Participation of the microsomal triglyceride transfer protein in lipoprotein assembly in Caco-2 cells: interaction with saturated and unsaturated dietary fatty acids

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Abstract This study was designed to gain insight into the role of microsomal triglyceride transfer protein (MTP) in the association of apolipoprotein (apo) B with lipid during intestinal lipoprotein assembly. The MTP-inhibiting compound BMS-200150 (Jamil et al. 1996. *Proc. Natl. Acad. Sci. USA* **93:** 11991–11995) was used to inhibit the lipid transfer activity of MTP in Caco-2 cells. MTP inhibition reduced the number of apoB-containing lipoproteins that were secreted from the cells. Secretion of apoB-100 appeared to be more sensitive to BMS-200150 than apoB-48 secretion, which appeared to be relatively insensitive. BMS-200150 caused a decrease in the triglyceride content of the secreted lipoproteins, compared with control incubations without MTP inhibition. This indicated that, in Caco-2 cells, MTP is not only involved in the first step of lipoprotein synthesis, i.e., the rescue of apoB from intracellular degradation through early lipidation of the protein, but also in further steps involving the association of lipoproteins with triglycerides. When 0.5 mM oleic acid (18:1) was used to stimulate cellular lipid synthesis, secreted lipoproteins were predominantly of chylomicron/VLDL density and their secretion could be efficiently inhibited with BMS-200150. With 0.5 mM palmitic acid (16:0), lipoproteins of distinct densities (i.e., chylomicron/VLDL and IDL/LDL) were secreted by Caco-2 cells, as reported before (van Greevenbroek et al. 1995. *J. Lipid Res.* **36:** 13–24). Secretion of the lipoproteins at chylomicron/VLDL density was strongly reduced by inhibition of MTP activity by BMS-200150, whereas the IDL/LDL density lipoproteins were relatively insensitive. In conclusion, specific inhibition of MTP activity in Caco-2 cells with BMS-200150 resulted in reduced secretion of apoB-containing lipoproteins (predominantly apoB-100) by Caco-2 cells and furthermore reduced the triglyceride content of these lipoproteins. MTP inhibition preferentially reduced the secretion of triglyceride-rich lipoproteins ($d < 1.006$ g/ml).—**van Greevenbroek, M. M. J., M. G. Robertus-Teunissen, D. W. Erkelens, and T. W. A. de Bruin.** Participation of the microsomal triglyceride transfer protein in lipoprotein assembly in Caco-2 cells: interaction with saturated and unsaturated dietary fatty acids. *J. Lipid Res.* 1998. **39:** 173–185.

Supplementary key words intestine • apolipoprotein B • fatty acid • lipoprotein • chylomicron • microsomal triglyceride transfer protein

It is presently accepted that the general mechanism of the intracellular assembly of apolipoprotein (apo) B-containing lipoproteins in enterocytes and hepatocytes includes association of apoB with lipid in a multi-step process. The first step in the assembly is the co-translational lipidation of apoB at the endoplasmic reticulum. This protects the protein from early intracellular degradation and results in the formation of a primordial intracellular lipoprotein (1, 2). Co-translational lipidation is mediated by microsomal triglyceride transfer protein (MTP). MTP is present in the lumen of the endoplasmic reticulum of enterocytes and hepatocytes and physically interacts with apoB during lipoprotein assembly (3). MTP is a heterodimer that consists of a 97 kDa large sub-unit and a smaller sub-unit of 58 kDa, identified as protein disulfide isomerase (PDI) (4, 5). The large sub-unit possesses the lipid transfer activity or confers the lipid transfer activity on the complex (6). In the absence of MTP, no lipidation of apoB occurs on the endoplasmic reticulum membrane and, as a result, apoB will be rapidly degraded (7). The molecular

Abbreviations: apoB, apolipoprotein B; BSA, bovine serum albumin; d, density; DMEM, Dulbecco's modified Eagle's minimum essential medium; FCS, fetal calf serum; HDL, high density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; MTP, microsomal triglyceride transfer protein; PDI, protein disulfide isomerase; VLDL, very low density lipoprotein.

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mechanisms behind the further steps of intracellular association of apoB with triglycerides are at present not clear. These may include fusion of the primordial lipoprotein with preformed triglyceride-rich droplets in the endoplasmic reticulum or sequential addition of triglycerides and apoproteins as the lipoprotein moves along the secretory pathway. It is accepted that MTP is involved in the first step of lipoprotein assembly, but its potential role in subsequent steps has not yet been elucidated (see ref. 1 for review), although it has been suggested that MTP is not obligatory in the conversion of HDL apoB-48 to VLDL in McArdle 7777 cells (8).

Most data concerning the assembly of intestinal lipoproteins are derived from experiments using the Caco-2 cell line as a model of the human enterocyte. Although the Caco-2 cell line has its specific limitations (9), it is evident from the results of other authors (10, 11) and from our own group (12, 13) that Caco-2 cells are capable of polarized secretion of lipoproteins at chylomicron/VLDL density after stimulation of cellular lipid synthesis. In the present study, we used Caco-2 cells on microporous membranes to gain further insight in the role that MTP plays in the assembly of intestinal lipoproteins.

