of cholesterol. The first event of this cascade was the most difficult to explain. Indeed, how might tocopherols affect CYP27A1 expression? Because tocopherols inhibited endogenous cholesterol synthesis, we hypothesized that intracellular cholesterol concentrations might regulate CYP27A1 expression. The experiment employed to verify this hypothesis showed that adding cholesterol to Caco-2 cells significantly increased CYP27A1 activity, validating our hypothesis. Such a regulation of CYP27A1 has already been observed in Caco-2 cells [27]; however, the mechanism behind the regulation remains to be determined.

The cholesterol-mediated down-regulation of CYP27A1 expression by tocopherols has led us to verify its physiological consequences in terms of CYP27A1 activity and endogenous oxysterol concentrations. Because we found that both CYP27A1 activity and oxysterol concentrations were decreased by tocopherols, we then hypothesized that the oxysterol-dependent LXR transcription factor activity could be modulated by tocopherols. Of note, the indirect effect of tocopherols on LXR activity can also be mediated by cholesterol intermediaries, the concentration of which probably decreases within the cells as a consequence of cholesterol synthesis inhibition. Indeed, several cholesterol intermediaries are LXR ligands [35].

The experiments conducted to study the direct effect of tocopherols on LXR expression, in addition to the above-described effect of tocopherols on LXR activity, showed that both LXRα and LXRβ were down-regulated by tocopherol treatment. If the LXRα down-regulation can be explained by its autoregulation [36], the regulation of LXRβ remains more elusive. However, the down-regulation of LXRs by tocopherols, together with the decrease of oxysterols, has led us to hypothesize that genes regulated by this nuclear receptor, including ABCA1, were affected by tocopherol treatments. The experiments undertaken to verify this hypothesis were conclusive. Indeed, they showed a down-regulation of ABCA1, ABCG1 and SREBP1c by tocopherols. We thus conclude that α- and γ-tocopherol can diminish cholesterol secretion by a mechanism which involves a down-regulation of ABCA1 *via* LXR. This mechanism is supported by the results of a study which have shown that LXR/RXR activation enhances the basolateral cholesterol efflux in Caco-2 cells [37]. Moreover, even if the role of ABCG1 in intestinal cholesterol efflux has never been proven, it is probable that the decrease of this transporter results in the decrease of cholesterol efflux. Indeed, it is known that ABCG1 promotes macrophage cholesterol efflux to HDL [38], and the same function of ABCG1 has been proposed in the intestine [24].

Several studies have attempted to understand the regulation of ABCA1 and/or ABCG1 in monocytes by redox balance [39,40], antioxidants [41] or tocopherol [42], but this is the first time that the effect of tocopherols on ABCA1 and ABCG1 expression has been studied in enterocytes. Interestingly, experiments using statins, known to increase the SREBP2 cleavage process, resulted in an increase of oxysterols and ABCA1, together with an increase of cholesterol efflux from macrophages [43,44]. These results fit perfectly with our proposed model of regulation.

In conclusion, the results obtained support a significant effect of α- and γ-tocopherol on intestinal cholesterol metabolism. The underlying mechanisms have been partially elucidated and involve an inhibitory effect of tocopherols on the *de novo* cholesterol synthesis within the enterocytes which is due to a down-regulation of several genes implicated in the cholesterol synthesis pathway. A consequent inhibitory effect of tocopherols on the cholesterol apo-AI-mediated efflux was observed, which involves oxysterols, CYP27A1, LXR and ABCA1. A scheme, based on our data and on the data available in the literature, has been drawn to show the cascade of events (Fig. 5). Such mechanism could explain the inhibitory effect of tocopherols on blood HDL-cholesterol previously observed in human studies [45–47]. Moreover, in physiopathological state, such as insulin resistance and diabetes, where the intestinal cholesterol synthesis is increased [48], the effect of tocopherols could limit, at least in part, cholesterol overproduction. This data reinforces the hypothesis that vitamin E strongly impacts cholesterol metabolism in the intestine.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2009.10.008.

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