Apple procyanidins decrease cholesterol esterification and lipoprotein secretion in Caco-2/TC7 enterocytes

Romain Vidal,* Sandra Hernandez-Vallejo,* Thomas Pauquai,* Odile Texier,† Monique Rousset,* Jean Chambaz,* Sylvie Demignot,* and Jean-Marc Lacorte[1],*

Unité Mixte de Recherche 505,* Institut National de la Santé et de la Recherche Médicale-Université Pierre et Marie Curie/Ecole Pratique des Hautes Etudes, 75006 Paris, France; and Laboratoire de Pharmacognosie,† Faculté de Pharmacie, 63001 Clermont-Ferrand, France

Abstract Decrease of plasma lipid levels by polyphenols was linked to impairment of hepatic lipoprotein secretion. However, the intestine is the first epithelium that faces dietary compounds, and it contributes to lipid homeostasis by secreting triglyceride-rich lipoproteins during the postprandial state. The purpose of this study was to examine the effect of apple and wine polyphenol extracts on lipoprotein synthesis and secretion in human Caco-2/TC7 enterocytes apically supplied with complex lipid micelles. Our results clearly demonstrate that apple, but not wine, polyphenol extract dose-dependently decreases the esterification of cholesterol and the enterocyte secretion of lipoproteins. Apple polyphenols decrease apolipoprotein B (apoB) secretion by inhibiting apoB synthesis without increasing the degradation of the newly synthesized protein. Under our conditions, cholesterol uptake, apoB mRNA, and microsomal triglyceride protein activity were not modified by apple polyphenols. The main monomers present in our mixture did not interfere with the intestinal lipid metabolism. By contrast, apple procyanidins reproduced the inhibition of both cholesteryl ester synthesis and lipoprotein secretion. Overall, our results are compatible with a mechanism of action of polyphenols resulting in impaired lipid availability that could induce the inhibition of intestinal lipoprotein secretion and contribute to the hypolipidemic effect of these compounds in vivo.—Vidal, R., S. Hernandez-Vallejo, T. Pauquai, O. Texier, M. Rousset, J. Chambaz, S. Demignot, and J-M. Lacorte. **Apple procyanidins decrease cholesterol esterification and lipoprotein secretion in Caco-2/TC7 enterocytes. *J. Lipid Res.* 2005.** 46: **258–268.**

Supplementary key words polyphenol • intestine • dietary lipids • apolipoprotein B

Fruit and vegetable consumption is consistently associated with a decrease in lipemia along with a decrease in the risk for cardiovascular diseases and stroke, diabetes, and obesity (1–3). Such effects are attributed to dietary fibers and phenolic compounds. Polyphenols, which are abundant in fruits, vegetables, and beverages such as tea and red wine, are very heterogenous, and their main classes are defined from the nature of their carbon skeletons (4). Flavonoids, the most abundant polyphenols in our diet, can be divided into several classes: flavones, flavonols, isoflavones, anthocyanins, flavanols, procyanidins, and flavanones. The protective effect of flavonoids against cardiovascular diseases has been attributed to several mechanisms, including inhibition of LDL oxidation (5) and of platelet aggregation (6) and improved endothelial function (7). More recently, it has been shown that flavonoids could interfere with the synthesis and secretion of triglyceride-rich lipoproteins (TRLs) in hepatocytes (8–10).

In addition to hepatocytes, enterocytes are able to synthesize intestine-specific TRLs, namely chylomicrons, the secretion of which ensures the delivery of dietary lipids to the body. TRLs are composed of a core of neutral lipids, mostly triglyceride (TG) but also cholesteryl esters, surrounded by a monolayer of amphipathic lipids such as phospholipids and cholesterol. Apolipoprotein B (apoB) plays a major structural role in the assembly of TRLs and is required for their secretion. In humans, hepatic TRLs (i.e., VLDLs) contain an apoB-100 isoform, whereas intestinal TRLs (i.e., chylomicrons) contain an apoB-48 isoform, resulting from apoB mRNA editing. TRL assembly has been mostly characterized in hepatocytes as a two-step process: the formation of a lipid-poor apoB particle, followed by its fusion with a TG-rich apoB-free lipid droplet under the action of the microsomal triglyceride protein (MTP) (for review, see 11, 12). However, major

Manuscript received 2 June 2004 and in revised form 29 October 2004 and in re-revised form 17 November 2004.

Published, JLR Papers in Press, December 1, 2004.
DOI 10.1194/jlr.M400209-JLR200

Abbreviations: apoB, apolipoprotein B; Cys/Met, cysteine/methionine mix; TG, triglyceride; TRL, triglyceride-rich lipoprotein; XTT, sodium 3′-[(1-phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate.

[1] To whom correspondence should be addressed.
e-mail: jean-marc.lacorte-u505@bhdc.jussieu.fr

differences in TRL formation exist between hepatocytes and enterocytes, including the mode of fatty acid supply. In hepatocytes, fatty acids (FA) are brought by plasma to the basolateral pole of the cell. In enterocytes, dietary lipids, after their emulsion by bile and their subsequent digestion by pancreatic enzymes, are provided at the apical pole of enterocytes as complex lipid micelles made of FAs, monoacylglycerol, lysophosphatidylcholine, cholesterol, and biliary salts (13). After absorption, FA and monoacylglycerol are used for intracellular de novo synthesis of TG. Concomitantly, absorbed cholesterol is esterified by ACAT, but its requirement for chylomicron assembly and secretion is still debated (14).

Although postprandial lipemia is a physiological phenomenon that occurs several times a day after each ingestion of dietary fat, clinical data show a correlation between postprandial lipemia and the progression of coronary artery disease (15). Indeed, exaggerated postprandial hyperlipidemia, resulting from an excess of dietary sources or an ineffective clearance of TRLs, leads to an accumulation of TRL remnants in plasma that may have adverse effects on vascular endothelium (16). In this context, it is obvious that modulation of intestinal lipoprotein secretion should play an important role in the control of cardiovascular disease risk factors.

Apples and wine having been demonstrated to reduce plasma lipid levels and atherosclerosis progression (17–19), the objective of our study was to examine the potential role of polyphenols on the synthesis and secretion of intestinal lipoproteins using human enterocyte Caco-2/TC7 cells (20). To get closer to physiology in analyzing intestinal lipid metabolism, Caco-2/TC7 cells were cultured on microporous filters that delineate the apical and basal compartments. Dietary lipids were supplied in the apical compartment as complex micelles (21) mimicking the postprandial duodenal content after a fat-rich meal (13).

Our results clearly demonstrate that a procyanidin-enriched fraction isolated from apple polyphenol extract decreases the esterification of cholesterol and impairs intestinal lipoprotein secretion. Such results may account for the protective effect of polyphenols against postprandial hypertriglyceridemia observed in vivo.

MATERIALS AND METHODS

Materials

Unless indicated otherwise, all chemicals were purchased from Sigma (Saint-Quentin-Fallavier, France). Applephenon C100, an apple (*Pyrus malus*) polyphenol extract, was from Jan Dekker France (St. Germain en Laye, France). Wine polyphenols were supplied by Vin et Santé (Onivins, Paris). Chlorogenic acid, phloridzin, (−)-epicatechin, and (+)-catechin were from Extrasynthese (Genay, France). 1-^{14}C-labeled oleic acid and [1,2-^{3}H(N)] cholesterol were purchased from ICN (Orsay, France), and [^{35}S]cysteine/methionine mix (Cys/Met) was from Perkin-Elmer (Courtaboeuf, France). The rabbit anti-human apoB antibodies were generously provided by Dr A. Mazur (Institut National de la Recherche Agronomique, Theix, France), and the peroxidase-conjugated goat anti-rabbit antibody was purchased from Vector Laboratories (Burlingame, CA). CP-113818 was generously provided by Dr. O. Francone (Pfizer, Groton, USA) and N. Fournier (Faculté de Pharmacie, Chatenay-Malabry, France).

Cell culture

Caco-2/TC7 cells (20) were routinely cultured in DMEM (25 mM glucose and Glutamax) from Gibco-BRL (Life Technologies, Cergy Pontoise, France) supplemented with 20% heat-inactivated fetal calf serum (Abcys, France), 1% nonessential amino acids (Gibco-BRL), penicillin (100 IU/ml), and streptomycin (10 μg/ml; Gibco-BRL) and maintained under a 10% CO_2 atmosphere at 37°C. Cells were used between passages 13 and 34. For experiments, cells were seeded at a density of 50×10^3 cells/cm^2 on microporous (1 μm pore size) polyethylene terephthalate (PET) membranes (Falcon, Becton Dickinson, Meylan, France). The medium was changed in both compartments 2 days after seeding and daily thereafter. Transepithelial electrical resistance was regularly measured using a Millicell-ERS apparatus (Millipore, St. Quentin en Yvelines, France) to assess confluence, which is usually reached 1 week after seeding. After confluence, cells were switched to asymmetric conditions with a serum-free medium in the upper compartment for 1 week more. Polyphenols were dissolved in ethanol and supplied on the apical side of the cell culture such that the maximum final concentration of ethanol was 1%. The number of surviving cells was measured by the sodium 3′-[(1-phenylaminocarbonyl)-3,4-tetrazolium]bis(4-methoxy-6-nitro) benzene sulfonic acid hydrate (XTT) assay (Cell Proliferation Kit II; Roche Biochemicals, Meylan, France) after a 24 h incubation period.