Recently, the presence of MTP has been confirmed in Caco-2 cells (14). In the present report, we used a specific inhibitor of MTP-mediated lipid transport i.e., BMS-200150 (15) to inhibit the activity of MTP in Caco-2 cells. BMS-200150 has been identified in a screening for compounds with MTP inhibiting activity as a potent inhibitor of MTP-mediated transfer of lipid between small unilamellar vesicles in vitro with an IC_{50} of 0.6 μM. In HepG2 cells, secretion of apoB is decreased by BMS-200150 in direct proportion to inhibition of MTP with an IC_{50} of 1.8 μM (15). Dietary fatty acid species may vary in their effect on the secretion of lipoproteins into the lymph. As MTP has been postulated to be involved in translocation of triglycerides into lipoproteins, we asked the question whether a variable preference of MTP for transfer of triglycerides synthesized during incubations with various fatty acids regulates the density of lipoproteins that are secreted. Palmitic (16:0) and oleic (18:1) acid are two major constituents of human dietary fat. We have shown in earlier publications (12, 13) that Caco-2 cells incubated with oleic acid synthesize lipoproteins at density (d) <1.006 g/ml, whereas stimulation of lipoprotein secretion with palmitic acid results in secretion of lipoproteins at two distinctly different density ranges (i.e., chylomicron/VLDL; $d < 1.006$ g/ml and IDL/LDL; $1.009 < d < 1.068$ g/ml). We determined the effects of inhibition of MTP activity with BMS-200150 on lipoprotein secretion with either unsaturated fatty acids (oleic acid) or with saturated fatty acids (palmitic acid) present as stimulator of cellular triglyceride synthesis in the incubation medium.

The next question we sought to answer in this report is to distinguish between two different hypotheses on the role that MTP plays in the assembly of lipoproteins in Caco-2 cells. First, that MTP activity is only required in the initial step of lipoprotein assembly in the cell, lipidation of apoB on the endoplasmic reticulum membrane (model 1). The alternative hypothesis is that MTP activity not only mediates the first step of assembly, but is also required in additional steps in the lipidation of the primordial lipoprotein during lipoprotein assembly (model 2). To distinguish between these two hypotheses, experiments were performed in which the triglyceride secretion was restricted to 30% of normal values using MTP inhibition with BMS-200150 as a tool. This inhibition was partial, not complete, to allow a reduced secretion of lipoproteins by the cells. From the number of lipoproteins secreted during incubation with the MTP inhibitor, and from their triglyceride contents, we deduced the role that MTP plays in the intracellular assembly of lipoproteins in the cells. If model 1 is true, then inhibition of MTP activity in the cells causes a decreased co-translational lipidation of apoB on the endoplasmic reticulum. This would result in an increased intracellular degradation of apoB and secretion of fewer lipoproteins into the medium. Fewer cellular triglycerides will then be used for the rescue of apoB during inhibition of MTP activity and equal or even higher amounts of cellular triglycerides will be available for further lipidation of the lipoproteins along the secretory pathway. The triglyceride-enriched lipoproteins that then are secreted from these cells will have identical or even lesser densities than lipoproteins secreted in the absence of the inhibitor. If model 2 is true, inhibition of MTP activity with BMS-200150 would first reduce initial lipidation of apoB and thus reduce lipoprotein secretion. Additionally, it would decrease the further association of the nascent dense lipoproteins with lipid in the endoplasmic reticulum lumen, leading to more dense lipoproteins in the medium.

Finally, it was our aim to distinguish between the effects of offering a reduced amount of intracellular lipid in the cells and, on the other hand, the effect of specific MTP inhibition with BMS-200150. Therefore, additional experiments were performed in which cells were incubated with low concentrations of fatty acids to obtain a condition of reduced supply of lipids (especially triglycerides) in the cells, not via specific inhibition of MTP, but by a reduction in total amount of intracellular lipids.

EXPERIMENTAL PROCEDURES

Materials

[1-^{14}C]oleic acid (2.1 GBq/mmol) was purchased from New England Nuclear (Boston, MA). Palmitic acid and oleic acid (both 99% pure by gas chromatography) and essential fatty acid-free BSA were obtained from Sigma (St. Louis, MO). Isopropanol (HPLC grade) was purchased from Riedel de Haehn (Seelze, Germany). BMS-200150 was a kind gift from the Bristol-Myers Squibb company (Bristol-Myers Squibb, Princeton, NJ).

Cell culture

Caco-2 cells (16) of intermediate passages (p70–p90) were cultured in DMEM supplemented with 20% heat inactivated FCS and 1% nonessential amino acids (Gibco BRL, Grand Island, NY). Cells were subcultured weekly in 75 cm^2 culture flasks (Costar, Cambridge, MA) as described (17). For experiments, cells were cultured in medium supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco BRL) on microporous membranes of 0.45 μm pore size and 24.5 mm diameter (Transwel COL™, Costar, Cambridge, MA). Cells were seeded at a surface dilution of 1:3 from stock cultures in 75 cm^2 flasks. Medium was changed on days 3, 6, 8, 10, and 13 after seeding. Confluence of the monolayers was visually determined by phase-contrast microscopy. Experiments were performed on days 14 or 15 after plating, when monolayers were 5–7 days post-confluence.

Assay conditions for incubation of cells with fatty acids and MTP inhibitor

Fatty acids were complexed to fatty acid-free BSA in serum-free (SF) DMEM as described (12). Final concentrations of fatty acids in the incubation media did not exceed 0.5 mM unless stated otherwise. Fatty acid:BSA ratio is 7.5:1 (mol/mol) in all incubations. When used, [1-^{14}C]oleic acid was added to the fatty acid stock solutions. The contribution of radiolabeled fatty acid tracer to the total amount of fatty acid in the apical incubation media did not exceed 0.25 mol%, except for the experiment described in Fig. 4 (1 mol%). The fatty acid/BSA complexes were diluted in SF DMEM when fatty acid concentrations lower than 0.5 mM were used, in order to maintain a constant fatty acid:BSA ratio in all incubations.

A 16 μM stock solution of the MTP-inhibiting compound BMS-200150 (15) was prepared in DMSO and stored in aliquots at −80°C. DMSO concentration in all incubation media was 0.05% (vol/vol), independent of the concentrations of BMS-200150 used. Confluent Caco-2 monolayers were rinsed three times with SF DMEM and preincubated for 30 min at 37°C with SF DMEM containing BMS-200150 to allow equilibration of the inhibitor with MTP in the cells. For experiments, cells were incubated with 1.5 ml apical medium (SF DMEM containing both fatty acid/BSA complexes and the MTP inhibitor) and 3.0 ml basolateral medium (SF DMEM containing the inhibitor). Cells were incubated with *a*) 0.5 mM fatty acid without BMS-200150 (control), *b*) 0.5 mM fatty acid without BMS-200150 (inhibition of MTP), or *c*) 0.1 or 0.05 mM fatty acid without BMS-200150 (reduced lipid availability); fatty acids used were either oleic or palmitic acid.

The experimental interventions were chosen to obtain a similar degree of inhibiting secretion of cellular triglycerides either by specifically inhibiting MTP activity, or by reducing the total amount of cellular lipid that is available for lipoprotein synthesis. In those incubations which were designed to reach reduced lipid availability, fatty acid concentrations utilized were 0.1 mM in non-radiolabeled incubations (this was the minimal amount of fatty acid necessary to obtain measurable levels of triglyceride mass); 0.05 mM fatty acids were utilized in incubations with a radiolabeled tracer present. After an incubation for 18 h, apical and basolateral media were collected and protease inhibitors were added (EDTA and epsilon aminocaproic acid, final concentrations 2 mM). Media were centrifuged (1200 *g*, 15 min) to remove cellular debris and assayed immediately. Cells on filters were rinsed with ice-cold PBS and lysed for 30 min in 300 μl apical and 200 μl basolateral lysis buffer (PBS containing 1% TX100, 0.1% SDS, 1 mM EDTA, 1 mM PMSF, 0.02% NaN_3) on ice. Lysates were collected and vortexed vigorously. Nuclei were pelleted (8000 *g*, 3 min) and cell lysates were transferred to new vials and analyzed immediately or stored at −20°C. Cellular cholesterol and triglycerides and triglycerides in the media were determined as described (13). For determination of secreted phospholipids, 1 ml of basolateral medium was extracted (18). The organic phase containing the phospholipids was washed with 3 ml of 20 mM acetic acid to remove all water-soluble phosphate from the organic phase and phospholipids were determined according to Rouser, Fleischer, and Yamamoto (19). Cell monolayers were rinsed with NaCl for determination of cellular phospholipids. Total lipid was extracted from cells on filters as described (13) and cellular phospholipids were determined (19). Cellular levels of MTP mRNA were assayed in a total RNA fraction which was extracted from the Caco-2 monolayers on filters using TRIzol reagent (Gibco BRL, Grand Island, NY). Three hundred μl of TRIzol reagent was added to the apical side and 200 μl to the basolateral side of the monolayers. RNA was extracted

from these cell lysates according to manufacturer's instructions. MTP mRNA levels in these samples were determined using a RNAse protection assay, as described (20). MTP protein in the cells was detected by Western blotting of a total protein fraction of Caco-2 cells that had been incubated with 0.5% BSA, 0.5 mM palmitic acid, or 0.5 mM oleic acid. Equal amounts of cell lysates were separated on 4–15% SDS PAGE, transferred to nitrocellulose and MTP protein was detected using a polyclonal rabbit-anti-MTP large subunit peptide antiserum ((21), courtesy Dr. Carol Shoulders, London, UK).

Quantification of apoB-48 and apoB-100

The amounts of apoB-100 and apoB-48 secreted into the basolateral medium were determined on SDS PAGE according to the method of Karpe and Hamsten (22) with some modifications. For delipidation, 1 ml basolateral medium was pipetted into 4 ml of methanol and vortexed immediately. Four ml of ice-cold diethylether was added, mixed well, and protein was pelleted by centrifugation for 48 min at 1°C (2500 *g*, zero brake rate). The supernatant was aspirated and a second ml of medium was delipidated in the same reaction tube repeating this procedure. Protein pellets were rinsed with 4 ml of ice-cold diethylether and pelleted for 32 min. Supernatant was aspirated and pellets were dried. Protein was allowed to dissolve in 75 μl sample buffer (0.15 M sodium phosphate, 12.5% glycerol, 2% SDS, 5% β-mercaptoethanol, 0.005% bromophenolblue) for 1 h at room temperature, transferred to small vials and heated to 80°C for 10 min, and stored at −20°C until analysis on gel (samples were never stored for more than 1 week). Proteins were separated on 5% running/3% stacking SDS PAGE (22) using the Bio-Rad Mini Protean II configuration (Bio-Rad, Hercules, CA). Twenty-five μl of sample was applied in duplicate to the gel. ApoB-100 (>95% pure) isolated from LDL was used as a standard (2.0 to 0.05 μg/lane). Gels were stained in colloidal Coomassie stain G250 (NOVEX, San Diego, CA) and the integrated optical densities (i.o.d.) of apoB-100 and apoB-48 were determined (23). Within and between assay coefficients of variation were 5% and 8%, respectively.