Micelle preparation and incubations

Lipids were brought to the cells as a complex lipid emulsion containing 0.6 mM oleic acid, 0.2 mM L-α-lysophosphatidylcholine, 0.05 mM cholesterol, 0.2 mM 2-mono-oleoylglycerol, and 2 mM taurocholic acid. Preparation of micelles was done as previously described (21). Where appropriate, micelles were supplemented with 2 μCi of [1-^{14}C]oleic acid (56 mCi/mmol; ICN) per milliliter final volume of medium and, according to the experiment, 2 μCi of [1,2-^{3}H(N)]cholesterol (35–50 mCi/mmol) per milliliter final volume of medium. Freshly prepared micelle-containing medium (1.5 ml) was placed in the upper compartment.

Analysis of lipids

Lipid extraction from media (1 ml) or sonicated cell lysates (0.1 ml adjusted to 1 ml with serum-free medium) was carried out as previously described (21) except that counting was performed in a Tri-Carb scintillation counter (Packard, Rungis, France).

Western blotting

Immediately after collection, cell culture medium (200 μl) was mixed with 20 μl of 10× PBS containing 10% Triton X-100, 50 mM EDTA, and 2 μl of a protease inhibitor cocktail (P8340; Sigma), then stored at −20°C until analysis. Culture medium (20 μl) was boiled for 4 min in Laemmli buffer and fractionated under reducing conditions on a 6% polyacrylamide gel. Proteins were transferred onto Bio-Rad nitrocellulose membranes for 2 h at 50 V using a mini-Trans-Blot Cell (Bio-Rad) in 25 mM Tris and 192 mM glycine. The membrane was soaked overnight at 4°C in TBS-T (20 mM Tris-HCl, pH 7.6, 137 mM NaCl, and 0.1% Tween 20) containing 10% nonfat dry milk. Blots were probed for 1 h at room temperature using rabbit polyclonal anti-apoB antibodies (1:10,000) and then peroxidase-conjugated goat anti-rabbit immunoglobulins and developed using ECL Western blotting reagents according to the manufacturer's instructions (Amersham-Pharmacia, Orsay, France). Films were scanned and quantified using Scion Image software.

Separation of lipoproteins

After incubation for 30 min in a Cys/Met-free medium (Gibco-BRL), cells were incubated during 24 h in a Cys/Met-free medium supplemented with 150 μCi/ml [^{35}S]Cys/Met in the apical compartment in the presence or absence of lipid micelles and of apple polyphenols. After collection, basolateral media were immediately adjusted to 0.005% gentamicin, 1 mM EDTA, 0.04% sodium azide, and 0.02% sodium(ethylmercurithio)-2 benzoate. Media (4 ml) were layered under 4 ml of 0.15 M NaCl and centrifuged at 10,000 *g* for 30 min at 20°C. The 1.5 ml top fraction, containing chylomicrons, was recovered and washed once by ultracentrifugation at 100,000 *g* for 18 h in a new tube containing 4 ml of culture medium containing 20% fetal calf serum and adjusted to 8 ml with 0.15 M NaCl. The chylomicron-depleted basolateral medium was adjusted to 8 ml with 0.15 M NaCl, and sequential ultracentrifugation was performed, as described previously (22), to isolate lipoproteins that float at the density range of the major human lipoprotein classes (VLDL, LDL, and HDL; i.e., 100,000 *g* and 10°C for 18 h at d = 1.006, for 20 h at d = 1.063, and for 40 h at d = 1.21, respectively). [^{35}S]Cys/Met-labeled fractions of d > 1.006 were dialyzed against PBS, and then all fractions were concentrated using YM10 centricons (Millipore) and adjusted to 120 μl. Samples (20 μl) were separated onto 6% SDS-PAGE gels, and the gels were processed for fluorography and/or placed against PhosphorImager screens for the quantification of individual bands corresponding to apoB-100 and apoB-48.

Pulse-chase analysis

After incubation for 1 h in a Cys/Met-free medium (Gibco-BRL), cells were pulsed for 30 min in Cys/Met-free medium supplemented with 200 μCi/ml [^{35}S]Cys/Met and lipid micelles in the apical compartment and in the presence or absence of polyphenols. After the pulse, cells were rinsed and then chased, for 3 h, in the presence of an excess of unlabeled methionine (10 mM) and cysteine (2 mM) in both compartments and with lipid micelles in the presence or absence of polyphenols in the apical compartment. Cell lysates were prepared as described above and centrifuged to remove cell debris. Basolateral medium was collected and adjusted to 2% protease inhibitor cocktail. For each sample to immunoprecipitate, 40 μl of a 30% suspension of protein A-Sepharose beads (Pharmacia) in buffer 1 (1% BSA and 0.1% sodium azide in 0.1 M sodium phosphate buffer, pH 8) was incubated for 2 h at room temperature with 200 μl of rabbit polyclonal anti-apoB antibodies (1:100 in the same buffer). The beads were washed twice in buffer 1 and once in buffer 2 (0.2% BSA, 1% Triton X-100, and 0.1% sodium azide in 0.1 M sodium phosphate buffer, pH 8), and then 200 μl of cell lysate containing 200 μg of proteins or 200 μl of basolateral medium was added. After 90 min of incubation at 4°C, the beads were washed four times with buffer 2, twice with 0.1 M phosphate buffer, pH 8, and once with 10 mM phosphate buffer, pH 8. The beads were boiled for 4 min in 50 μl of Laemmli buffer. Aliquots were counted in Optiphase Highsafe 2 scintillation fluid in a Beckman scintillation counter.

RNA extraction and semiquantitative RT-PCR analysis

Total RNA was prepared according to the manufacturer's instructions (Tri Reagent; Euromedex, France). cDNA was synthesized from 1 μg of total RNA in 20 μl using random hexamers and murine Moloney leukemia virus reverse transcriptase (Invitrogen, Cergy Pontoise, France) as recommended by the manufacturer. Synthesis of primers for ACAT1, ACAT2, MTP, and APOB gene (**Table 1**) was done by Proligo (Paris, France). Quantitative RT-PCR analyses were performed with 50 ng of reverse-transcribed total RNA in 1× LC FastStar DNA Master SYBR Green I buffer (Roche Diagnostics, Meylan, France), 3 mM $MgCl_2$, and 200 nM of each sense and antisense primer in a final volume of 20 μl using the LightCycler apparatus (Roche Diagnostics, Meylan, France). Quantification of HMG-CoA reductase and 18S rRNA gene expression was done using Assays-on-Demand and Predeveloped Taqman Assay Reagent, respectively, from Applied Biosystems (Les Ulis, France) and FastStart DNA Master Hybridization probes (Roche Diagnostics) as recommended by the manufacturers. Control Caco-2/TC7 lipid-induced total RNA was used to make standard curves. In summary, cDNA of control cells was diluted into 10-fold serial dilutions (10^{-1} to 10^{-6}), amplified along with samples, and plotted in arbitrary units to acquire quantitative data with the LightCycler software. After expression of data as percentage relative to control (micelles), mRNA levels of target genes were normalized to 18S rRNA and expressed in arbitrary units. All samples were assayed in duplicate, and the average value of the duplicates was used for quantification.

TABLE 1. Sequences of primers for quantitative RT-PCR

Gene	Orientation	Primer Sequence (5′→3′)
ACAT1	Sense	CCTGAGGAAGATGAAGACC
	Antisense	CTCTGCCTCTGCTGTCAAC
ACAT2	Sense	CGTCTGCAGAGGACAGAAG
	Antisense	GTGTCGGGTCCATTGTACC
MTP	Sense	TTCAGCACCTCAGGACTGC
	Antisense	GTCTGAGGTCTGAGCAGAG
APOB	Sense	CCCACAGCAAGCTAATG
	Antisense	GTCTGCAGTTGAGATAG

APOB, apolipoprotein B; MTP, microsomal triglyceride protein.

Preparation of procyanidin-enriched fraction

Procyanidin-enriched fraction was prepared from Applephenon C100. Procyanidin-enriched fraction was purified on a Sephadex LH-20 column (55 × 3 cm inner diameter, 100 μm; Pharmacia) from 1.7 g of Applephenon C100. The following eluents were successively applied: water (1,000 ml), methanol-water (1:1; 3,200 ml), and acetone-water (7:3; 220 ml). Procyanidin-enriched fraction (in the acetone-water eluent) and procyanidin-depleted fractions (in the methanol-water eluent) were dried by removing the solvents under reduced pressure and freeze-drying. A total of 169 mg was recovered from the procyanidin-enriched fraction. Bidimensional chromatography was carried out with high-performance cellulose plates on aluminum foil (10 × 10 cm, 0.1 mm; Merck). Eluent for procyanidins were *t*-butanol-acetic acid-water (3:1:1, v/v; eluent A) and acetic acid-water (6:94 p/v; eluent B). Plates were sprayed with a mixture of equal volumes of aqueous $K_3Fe(CN)_6$ (2%, m/v) and $FeCl_3$ (2%, m/v) to visualize phenolic compounds.

MTP activity assay

MTP activity was measured with an MTP assay kit according to the manufacturer's instructions with some modifications (Roar Biomedical, New York, NY). The assay is based on a transfer of fluorescence, attributable to MTP activity, between donor and acceptor vesicles. Caco-2/TC7 cells were scraped and sonicated in homogenization buffer containing 10 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, and 1/50 protease inhibitor cocktail. The MTP assay was performed by incubating 200 μg of protein of cell homogenates with 10 μl of donor solution and 10 μl of acceptor solution in a total volume of 250 μl and incubated for different times at 37°C. MTP activity was calculated by measuring fluorescence at 465 nm excitation wavelength and 538 nm emission wavelength every 20 min using the Fluostar Ascent FL (Labsystems S.A., Paris, France). Results are expressed in arbitrary units calculated as fluorescence intensity transferred per minute.

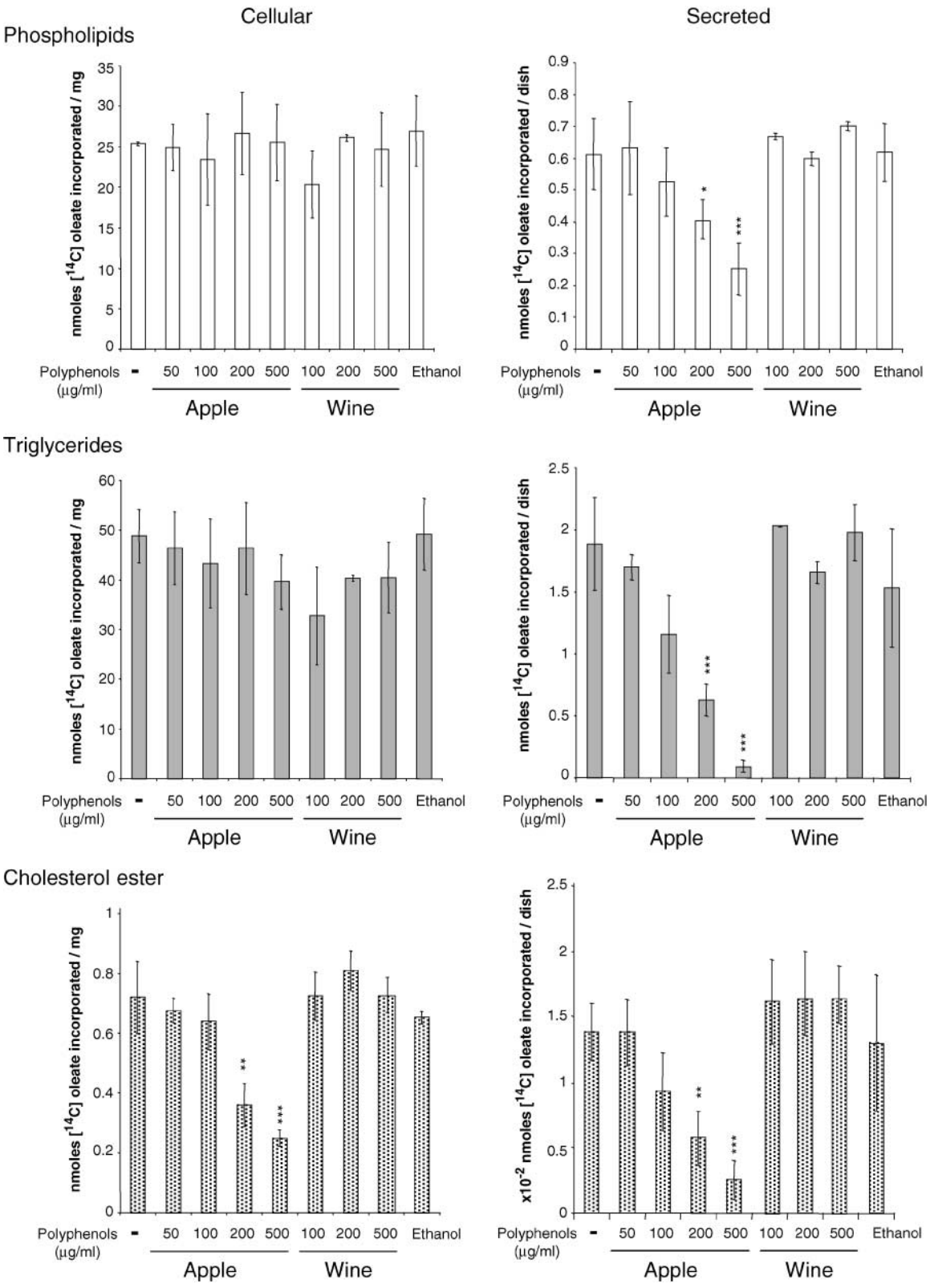

Fig. 1. Synthesis and secretion of triglycerides (TGs), phospholipids, and cholesteryl esters in Caco-2/TC7 cells incubated with apple or wine polyphenols. Caco-2/TC7 cells were cultured for 15 days on microporous membranes and in asymmetric conditions during the last 7 days. Complex micelles containing 2 μCi of [1-^{14}C]oleic acid per milliliter of medium and increasing concentrations of apple or wine polyphenols were added on the apical side of the cells for 24 h. Lipids were extracted from cells (cellular) and basal medium (secreted), separated by thin-layer chromatography, and radioactive spots corresponding to phospholipids, TGs, and cholesteryl esters were counted. Results are from three different experiments in duplicate and are expressed as nanomoles of [1-^{14}C]oleic acid incorporated into lipids per milligram of protein for cellular lipids and per dish for secreted lipids. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Values represent means ± SD.

Statistical analysis

Results are expressed as means ± SD. Statistical analysis was performed with Student's *t*-test for unpaired data.

RESULTS

Polyphenols were supplied at the apical side of Caco-2/TC7 cells, using concentrations similar to those reached in physiological conditions. Indeed, the maximal dose of apple or wine polyphenol extracts used in this study corresponded to the consumption of three apples or half a liter of wine per day, respectively (4). These concentrations did not induce any toxic effect, as assessed by XTT assay and lactate dehydrogenase activity measurement (data not shown).

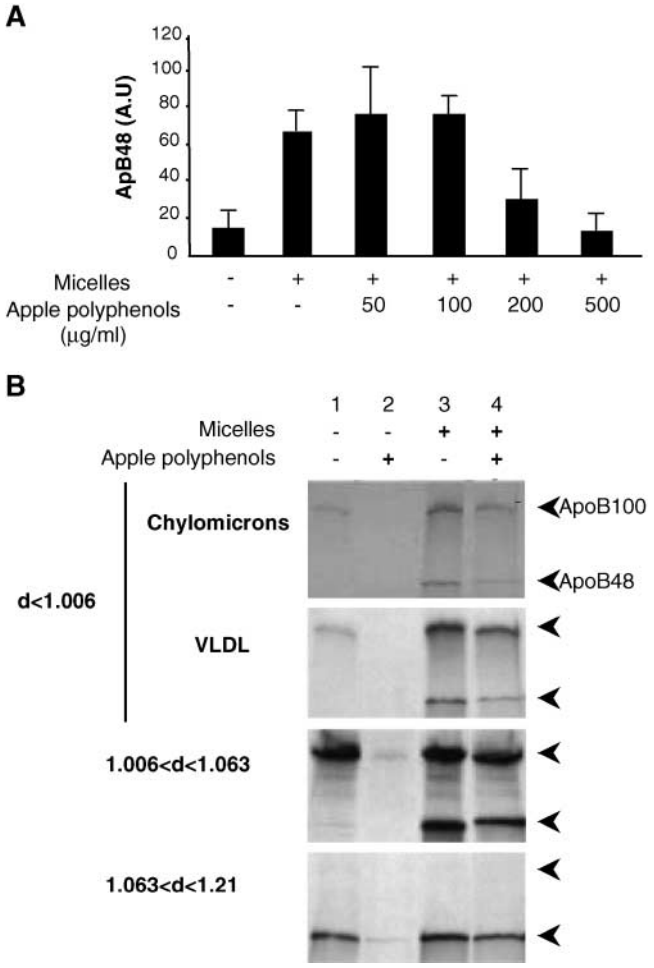

Fig. 2. Effect of apple polyphenols on apolipoprotein B-48 (apoB-48) and lipoprotein secretion by Caco-2/TC7 cells. A: Caco-2/TC7 cells were cultured as described for Fig. 1 and incubated for 24 h with or without micelles and increasing concentrations of apple polyphenol extract. Basal medium (20 µl) was subjected to 6% SDS-PAGE, and apoB was revealed by Western blotting. Quantification of apoB-48 was done by film scanning. Intensity of the bands was expressed as arbitrary units (A.U.). B: Caco-2/TC7 cells were incubated for 24 h in medium supplemented with [^{35}S]cysteine/methionine mix (Cys/Met; 100 µCi/ml) with or without micelles and 200 µg/ml apple polyphenols. Basal medium was collected, and lipoproteins were isolated by sequential ultracentrifugation. Each fraction was analyzed by 6% SDS-PAGE and fluorography to visualize ^{35}S-labeled apoB-100 and apoB-48. Different exposures were used to reveal chylomicrons and other lipoprotein fractions. Values represent means ± SD.