Composition of secreted lipoproteins

To determine the density of lipoproteins secreted by Caco-2 cells, lipoproteins present in the basolateral medium of cells incubated with [^{14}C]oleic acid (7.4 KBq/filter) present as tracer, were separated by ultracentrifugation in a d 1.006–1.250 g/ml KBr density gradient (12, 24). Fractions of 0.5 ml were aspirated, mixed with 3 ml of Ultima Gold (Packard, Meriden, CT) and counted in a Packard 1900 CA Tri Carb liquid scintillation counter.

Lipoproteins secreted into the basolateral medium in incubations without radiolabeled fatty acid were separated as described above for determination of the mass of triglycerides present in lipoproteins of different densities. One ml of fractions were aspirated and total lipid was extracted according to the method of Bligh and Dyer (18), followed by determination of triglyceride mass, as described (13).

Additional analyses were done to characterize the lipoproteins secreted in the distinct density fractions during incubations in the presence of palmitic acid. The amount of triglycerides and phospholipids present in lipoprotein fractions of chylomicron/VLDL and IDL/LDL densities were determined using 30 KBq [^{14}C]oleic acid to sufficiently label the lipids for detection. Density fractions of the basolateral media were extracted and neutral lipids in the extracts were separated on one-dimensional TLC (12). Triglyceride and phospholipid spots were identified and radioactivity was measured. After incubations without radiolabeled tracer, the two density fractions were delipidated, proteins were separated on 4–15% SDS PAGE and blotted on nitrocellulose using the phast-system configuration (Pharmacia, Uppsala, Sweden). ApoB-48 and apoB-100 were identified using the anti-human apoB monoclonal antibody 2D8 (25).

Cell morphology

Monolayers of Caco-2 cells were fixed at the end of the incubations in 0.1 M PIPES containing 2% paraformaldehyde and 0.2% glutaraldehyde. Ultrastructural morphology of the cells was studied after freeze substitution according to the method of van Genderen et al. (26).

Statistical analysis

All values are expressed as mean ± standard deviation (SD) unless stated otherwise. Mean differences between groups were calculated by unpaired Student's *t*-tests. Statistical significance was defined as $P < 0.005$ (two-tailed).

RESULTS

Polarized Caco-2 cells were incubated with 0.5% BSA, 0.5 mM oleic acid, or 0.5 mM palmitic acid and presence of MTP was demonstrated as follows. MTP protein was detected in cell lysates on Western blot (**Fig. 1**). Unexpectedly, no difference was seen in cellular levels of MTP in the incubations, despite the differences in basolateral triglyceride secretion in these incubations (20, 6, and 50 nmol/filter with palmitic acid,

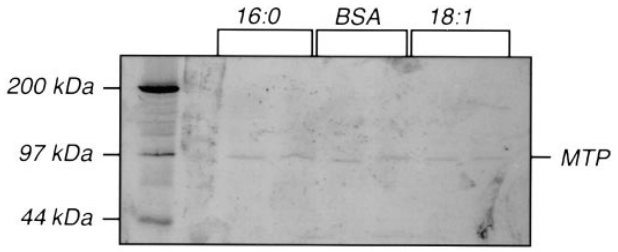

Fig. 1. MTP expression in Caco-2 cells. Immunoblot of total Caco-2 cell protein after 18 h incubation with 0.5% BSA, 0.5 mM palmitic acid (16:0), or 0.5 mM oleic acid (18:1). Equal amounts of cell lysate were separated on 4–15% SDS PAGE, transferred to nitrocellulose, and MTP was detected using a polyclonal rabbit anti-MTP large subunit peptide antiserum (21). Results of two independent experiments are shown in adjacent lanes.

BSA, and oleic acid, respectively). MTP mRNA was determined in Caco-2 cells (20) and, consistent with the results on MTP protein, there was no correlation between MTP mRNA levels and secretion of triglycerides, apoB-100, or apoB-48 (data not shown). To show the presence of functional MTP, Caco-2 cells were incubated with increasing concentrations of the specific MTP inhibitor BMS-200150 to determine whether lipoprotein secretion could be inhibited. BMS-200150 effectively inhibited secretion of lipoproteins from polarized Caco-2 cells with an IC_{50} of 2 μM for triglyceride secretion, measured in an 18 h incubation (**Fig. 2**). BMS-200150 did not affect the uptake of radiolabeled oleic acid, present as tracer during incubation with 0.5 mM palmitic or oleic acid (data not shown).

Basolateral triglyceride secretion, without inhibition of MTP, was 43.5 ± 4.7 nmol/filter in incubations with oleic acid in the apical medium and 16.2 ± 0.6 nmol/filter with palmitic acid (Fig. 2 panel A). The total mass of triglycerides secreted into the basolateral medium in incubations was 2 to 2.5-times higher with oleic acid than with palmitic acid, in all incubations. The inhibition of triglyceride secretion by BMS-200150 (expressed as percentage of the control incubations) was independent of the species of fatty acid used to stimulate cellular triglyceride synthesis. Incubation of cells with 0.8–3.0–6.0 μM BMS-200150 and 0.5 mM oleic acid, reduced triglyceride secretion to 70%–35%–21%, respectively, of the secretion with 0.5 mM oleic acid alone. Reduction of triglyceride secretion with 0.8–3.0–6.0 μM BMS-200150 in the presence of palmitic acid was 64%–44%–33%, respectively, compared with 0.5 mM palmitic acid. The BMS-200150 concentrations (0 to 6 μM) used in these experiments did not decrease the secretion of apoB-48 into the medium and had only a mild inhibiting effect on the secretion of apoB-100 (Fig. 2 panel B). Maximum inhibition of apoB-100 was 26% of control