Apple polyphenols decrease the intracellular incorporation of [1-^{14}C]oleic acid into cholesteryl ester and inhibit lipid secretion by Caco-2/TC7 cells

To evaluate the effect of apple or wine polyphenols on intestinal lipid metabolism and secretion, cells were supplied during 24 h, in the apical medium, with complex micelles containing [1-^{14}C]oleic acid and increasing concentrations of polyphenols. As shown in **Fig. 1**, 200 and 500 µg/ml apple polyphenols reduced the intracellular accumulation of [1-^{14}C]oleate incorporated into cholesteryl ester by 45% (0.38 ± 0.04 vs. 0.69 ± 0.11 nmol/mg protein; $P < 0.01$) and 64% (0.25 ± 0.03 vs. 0.69 ± 0.11 nmol/mg protein; $P < 0.001$), respectively. No difference was observed for the intracellular accumulation of newly synthesized phospholipids or TGs. By contrast, wine polyphenols exerted no effect on [1-^{14}C]oleate incorporation into cholesteryl ester, phospholipids, and TGs.

At the same time, apple polyphenols dose-dependently decreased the secretion of all classes of newly synthesized lipids, resulting at 500 µg/ml in a 77% decrease in cholesteryl esters (0.032 ± 0.008 vs. 0.14 ± 0.02 pmol/dish; $P < 0.001$), a

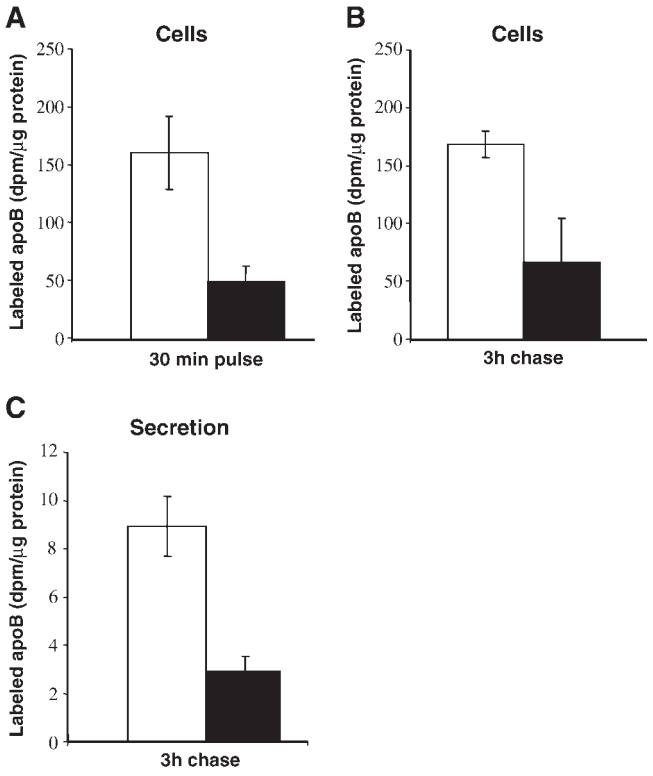

Fig. 3. Effect of apple polyphenols on the synthesis and degradation of apoB. Pulse-chase experiments of apoB in Caco-2/TC7 cells cultured with micelles and with (closed bars) or without (open bars) 500 µg/ml apple polyphenols. After 30 min of labeling with [^{35}S]Cys/Met and a 3 h chase, apoB was immunoprecipitated from 200 µg of cell extracts (A, B) and 200 µl of basal medium (C) and counted. Results correspond to one representative experiment of three independent determinations. Values represent means ± SD.

61% decrease in phospholipids (0.24 ± 0.07 vs. 0.61 ± 0.12 nmol/dish; $P < 0.001$), and a 92% decrease in TGs (0.16 ± 0.1 vs. 1.9 ± 0.4 nmol/dish; $P < 0.001$) in the basal medium. Again, no effect on the secretion of lipids was observed with wine polyphenols, regardless of concentration.

Apple polyphenols decrease apoB and lipoprotein secretion

Because lipids are secreted associated with apoB, we quantified by Western blotting the apoB secreted in the basal medium after 24 h of contact with micelles supplemented or not with apple polyphenols. Micelle supply induced a 4-fold increase in apoB-48 secretion in the basal medium (**Fig. 2A**). The addition of apple polyphenols dose-dependently decreased the secretion of apoB-48, compared with micelles added alone (65% and 81% at 200 and 500 μg/ml, respectively). To further characterize the effect of polyphenols on the pattern of secreted lipoproteins, cells were incubated for 24 h in the presence of [^{35}S]Cys/Met with or without micelles that were supplemented or not with 200 μg/ml apple polyphenols. Then, basolateral media were subjected to sequential ultracentrifugation, and the recovered fractions were analyzed by SDS-PAGE and fluorography. The results are shown in Fig. 2B. As reported elsewhere (23), micelle supply dramatically increased the secretion of TRLs (d < 1.006 g/ml) (Fig. 2B, compare lanes 1 and 3). For cells that were supplied with micelles (Fig. 2B, lanes 3 and 4), polyphenol addition resulted in the inhibition of both apoB-100 and apoB-48 secretion. Moreover, this effect was observed in all lipoprotein fractions. Interestingly, the inhibitory effect of polyphenols on apoB-100 and apoB-48 secretion was very efficient on cells that were not incubated with micelles. Indeed, the secretion of TRLs (d < 1.006 g/ml) was not detectable and that of lipoproteins floating at d > 1.006 g/ml was strongly impaired (Fig. 2B, lane 2).

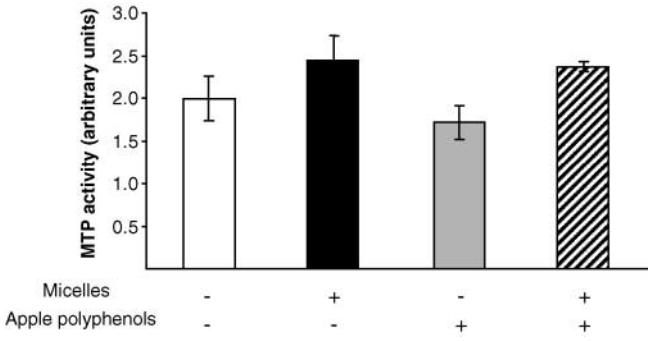

Fig. 4. Effect of micelles and apple polyphenols on microsomal triglyceride protein (MTP) activity. Caco-2/TC7 cells were incubated with or without micelles, apple polyphenols (200 μg/ml), or both. MTP activity was measured using an MTP assay kit (Roar Biomedical) as described in Materials and Methods. The reaction was started with 200 μg of proteins, and transfer of fluorescence from donor to acceptor particles was measured at 37°C every 20 min for 6 h. Results are expressed in arbitrary units corresponding to the fluorescence intensity transfer per minute. Values represent means ± SD from three experiments performed in triplicate.

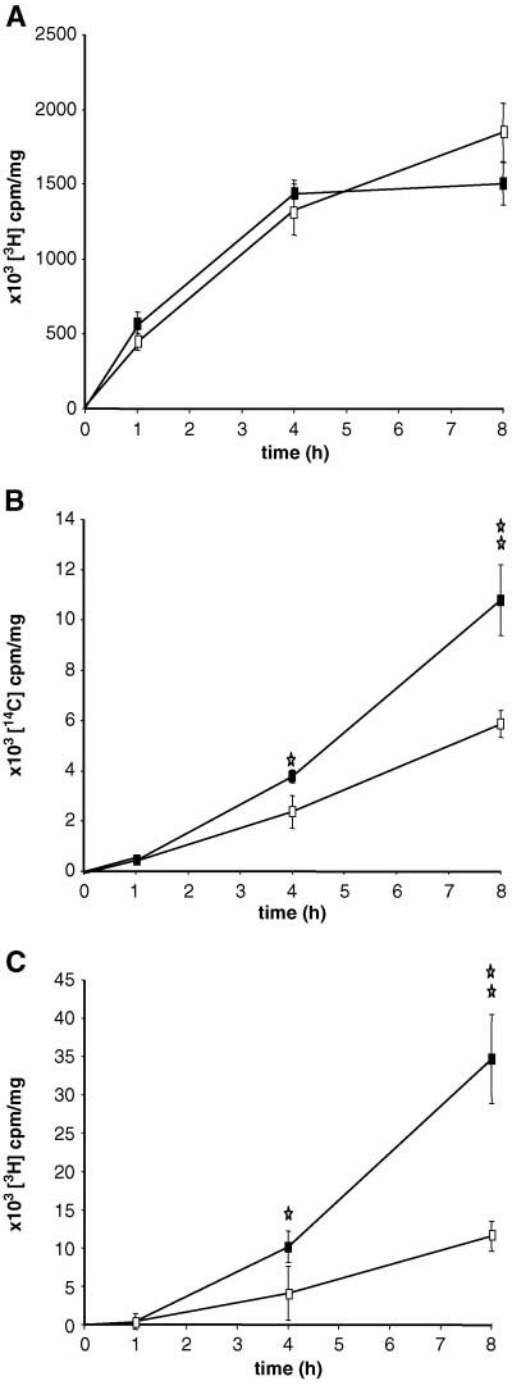

Fig. 5. Kinetics of cholesterol uptake and esterification. Cells were incubated for various times with micelles containing both [1,2-^{3}H(N)]cholesterol and [1-^{14}C]oleic acid and with (open squares) or without (closed squares) 200 μg/ml apple polyphenols. Lipids were extracted from cells. Free cholesterol and cholesteryl esters were separated by TLC. A: Cholesterol uptake was evaluated by measuring the accumulation of [1,2-^{3}H(N)]cholesterol in cell lysates. B and C: Cholesteryl ester synthesis was quantified by the incorporation of [^{14}C]oleic acid (B) or the accumulation of [^{3}H]cholesteryl ester (C). Values represent means ± SD of three experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$.

Apple polyphenols inhibit [^{35}S]Cys/Met incorporation into apoB

Previous studies have demonstrated that decreased apoB secretion could be attributable to a decrease of its synthesis or to an increase of its degradation (24, 25). To identify the points of control of apoB decrease under supplementation with apple polyphenols, pulse-chase experiments were performed. After a 30 min pulse, incorporation of [^{35}S]Cys/Met into apoB decreased by 60% when 500 μg/ml apple polyphenols was added on the apical medium (**Fig. 3A**). Over the 3 h chase, the newly synthesized apoB remained at the same level with or without apple polyphenols (Fig. 3B), indicating that apple polyphenols inhibit an early step of apoB synthesis and do not increase apoB degradation. As shown in Fig. 3C, after a 3 h chase, labeled apoB secretion was decreased by 66% in the presence of apple polyphenols, which is in the same range as inhibition of apoB synthesis (Fig. 3A) and inhibition of apoB-48 secretion over 24 h of treatment with apple polyphenols (Fig. 2A).

MTP activity is not affected by apple polyphenols

It is well known that lipid transfer by MTP enzyme is necessary for the lipidation of nascent apoB and that this enzyme is a major actor in the assembly and subsequent secretion of intestinal lipoproteins (26). As it was previously shown that MTP activity was inhibited by flavonoids such as naringenin, hesperitin, and quercetin (9, 27), we tested the possibility that apple polyphenols impair MTP activity in our model. MTP activity was measured after 24 h of incubation with or without 200 μg/ml apple polyphenols and micelles in the apical compartment. As shown in **Fig. 4**, no obvious change was induced by micelle and/or apple polyphenol supply. Similar results were obtained after 2, 4, and 8 h of incubation (data not shown), indicating that changes in MTP activity did not account for the decrease in TRL secretion that was induced by apple polyphenols.

Apple polyphenols inhibit cholesterol esterification but not cholesterol uptake

Because reports have suggested that the level of cholesteryl esters might regulate apoB secretion in Caco-2 cells (28, 29), we investigated whether apple polyphenols could interfere with cholesterol uptake and/or cholesterol esterification and in turn hinder lipoprotein secretion. As quantified by intracellular [1,2-^{3}H(N)]cholesterol (**Fig. 5A**), apple polyphenols did not impair the intracellular accumulation of free cholesterol from micelles, suggesting that apple polyphenols did not interfere with cholesterol uptake or catabolism. In contrast, the incorporation of [1-^{14}C] oleic acid into cellular cholesteryl esters was decreased by 38% (2,375 ± 643 vs. 3,779 ± 248 cpm/mg protein; $P < 0.05$) after 4 h and by 47% (5,863 ± 541 vs.

TABLE 2. Relative gene expression in Caco-2/TC7 cells quantified by real-time PCR

Gene	Micelles	Micelles and Apple Polyphenols
	arbitrary units	
ACAT1	1 ± 0.22	1.23 ± 0.18
ACAT2	1 ± 0.003	0.84 ± 0.15
MTP	1 ± 0.04	1.02 ± 0.25
APOB	1 ± 0.04	0.92 ± 0.28
HMG-CoA reductase	1 ± 0.07	1.06 ± 0.18

Caco-2/TC7 cells were grown on filters for 15 days and then incubated with micelles or micelles and apple polyphenols for 24 h. Quantification of gene expression was done by real-time RT-PCR. The mRNA abundance was expressed as the ratio between the target gene mRNA and 18S rRNA. Values represent means ± SD from three experiments in duplicate.

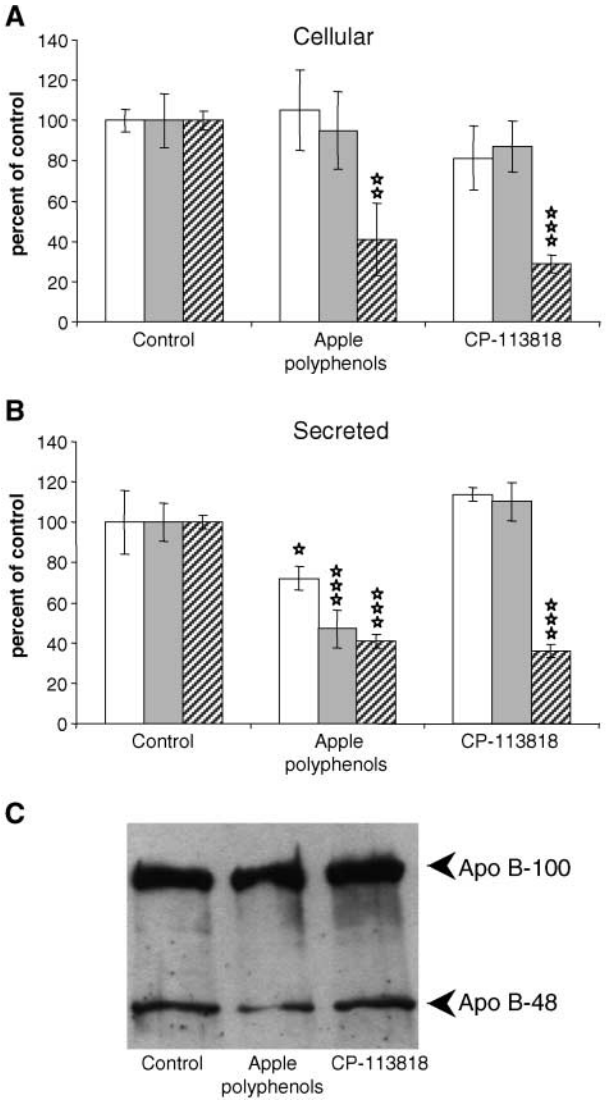

Fig. 6. Effect of CP-113818, an ACAT inhibitor, on cholesteryl ester synthesis and apoB secretion. Caco-2/TC7 cells were cultured as described for Fig. 1 and incubated with micelles containing [^{14}C] oleic acid (control) supplemented or not with 200 μg/ml apple polyphenols or 40 nM CP-113818. Lipids were extracted from cells (A) and basal media (B), separated by TLC, and [1-^{14}C]oleic acid incorporated into phospholipids (open bars), TGs (gray bars), and cholesteryl ester (hatched bars) was counted. Data are expressed as a percentage of control (set at 100%). Values represent means ± SD of two different experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. C: A representative SDS-PAGE image of apoB revealed by Western blotting from 20 μl of basal medium.

10,791 ± 1,409 cpm/mg protein; $P < 0.01$) after 8 h of incubation (Fig. 5B). Moreover, measurement of [1,2-^{3}H(N)] cholesteryl esters showed a 68% inhibition (11,528 ± 1,933 vs. 34,692 ± 5,798 cpm/mg protein; $P < 0.01$) at 8 h (Fig. 5C). Altogether, these data suggest that ACAT activity was inhibited by polyphenols. Wilcox and colleagues (9) recently demonstrated that flavonoids decreased ACAT activity as well as ACAT2 gene expression in HepG2 cells. We therefore quantified ACAT mRNA levels by relative quantitative real-time RT-PCR. As shown in **Table 2**, the addition of apple polyphenols in the apical compartment did not induce any modification of ACAT2, ACAT1, MTP, apoB, and HMG-CoA reductase mRNAs, suggesting differences between hepatocytes and enterocytes. Thus, in the enterocytes, our results imply that ACAT is inhibited by polyphenols at the activity level.

Inhibition of ACAT activity is not sufficient to decrease intestinal lipoprotein secretion

To evaluate the effect of cholesterol esterification on intestinal lipoprotein secretion, Caco-2/TC7 cells were incubated with micelles in the presence or absence of CP-113818, an ACAT inhibitor that was shown to inhibit ACAT intestinal activity (30). CP-113818, used at 40 nM in the range of IC_{50} of ACAT activity in human intestine (30), inhibited the incorporation of [1-^{14}C]oleic acid in cholesteryl esters by 71% ($P < 0.001$) (**Fig. 6A**). The nonsignificant decrease in intracellular newly synthesized TGs and phospholipids demonstrated the specificity of the CP-113818 inhibitor when used at this concentration. Moreover, CP-113818 decreased the secretion of cholesteryl esters by 61%, whereas that of TGs, phospholipids, and apoB remained unchanged (Fig. 6B, C), suggesting that inhibition of cholesteryl ester synthesis was not sufficient to inhibit the secretion of lipoproteins in Caco-2/TC7 cells.

Procyanidin-enriched fraction reproduces the effects of apple polyphenol extract

The apple polyphenol extract that we used contained procyanidins, chlorogenic acid, caffeic acid, (−)-epicatechin, (+)-catechin, and phloretin-2′-glucoside (40–45%, 15–20%, 7–8%, 3–5%, 4–6%, and 3–4%, respectively). To identify the molecule(s) that could be involved in the control of intestinal lipoprotein metabolism, we carried out experiments with the different components of the extract (i.e., monomers or procyanidins). As procyanidin molecules differ in fruit and vegetable species, we performed procyanidin enrichment from total apple polyphenol extract using a Sephadex LH-20 column. All molecules were used at a concentration equivalent to that present in 200 μg/ml apple polyphenol extract. As shown in **Table 3**, chlorogenic acid, phloridzin, (−)-epicatechin, and (+)-catechin, supplied alone or mixed together, did not modify the synthesis and secretion of cholesteryl esters, TGs, and phospholipids. By contrast, the procyanidin-enriched fraction purified from apple polyphenols, but not the procyanidin-depleted fraction, induced an inhibition of both cholesteryl ester synthesis and lipid secretion, similar to the pattern obtained with the total apple polyphenol extract (**Fig. 7**).