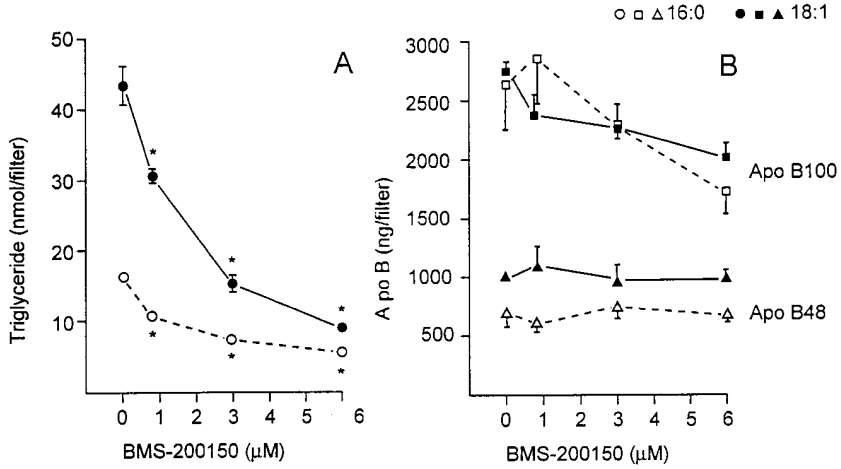

Fig. 2. Secretion of triglycerides, apoB-100 and apoB-48 in the basolateral medium of the Caco-2 cells incubated with increasing concentrations of BMS-200150. Cells were incubated for 18 h with 0–0.8–3.0–6.0 μM BMS-200150 in the presence of either 0.5 mM oleic acid (closed symbols) or 0.5 mM palmitic acid (open symbols) in the apical incubation medium for stimulation of lipid synthesis. Inhibition of triglyceride secretion (panel A) was significant with all concentrations of BMS-200150 used (*$P < 0.01$), inhibition of apoB-100 secretion (panel B) was significant with 6 μM BMS-200150 and 0.5 μM oleic acid in the apical medium (*$P < 0.01$). Inhibition of apoB-100 secretion with 6 mM BMS-200150 and palmitic acid was borderline significant ($P = 0.096$). The inhibition of the secretion of triglycerides was more pronounced than the inhibition of apoB-100 secretion i.e., 75% for triglycerides and 30% for apoB-100 with 6 μM BMS-200150. No effect was seen of BMS-200150 on apoB-48 secretion (panel B). Data are the average of at least three different measurements ± SEM.

TABLE 1. Size of secreted lipoproteins

BMS-200150	Triglycerides/Total apoB Ratio: Oleic Acid	Triglycerides/Total apoB Ratio: Palmitic Acid	n
μM	*nmol/mg*		
0	11625 ± 1427	4961 ± 1005	6
0.8	8833 ± 1211	3046 ± 395	6
3.0	4835 ± 1490[a]	2374 ± 148	3
6.0	2965 ± 291[a]	2242 ± 208	3

The size of the lipoproteins recovered in the basolateral medium was estimated by calculating the ratio of secreted triglycerides (nmol/filter) and the total apoB mass. Total apoB mass is the sum of apoB-48 (mg/filter) and apoB-100 (mg/filter) determined on SDS PAGE as described in Experimental Procedures. This ratio decreased with increasing BMS-200150 concentrations, indicating secretion of more dense lipoproteins ([a]$P < 0.05$).

($P < 0.01$) with oleic acid and 34% ($P = 0.1$) with palmitic acid. ApoB-48 secretion was 101% and 96% of control in the presence of either oleic acid or palmitic acid and 6 μM of MTP inhibitor. When concentrations of BMS-200150 were increased to 8 μM or more, a small inhibition of apoB-48 secretion was observed, but this was always less than the inhibitory effect on apoB-100 secretion (data not shown). As an estimate of the size of the secreted lipoproteins, we calculated the ratio of triglycerides/apoB (nmol/mg) in the basolateral medium (**Table 1**). This ratio was lower with increasing concentrations of BMS-200150, suggesting that decreasing MTP activity resulted in the secretion of more dense lipoproteins.

Phospholipids and triglycerides in cells and media

After incubations with 0.5 mM fatty acid with or without 3 μM BMS-200150 or during reduced cellular lipid availability (0.1 mM fatty acid), total cellular and secreted phospholipids and triglycerides were determined (**Table 2**). Intracellular triglyceride concentrations were higher after incubation with oleic acid than with palmitic acid, both in control incubations and during inhibition with oleic acid than with palmitic acid, both in control incubations and during inhibition of MTP. A 70% decrease of triglyceride secretion with BMS-200150 did not cause accumulation of cellular triglycerides in Caco-2 cells. In fact, 3 mM BMS-200150 caused a decrease in total (cellular plus basolateral) triglyceride mass in the incubations to 124 ± 18 nmol/filter, compared with 177 ± 23 controls with oleic acid ($P = 0.03$), and to 78 ± 12 nmol/filter compared with 89 ± 9 ($P = 0.08$) in controls with palmitic acid. Intracellular triglycerides in incubations with 0.1 mM fatty acid did not differ between oleic acid (64 ± 4 nmol/filter) and palmitic acid (75 ± 12 nmol/filter), probably because cellular triglyceride synthesis in both incubations is minimal (for comparison: basal cellular triglyceride levels in incubations with BSA without fatty acid is 55–60 nmol/filter).