DISCUSSION

Phenolic compounds that are present in fruits and vegetables have hypolipidemic properties in animal models fed a high-fat and/or a high-cholesterol diet (31–33). Studies performed in the liver attributed these beneficial effects to a decrease in the assembly and secretion of hepatic apoB-containing lipoproteins (8, 34, 35). However, it must be emphasized that intestinal epithelium, the first tissue to face dietary compounds, plays a pivotal role in lipid

TABLE 3. Effects of flavonoid monomers on lipid synthesis and basal secretion in Caco-2/TC7 cells

	Intracellular			Secreted		
Polyphenols	Phospholipids	Triglycerides	Cholesteryl Ester	Phospholipids	Triglycerides	Cholesteryl Ester
			%			
Control	100 ± 10	100 ± 5	100 ± 10	100 ± 3	100 ± 3	100 ± 3
Apple extract (200 μg/ml)	95 ± 18	77 ± 20	49 ± 5[a]	66 ± 15[a]	47 ± 21[b]	41 ± 13[c]
Chlorogenic acid	102 ± 5	101 ± 16	85 ± 15	85 ± 17	96 ± 6	72 ± 6
Phloridzin	90 ± 15	78 ± 16	72 ± 17	97 ± 5	90 ± 4	80 ± 4
(−)-Epicatechin	88 ± 24	84 ± 22	69 ± 26	96 ± 8	94 ± 9	77 ± 9
(+)-Catechin	106 ± 3	96 ± 3	79 ± 19	94 ± 9	92 ± 6	76 ± 6
Mix monomers	74 ± 7	99 ± 1	74 ± 7	103 ± 5	99 ± 8	80 ± 8

Caco-2/cells were grown on filters for 15 days and then incubated with micelles or micelles and apple polyphenols for 24 h. [1-^{14}C]oleic acid was used as tracer and was incorporated into micelles. Monomers of flavonoids were supplied at concentrations equivalent to those present in 200 μg/ml apple polyphenols [chlorogenic acid, 40 μg/ml; phloridzin, 12 μg/ml; (−)-epicatechin, 16 μg/ml; (+)-catechin, 10 μg/ml). Mix monomers contained chlorogenic acid, phloridzin, (−)-epicatechin, and (+)-catechin. Lipids were extracted from basal medium and cells. Lipids were separated by thin-layer chromatography, and [^{14}C]oleic acid incorporated into triglycerides, cholesteryl esters, and phospholipids was counted. Results are expressed as a percentage of the intracellular lipid content or of secreted lipids compared with cells incubated with micelles. Values represent means ± SD from two experiments in triplicate.

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

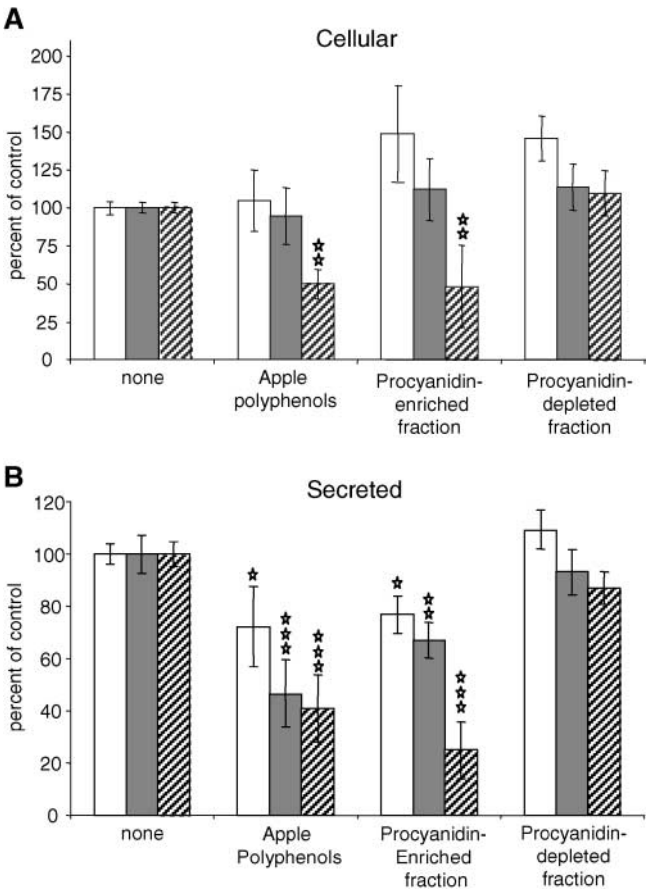

Fig. 7. Effect of apple procyanidin-enriched fraction on cholesteryl ester synthesis and lipid secretion. Caco-2/TC7 cells were incubated for 24 h with micelles containing [1-^{14}C]oleic acid supplemented or not with 200 μg/ml apple polyphenols, 90 μg/ml apple procyanidin-enriched fraction, or 110 μg/ml apple procyanidin-depleted fraction. Lipids were extracted from cells (A) or basal medium (B), separated by TLC, and [^{14}C]oleic acid incorporated into phospholipids (open bars), TGs (gray bars), and cholesteryl esters (hatched bars) was counted. Data are expressed as a percentage of control (set at 100%). Values represent means ± SD of two different experiments performed in triplicate. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

homeostasis through TRL synthesis and secretion during the postprandial state. In the present study, we demonstrate that although polyphenols from wine had no effect, a procyanidin-enriched fraction isolated from apple polyphenols decreased the esterification of cholesterol and the secretion of apoB-containing lipoproteins by the human Caco-2/TC7 enterocytes apically supplied with lipid micelles.

We have shown that in intestinal cells, apple polyphenols decreased intracellular cholesteryl ester, measured as the accumulation of [1,2-^{3}H(N)]cholesteryl ester or the incorporation of [1-^{14}C]oleic acid, without changing cholesterol uptake, most likely through an inhibition of cholesterol esterification, as reported for flavonoids such as taxifolin, naringenin, and hesperitin in HepG2 cells (8, 9). Two ACAT genes, ACAT1 and ACAT2, have been identified that differ by their tissue expression and presumably their cellular function (36). Wilcox et al. (9) have shown that naringenin and hesperitin inhibit ACAT activity in AC29 cells stably transfected with either ACAT1 or ACAT2 cDNA. ACAT2 mRNA was significantly reduced when HepG2 cells were incubated with naringenin and hesperitin, whereas ACAT1 mRNA was unaffected (9). We did not observe any change in ACAT1 and ACAT2 mRNA levels under our conditions. This suggests that, in enterocytes, the polyphenol-induced decrease in cholesterol esterification does not occur through the control of ACAT gene expression. Altogether, these results demonstrate that polyphenols interfere with the esterification of both endogenous and newly absorbed cholesterol.

The second effect of apple polyphenols was to decrease the secretion of apoB-100- and apoB-48-containing lipoproteins. This inhibition concerned all of the lipoprotein fractions and was dramatic in the absence of micelle supply. The requirement of apoB-100 for TGs to escape intracellular degradation (37) indicates that apoB-100-containing lipoproteins mainly float at $1.006 < d < 1.063$ mg/ml in the absence of exogenous lipid supply. By contrast, apoB-48 is almost exclusively secreted as lipid-poor lipoproteins [i.e., "HDL-like" lipoproteins, as reported by Liao and Chan (38)], corresponding to its lesser dependence on TGs. In the presence of polyphenols, both apoB-100- and apoB-48-containing lipoproteins were barely detectable, which could indicate a blockade of apoB synthesis. However, in the presence of micelles, polyphenol addition resulted in the secretion in apoB-100- and apoB-48-containing lipoproteins, which were recovered essentially in TG-enriched fractions. We demonstrated that apple polyphenols inhibited the synthesis of apoB but did not change the degradation of the newly synthesized protein. This inhibition could occur during the initial steps of synthesis, as described for the action of insulin on hepatic apoB (39, 40). Furthermore, it has been reported in HepG2 cells that lipid availability or transfer to apoB is required for the completion of its translation and translocation across the endoplasmic reticulum membrane (41, 42).

In this regard, the amount of intracellular cholesteryl esters has been proposed to control apoB secretion in HepG2 and Caco-2 cells (29, 43). In contrast, we show that the amount of secreted apoB-48 in the basal medium was not decreased by the ACAT inhibitor CP-113818, despite its efficient inhibition of cholesterol esterification. This suggests that inhibition of TRL secretion could be a specific effect of some molecules identified as inhibitors of ACAT activity. Wilcox et al. (43) have shown that DuP 128, an ACAT inhibitor, failed to significantly alter apoB secretion in HepG2 cells in the presence of oleic acid but that CI-1011, another ACAT inhibitor, did. Altogether, these results demonstrate that the decrease of intracellular cholesterol esterification is not sufficient to explain by itself the inhibition of apoB-containing lipoprotein secretion.