The efficiency of secretion of triglycerides by the cells was defined as the amount of triglycerides that was recovered in the basolateral medium expressed as percentage of total (intracellular plus basolateral) triglycerides in the incubations. Both the mass of triglycerides

TABLE 2. Intracellular and secreted mass of the major lipid components of lipoproteins

	Oleic Acid Incubations			Palmitic Acid Incubations		
	0.5 mM Fatty Acid	0.1 mM Fatty Acid	0.5 mM Fatty Acid + 3 μM BMS-200150	0.5 mM Fatty Acid	0.1 mM Fatty Acid	0.5 mM Fatty Acid + 3 μM BMS-200150
Cellular triglycerides (nmol/filter)	102 ± 12	65 ± 4[b]	102 ± 18[a]	80 ± 23	74 ± 12	62 ± 11
	(100%)	(−36%)	(0%)	(100%)	(−7%)	(−22%)
Secreted triglycerides (nmol/filter)	57.9 ± 12.0[a]	16.7 ± 1.6[a,b]	18.3 ± 3.3[a,b]	27.6 ± 8.6	11.0 ± 0.6[b]	7.9 ± 2.9[b]
	(100%)	(−71%)	(−68%)	(100%)	(−60%)	(−71%)
Secretion efficiency	36.1 ± 5.1[a]	20.4 ± 2.4[a,b]	15.5 ± 3.8[a,b]	25.7 ± 1.9	13.2 ± 2.0[b]	10.9 ± 2.3[b]
	(100%)	(−43%)	(−57%)	(100%)	(−48%)	(−57%)
Cellular phospholipids (nmol/filter)	201 ± 31	237 ± 9	166 ± 12	215 ± 61	241 ± 7	175 ± 15
	(100%)	(+18%)	(−17%)	(100%)	(+12%)	(−19%)
Secreted phospholipids (nmol/filter)	12.9 ± 1.2[a]	5.6 ± 1.1[b]	5.8 ± 1.3[a,b]	6.1 ± 0.5	3.3 ± 0.3	3.0 ± 0.8[b]
	(100%)	(−57%)	(−55%)	(100%)	(−46%)	(−51%)
Secretion efficiency	6.2 ± 1.0[a]	2.3 ± 0.3[a,b]	3.3 ± 0.5[a,b]	2.9 ± 0.7	1.4 ± 0.1[b]	1.7 ± 0.4[b]
	(100%)	(−63%)	(−47%)	(100%)	(−52%)	(−41%)

Mass of triglycerides and phospholipids was determined in cells and media after incubation with 0.5 mM either oleic or palmitic acid without BMS-200150 (0.5 mM, n = 6), with 3 μM BMS-200150 (BMS, n = 3), or with 0.1 mM oleic or palmitic acid without BMS-200150 (0.1 mM, n = 3). Secretion efficiency was calculated for triglyceride and phospholipid secretion (secretion efficiency: mass in basolateral medium/mass in cells plus basolateral medium × 100%). Data expressed as percentage of change compared with the 0.5 mM fatty acid values are given in parentheses (positive % represent increases, negative % represent decreases vs. 0.5 mM fatty acid).

[a] Differences observed between incubations with oleic vs. palmitic acid: $P < 0.05$, 0.5 mM vs. 0.5 mM, 0.1 mM vs. 0.1 mM, and BMS vs. BMS. Borderline significant differences were seen in cellular triglycerides in 0.5 mM fatty acid (oleic vs. palmitic acid, $P = 0.07$), and in phospholipids secreted into the medium with 0.1 mM fatty acid (oleic vs. palmitic acid. $P = 0.06$).

[b] Differences were also significant between 0.1 mM fatty acid or BMS vs. the 0.5 mM incubations with the corresponding fatty acid, $P < 0.05$.

(nmol/filter) secreted into the basolateral medium and the triglyceride secretion efficiency (% of total triglycerides) were higher in incubations with oleic acid (57.9 nmol/filter and 36.1 ± 5.1%, respectively) than with palmitic acid (27.6 ± 8.6 nmol/filter and 25.7 ± 1.9%, respectively). Both inhibition of MTP activity and reduced cellular lipid availability resulted in a strong reduction of the secretion efficiency of the cells.

Secretion of phospholipids (nmol/filter) was also higher with oleic acid (12.9 ± 1.2 nmol/filter), than with palmitic acid (6.1 ± 0.5 nmol/filter), paralleling data on triglyceride secretion (Table 2). Phospholipid secretion efficiency was much lower than the secretion efficiency of triglycerides. Phospholipids are a functional component of cells and thus only a small percentage of cellular phospholipids is secreted. Both inhibition of MTP activity and reduced lipid availability caused a decrease in the secretion efficiency of phospholipids.

Density of secreted lipoproteins

To characterize the effect of inhibition of MTP on lipoprotein density, lipoproteins secreted into the basolateral medium were separated on a discontinuous KBr gradient (**Fig. 3**, panel E). Cells were incubated with 0.5 mM fatty acid (oleic or palmitic acid) and 0, 0.8, or 3 μM BMS-200150. As [^{14}C]oleic acid is incorporated into both triglycerides and phospholipids in cells, we were unable to discriminate between these lipid classes in the density fractions when radiolabeled fatty acid was used. It was therefore inconclusive whether differences in total radioactivity in the density fractions were the result of a redistribution of tracer among the lipid classes in the secreted lipoproteins or represented actual changes in the amount of lipoprotein-associated triglycerides. To characterize possible differences in lipoprotein composition as an underlying cause of the shift in lipoprotein density, the mass of triglycerides in the density fractions of the basolateral media of non-radiolabeled incubations was determined. The mass of triglycerides (nmol) was determined in gradient fractions and expressed as % of total triglycerides recovered in the gradient (Fig. 3, panels C and D). Likewise, when radiolabeled tracer was used, total radioactivity in the fractions was determined and expressed as % of total dpm in the gradient (Fig. 3, panels A and B). Triglyceride secretion in incubations with 0.5 mM fatty acid was inhibited with BMS-200150. Three μM caused 70% inhibition of triglyceride secretion (Fig. 2, panel A). To obtain levels of basolateral triglyceride secretion that were also 60–70% lower than the control incubations with 0.5 mM fatty acid, cells were incubated with a low concentration (0.1 mM) of fatty acid without the MTP inhibitor.

Approximately 60% of secreted lipoproteins were recovered at $d < 1.006$ g/ml when oleic acid was used to stimulate cellular lipid synthesis. Both inhibition of MTP activity and decreased total lipid availability caused a substantial decrease of these triglyceride-rich lipoproteins present at $d < 1.006$ g/ml (Fig. 3, panels A and C). With BMS-200150, this reduction was both absolute (lower amount of radioactive label and nmol triglycerides in the $d < 1.006$ g/ml fraction) and relative (only 20–40% of total secreted lipoproteins was recovered at $d < 1.006$ g/ml). When palmitic acid was used to stimulate lipid synthesis, lipoproteins secreted in the two density fractions (chylomicron/VLDL; $d < 1.006$ g/ml and IDL/LDL; $1.009 < d < 1.063$ g/ml) were affected in different ways (Fig. 3, panels B and D). The limitation of total cellular lipid availability resulted in a decrease of lipoproteins in the IDL/LDL fraction, but had less effect on lipoproteins secreted at $d < 1.006$ (Fig. 3, panels B and D); the amount of lipoproteins secreted into the two density fractions expressed as percentage of total secreted lipoproteins in the gradient was 20–40% versus 20–40% in the chylomicron/VLDL fraction, and 50–60% versus 20–30% in the IDL/LDL fraction in control versus 0.1 mM palmitic acid, respectively). BMS-200150 caused exactly the opposite effect, which was reflected by a specific reduction of lipoproteins at $d < 1.006$ (the amount of lipoproteins secreted into the two density fractions expressed as percentage of total secreted lipoproteins in the gradient was 20–40% versus 2–10% in the chylomicron/VLDL fraction, and 50–60% versus 50–60% in the IDL/LDL fraction in control versus BMS-200150 incubations, respectively). Additionally, incubation with BMS-200150 resulted in a shift of the IDL/LDL density peak toward higher density (1.02 g ml to 1.03 g/ml, Fig. 3, panels B and D). The incorporation of ^{14}C-labeled fatty acid in the IDL/LDL density peak was relatively high compared with control. Combined with the lower mass of secreted triglycerides (Fig. 3, panel D), and the shift of density of the peak of d 1.02 g/ml to d 1.03 g/ml, the present data provide evidence of a change in lipoprotein composition. This compositional change, presumably a lower triglycerides/phospholipids ratio of the lipoproteins, was studied in further detail.

Compositional changes in lipoprotein

The ratio of total basolateral triglyceride and phospholipid mass in the media was higher with oleic acid than with palmitic acid, and also higher in controls than in the presence of BMS-200150. These data, however, do not provide information on the composition of the lipoproteins in the two separate density fractions that were isolated from medium in the incubations with palmitic acid. As the mass of phospholipids in these fractions was too low to be detected, [^{14}C]oleic