Impairment of MTP activity has been demonstrated to be involved in the decrease of apoB secretion (12). Casaschi et al. (27), using Caco-2 cells grown on filters under a supply of oleic acid complexed to BSA, have shown that quercetin, a flavonoid, inhibited apoB secretion along with MTP activity and TG synthesis. However, we did not observe any decrease in MTP activity in Caco-2/TC7 cells incubated for 2 to 24 h with apple polyphenols, indicating

that inhibition of MTP could not explain the decrease of TRL secretion. An alternative explanation could be a blockade upstream of the transfer of TGs to apoB. Indeed, secreted TGs derive from lipolysis of cytosolic stores followed by their reesterification in the endoplasmic reticulum membrane and MTP-dependent transfer to the endoplasmic reticulum lumen (44). In hepatocytes, inhibition of the cytosolic TG lipolysis step results in a dramatic decrease in the secretion of TGs, cholesteryl esters, and phospholipids (45). Furthermore, it has been shown that VLDL secretion depends on a verapamil-sensitive mechanism for TG accumulation in the endoplasmic reticulum (46). Accordingly, our results could be explained by a blockade of TG availability in the endoplasmic reticulum lumen, upstream of MTP-mediated transfer, resulting in an impairment of apoB synthesis. Another hypothesis suggests that polyphenols could impair the secretory process itself.

Another important finding in our study was that the procyanidin-enriched fraction from apple polyphenols was able to reproduce the effects of the total extract. Procyanidins are oligomeric structures made of flavanols, catechin, and epicatechin. They are present in plants and have attracted increasing attention in the fields of nutrition and medicine for their potential health benefits (47). To explain the biological effects of procyanidins, it was assumed that these molecules are bioavailable in their target tissues. However, it must be emphasized that, among all the oligomeric procyanidin structures, only monomers and dimers can be transferred through the intestinal barrier, the first epithelium that is met by these compounds. In our study, monomers were unable to reduce cholesteryl ester synthesis and lipoprotein secretion, suggesting that dimers or larger oligomers interfered with intestinal lipoprotein metabolism. Dimeric procyanidins were found to inhibit nuclear factor κB activation and interleukin-2 (48) release when Jurkat cells were incubated with PMA (49). Recently, Eng et al. (50) demonstrated that procyanidin dimers specifically inhibit estrogen biosynthesis through their binding to the active site of aromatase. In addition, they showed that a mixture of procyanidin dimers was more active than individual molecules and that mice fed daily with this mixture had significant reductions in androgen-dependent tumor growth. Moreover, other reports have shown that the relaxing activity of procyanidins in rat and human artery endothelium tended to increase as a function of polymerization degree (51, 52). Further studies are needed to analyze whether procyanidin polymers exert their inhibiting effects in our system through a signaling pathway, which remains to be determined. Finally, the differences observed between the effects of apple and wine polyphenols could depend on procyanidin content. Indeed, apple polyphenol extract contains 10% B1 and B2 procyanidin dimers, compared with 1.5% in wine polyphenol extract. These differences could explain why wine polyphenols do not display the effects of apple polyphenols.

Exaggerated postprandial hyperlipemia has been found to be associated with cardiovascular disease, probably by accumulation of atherogenic remnants of TRLs in the postprandial state. Therefore, the prevention of postprandial hyperlipemia could be of importance in decreasing the risk factor of cardiovascular disease. Recently, atorvastastin, a potent HMG-CoA reductase inhibitor, was shown to improve postprandial lipoprotein metabolism by decreasing the incremental area under the curve for cholesterol and TG associated with TRL in hypertriglyceridemic patients (53). A number of new agents are in development that target the inhibition of lipid absorption (54), transport (55), and esterification (56). In this respect, procyanidins are interesting candidates, because we demonstrate in this study that these natural molecules are able to inhibit cholesterol esterification and intestinal lipoprotein secretion in nutritional amounts.

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale and Vin et Santé. The authors thank P. André for providing the apple polyphenol extract (Applephenon C100) and O. Francone and N. Fournier for providing CP-113818. The authors also thank A. Scalbert for helpful discussions. R.V. was supported by Agence Pour la Recherche et l'Information en Fruits et Légumes frais (APRIFEL).

REFERENCES

1. Ness, A. R., and J. W. Powles. 1997. Fruit and vegetables, and cardiovascular disease: a review. *Int. J. Epidemiol.* **26:** 1–13.
2. Hu, F. B., and W. C. Willett. 2002. Optimal diets for prevention of coronary heart disease. *J. Am. Med. Assoc.* **288:** 2569–2578.
3. Bazzano, L. A., M. K. Serdula, and S. Liu. 2003. Dietary intake of fruits and vegetables and risk of cardiovascular disease. *Curr. Atheroscler. Rep.* **5:** 492–499.
4. Scalbert, A., and G. Williamson. 2000. Dietary intake and bioavailability of polyphenols. *J. Nutr.* **130 (Suppl.):** 2073–2085.
5. Frankel, E. N., J. Kanner, J. B. German, E. Parks, and J. E. Kinsella. 1993. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet.* **341:** 454–457.
6. Gryglewski, R. J., R. Korbut, J. Robak, and J. Swies. 1987. On the mechanism of antithrombotic action of flavonoids. *Biochem. Pharmacol.* **36:** 317–322.
7. Stein, J. H., J. G. Keevil, D. A. Wiebe, S. Aeschlimann, and J. D. Folts. 1999. Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation.* **100:** 1050–1055.
8. Theriault, A., Q. Wang, S. C. Van Iderstine, B. Chen, A. A. Franke, and K. Adeli. 2000. Modulation of hepatic lipoprotein synthesis and secretion by taxifolin, a plant flavonoid. *J. Lipid Res.* **41:** 1969–1979.
9. Wilcox, L. J., N. M. Borradaile, L. E. de Dreu, and M. W. Huff. 2001. Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. *J. Lipid Res.* **42:** 725–734.
10. Borradaile, N. M., L. E. de Dreu, L. J. Wilcox, J. Y. Edwards, and M. W. Huff. 2002. Soya phytoestrogens, genistein and daidzein, decrease apolipoprotein B secretion from HepG2 cells through multiple mechanisms. *Biochem. J.* **366:** 531–539.
11. Davidson, N. O., and G. S. Shelness. 2000. APOLIPOPROTEIN B: mRNA editing, lipoprotein assembly, and presecretory degradation. *Annu. Rev. Nutr.* **20:** 169–193.
12. Hussain, M. M., J. Shi, and P. Dreizen. 2003. Microsomal triglyceride transfer protein and its role in apoB-lipoprotein assembly. *J. Lipid Res.* **44:** 22–32.
13. Hernell, O., J. E. Staggers, and M. C. Carey. 1990. Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. II. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry.* **29:** 2041–2056.
14. Rudel, L. L., R. G. Lee, and T. L. Cockman. 2001. Acyl coenzyme A:cholesterol acyltransferase types 1 and 2: structure and function in atherosclerosis. *Curr. Opin. Lipidol.* **12:** 121–127.

15. Hyson, D., J. C. Rutledge, and L. Berglund. 2003. Postprandial lipemia and cardiovascular disease. *Curr. Atheroscler. Rep.* **5:** 437–444.
16. de Koning, E. J., and T. J. Rabelink. 2002. Endothelial function in the post-prandial state. *Atheroscler. Suppl.* **3:** 11–16.
17. Mee, K. A., and D. L. Gee. 1997. Apple fiber and gum arabic lowers total and low-density lipoprotein cholesterol levels in men with mild hypercholesterolemia. *J. Am. Diet. Assoc.* **97:** 422–424.
18. Hayek, T., B. Fuhrman, J. Vaya, M. Rosenblat, P. Belinky, R. Coleman, A. Elis, and M. Aviram. 1997. Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arterioscler. Thromb. Vasc. Biol.* **17:** 2744–2752.
19. Aprikian, O., V. Duclos, S. Guyot, C. Besson, C. Manach, A. Bernalier, C. Morand, C. Remesy, and C. Demigne. 2003. Apple pectin and a polyphenol-rich apple concentrate are more effective together than separately on cecal fermentations and plasma lipids in rats. *J. Nutr.* **133:** 1860–1865.
20. Chantret, I., A. Rodolosse, A. Barbat, E. Dussaulx, E. Brot-Laroche, A. Zweibaum, and M. Rousset. 1994. Differential expression of sucrase-isomaltase in clones isolated from early and late passages of the cell line Caco-2: evidence for glucose-dependent negative regulation. *J. Cell Sci.* **107:** 213–225.
21. Morel, E., S. Demignot, D. Chateau, J. Chambaz, M. Rousset, and F. Delers. 2004. Lipid-dependent bidirectional traffic of apolipoprotein B in polarized enterocytes. *Mol. Biol. Cell.* **15:** 132–141.
22. Boisfer, E., G. Lambert, V. Atger, N. Q. Tran, D. Pastier, C. Benetollo, J. F. Trottier, I. Beaucamps, M. Antonucci, M. Laplaud, S. Griglio, J. Chambaz, and A. D. Kalopissis. 1999. Overexpression of human apolipoprotein A-II in mice induces hypertriglyceridemia due to defective very low density lipoprotein hydrolysis. *J. Biol. Chem.* **274:** 11564–11572.
23. Chateau, D., T. Pauquai, F. Delers, M. Rousset, J. Chambaz, and S. Demignot. 2005. Lipid micelles stimulate the secretion of triglyceride-enriched apolipoprotein B48-containing lipoproteins by Caco-2 cells. *J. Cell Physiol.* **202:** 767–776.
24. Sparks, J. D., and C. E. Sparks. 1990. Insulin modulation of hepatic synthesis and secretion of apolipoprotein B by rat hepatocytes. *J. Biol. Chem.* **265:** 8854–8862.
25. Fisher, E. A., M. Pan, X. Chen, X. Wu, H. Wang, H. Jamil, J. D. Sparks, and K. J. Williams. 2001. The triple threat to nascent apolipoprotein B. Evidence for multiple, distinct degradative pathways. *J. Biol. Chem.* **276:** 27855–27863.
26. Hussain, M. M. 2000. A proposed model for the assembly of chylomicrons. *Atherosclerosis.* **148:** 1–15.
27. Casaschi, A., Q. Wang, K. Dang, A. Richards, and A. Theriault. 2002. Intestinal apolipoprotein B secretion is inhibited by the flavonoid quercetin: potential role of microsomal triglyceride transfer protein and diacylglycerol acyltransferase. *Lipids.* **37:** 647–652.
28. Kam, N. T., E. Albright, S. N. Mathur, and F. J. Field. 1989. Inhibition of acylcoenzyme A:cholesterol acyltransferase activity in CaCo-2 cells results in intracellular triglyceride accumulation. *J. Lipid Res.* **30:** 371–377.
29. Pal, S., E. Allister, A. Thomson, and J. C. Mamo. 2002. Cholesterol esters regulate apoB48 secretion in CaCo2 cells. *Atherosclerosis.* **161:** 55–63.
30. Marzetta, C. A., Y. E. Savoy, A. M. Freeman, C. A. Long, J. L. Pettini, R. E. Hagar, P. B. Inskeep, K. Davis, A. F. Stucchi, R. J. Nicolosi and E. S. Hamanaka. 1994. Pharmacological properties of a novel ACAT inhibitor (CP-113,818) in cholesterol-fed rats, hamsters, rabbits, and monkeys. *J. Lipid Res.* **35:** 1829–1838.
31. Yugarani, T., B. K. Tan, M. Teh, and N. P. Das. 1992. Effects of polyphenolic natural products on the lipid profiles of rats fed high fat diets. *Lipids.* **27:** 181–186.
32. Kirk, E. A., P. Sutherland, S. A. Wang, A. Chait, and R. C. LeBoeuf. 1998. Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C57BL/6 mice but not LDL receptor-deficient mice. *J. Nutr.* **128:** 954–959.
33. Gorinstein, S., E. Bartnikowska, G. Kulasek, M. Zemser, and S. Trakhtenberg. 1998. Dietary persimmon improves lipid metabolism in rats fed diets containing cholesterol. *J. Nutr.* **128:** 2023–2027.
34. Borradaile, N. M., K. K. Carroll, and E. M. Kurowska. 1999. Regulation of HepG2 cell apolipoprotein B metabolism by the citrus flavanones hesperetin and naringenin. *Lipids.* **34:** 591–598.
35. Pal, S., N. Ho, C. Santos, P. Dubois, J. Mamo, K. Croft, and E. Allister. 2003. Red wine polyphenolics increase LDL receptor expression and activity and suppress the secretion of apoB100 from human HepG2 cells. *J. Nutr.* **133:** 700–706.
36. Chang, T. Y., C. C. Chang, S. Lin, C. Yu, B. L. Li, and A. Miyazaki. 2001. Roles of acyl-coenzyme A:cholesterol acyltransferase-1 and -2. *Curr. Opin. Lipidol.* **12:** 289–296.
37. Olofsson, S. O., L. Asp, and J. Boren. 1999. The assembly and secretion of apolipoprotein B-containing lipoproteins. *Curr. Opin. Lipidol.* **10:** 341–346.
38. Liao, W., and L. Chan. 2000. Apolipoprotein B, a paradigm for proteins regulated by intracellular degradation, does not undergo intracellular degradation in CaCo2 cells. *J. Biol. Chem.* **275:** 3950–3956.
39. Sparks, J. D., R. Zolfaghari, C. E. Sparks, H. C. Smith, and E. A. Fisher. 1992. Impaired hepatic apolipoprotein B and E translation in streptozotocin diabetic rats. *J. Clin. Invest.* **89:** 1418–1430.
40. Adeli, K., and A. Theriault. 1992. Insulin modulation of human apolipoprotein B mRNA translation: studies in an in vitro cell-free system from HepG2 cells. *Biochem. Cell Biol.* **70:** 1301–1312.
41. Mitchell, D. M., M. Zhou, R. Pariyarath, H. Wang, J. D. Aitchison, H. N. Ginsberg, and E. A. Fisher. 1998. Apoprotein B100 has a prolonged interaction with the translocon during which its lipidation and translocation change from dependence on the microsomal triglyceride transfer protein to independence. *Proc. Natl. Acad. Sci. USA.* **95:** 14733–14738.
42. Pariyarath, R., H. Wang, J. D. Aitchison, H. N. Ginsberg, W. J. Welch, A. E. Johnson, and E. A. Fisher. 2001. Co-translational interactions of apoprotein B with the ribosome and translocon during lipoprotein assembly or targeting to the proteasome. *J. Biol. Chem.* **276:** 541–550.
43. Wilcox, L. J., P. H. Barrett, R. S. Newton, and M. W. Huff. 1999. ApoB100 secretion from HepG2 cells is decreased by the ACAT inhibitor CI-1011: an effect associated with enhanced intracellular degradation of apoB. *Arterioscler. Thromb. Vasc. Biol.* **19:** 939–949.
44. Gibbons, G. F., K. Islam, and R. J. Pease. 2000. Mobilisation of triacylglycerol stores. *Biochim. Biophys. Acta.* **1483:** 37–57.
45. Gilham, D., S. Ho, M. Rasouli, P. Martres, D. E. Vance, and R. Lehner. 2003. Inhibitors of hepatic microsomal triacylglycerol hydrolase decrease very low density lipoprotein secretion. *FASEB J.* **17:** 1685–1687.
46. Higashi, Y., H. Itabe, H. Fukase, M. Mori, Y. Fujimoto, and T. Takano. 2003. Transmembrane lipid transfer is crucial for providing neutral lipids during very low density lipoprotein assembly in endoplasmic reticulum. *J. Biol. Chem.* **278:** 21450–21458.
47. Moini, H., G. Rimbach, and L. Packer. 2000. Molecular aspects of procyanidin biological activity: disease preventative and therapeutic potentials. *Drug Metab. Drug Interact.* **17:** 237–259.
48. Spencer, J. P., H. Schroeter, B. Shenoy, S. K. Srai, E. S. Debnam, and C. Rice-Evans. 2001. Epicatechin is the primary bioavailable form of the procyanidin dimers B2 and B5 after transfer across the small intestine. *Biochem. Biophys. Res. Commun.* **285:** 588–593.
49. Mackenzie, G. G., F. Carrasquedo, J. M. Delfino, C. L. Keen, C. G. Fraga, and P. I. Oteiza. 2004. Epicatechin, catechin, and dimeric procyanidins inhibit PMA-induced NF-kappaB activation at multiple steps in Jurkat T cells. *FASEB J.* **18:** 167–169.
50. Eng, E. T., J. Ye, D. Williams, S. Phung, R. E. Moore, M. K. Young, U. Gruntmanis, G. Braunstein, and S. Chen. 2003. Suppression of estrogen biosynthesis by procyanidin dimers in red wine and grape seeds. *Cancer Res.* **63:** 8516–8522.
51. Fitzpatrick, D. F., B. Bing, D. A. Maggi, R. C. Fleming, and R. M. O'Malley. 2002. Vasodilating procyanidins derived from grape seeds. *Ann. NY Acad. Sci.* **957:** 78–89.
52. Aldini, G., M. Carini, A. Piccoli, G. Rossoni, and R. M. Facino. 2003. Procyanidins from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: new evidences for cardio-protection. *Life Sci.* **73:** 2883–2898.
53. Parhofer, K. G., E. Laubach, and P. H. Barrett. 2003. Effect of atorvastatin on postprandial lipoprotein metabolism in hypertriglyceridemic patients. *J. Lipid Res.* **44:** 1192–1198.
54. van Heek, M., C. Farley, D. S. Compton, L. Hoos, and H. R. Davis. 2001. Ezetimibe selectively inhibits intestinal cholesterol absorption in rodents in the presence and absence of exocrine pancreatic function. *Br. J. Pharmacol.* **134:** 409–417.
55. de Jong, A., J. Plat, and R. P. Mensink. 2003. Metabolic effects of plant sterols and stanols (Review). *J. Nutr. Biochem.* **14:** 362–369.
56. Delsing, D. J., E. H. Offerman, W. van Duyvenvoorde, H. van Der Boom, E. C. de Wit, M. J. Gijbels, A. van Der Laarse, J. W. Jukema, L. M. Havekes, and H. M. Princen. 2001. Acyl-CoA:cholesterol acyltransferase inhibitor avasimibe reduces atherosclerosis in addition to its cholesterol-lowering effect in ApoE*3-Leiden mice. *Circulation.* **103:** 1778–1786.