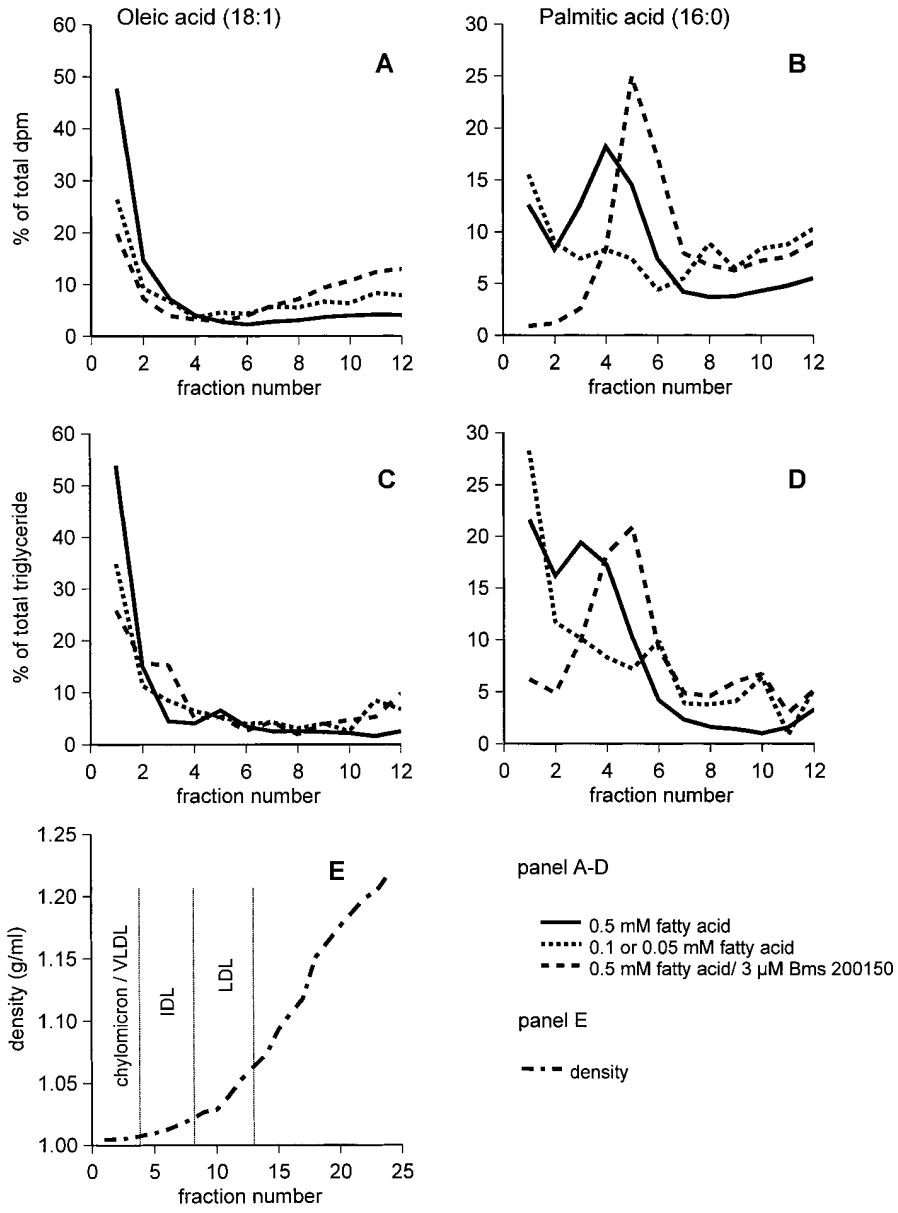

Fig. 3. Characteristics of secreted lipoproteins in density gradient analysis. Cells were incubated with 0.5 mM fatty acid with or without 3 μM BMS-200150 or with 0.1 mM fatty acid. Fatty acids used were oleic acid or palmitic acid. Incubations were performed either with 7.4 kBq [^{14}C]oleic acid present as tracer or without tracer. Lipoproteins secreted into the basolateral medium were separated on a d 1.006–1.250 g/ml KBr density gradient (density distribution in panel E). In radiolabeled experiments, 1.0 ml fractions were aspirated and radioactivity was quantified. In independent experiments without radiolabeled tracer, 1 ml fractions were aspirated, total lipid was extracted, and triglyceride mass was determined enzymatically. Data are expressed as % of total radioactivity (panels A and B) or as % of total triglyceride mass (panels C and D) in the gradient. Data in each panel are the mean of at least two independent experiments. Note the differences in scale in the incubations with oleic acid and with palmitic acid. Panels A and C (oleic acid): Density distribution of the lipoproteins recovered in the basolateral medium. Total radioactivity recovered in the gradient was 36600 ± 10140 dpm/filter (0.5 mM oleic acid), 29630 ± 2770 dpm/filter (0.05 mM oleic acid), or 11900 ± 2820 dpm/filter (0.5 mM oleic acid with 3 μM BMS-200150). Total triglycerides recovered in the gradient were 57.7 ± 0.5 nmol/filter (0.5 mM oleic acid), 15.2 ± 0.2 nmol/filter (0.1 mM oleic acid) and 22.0 ± 0.1 nmol/filter (0.5 mM oleic acid with 3 μM BMS-200150). Panels B and D (palmitic acid): Density distribution of secreted lipoproteins recovered in the basolateral medium. Two peaks of lipoproteins are present in the incubations with 0.5 mM palmitic acid, one of chylomicron/VLDL density and one of IDL/LDL density. Total radioactivity recovered in the gradient was 19120 ± 2760 dpm/filter (0.5 mM palmitic acid), 26520 ± 3430 dpm/filter (0.05 mM palmitic acid), or 11920 ± 490 dpm/filter (0.5 mM palmitic acid with 3 μM BMS-200150). Total triglycerides recovered in the gradient were 29.0 ± 6.7 nmol/filter (0.5 mM palmitic acid), 8.9 ± 1.0 nmol/filter (0.1 mM palmitic acid) and 11.6 ± 2.6 nmol/filter (0.5 mM palmitic acid with 3 μM BMS-200150). Panel E: Density (g/ml) in 0.5 ml fractions. Chylomicron VLDL; fractions 1–4, IDL; fractions 5–8, LDL; fractions 9–13.

acid was added as tracer in incubations with 0.5 mM palmitic acid with or without 3 μM inhibitor or with 0.1 mM palmitic acid. Lipoproteins of chylomicron/VLDL density (fraction 1; d < 1.006 g/ml) and of IDL/LDL density (fraction 2; 1.013 < d < 1.022 g/ml in controls, 1.022 < d < 1.029 g/ml with 0.1 mM palmitic acid or 3 μM BMS-200150, as a result of the density shift) were isolated and triglycerides and phospholipids in the two fractions were quantified (**Fig. 4**). In each of the three incubations, the triglycerides/phospholipids ratio was higher in fraction 1 than in fraction 2 (4.0 ± 0.3 vs. 2.3 ± 0.1, 6.0 ± 1.3 vs. 1.0 ± 0.1, and 3.3 ± 1.4 vs. 1.5 ± 0.2, respectively, in controls, 0.1 mM palmitic acid, and BMS-200150 incubations). Total triglycerides (dpm fraction 1 plus dpm fraction 2) were 4210 ± 390, 1280 ± 630, and 1240 ± 120 dpm in controls, with 0.1 mM palmitic acid, or with 3 μM BMS-200150, respectively. Total phospholipids (dpm fraction 1 plus dpm fraction 2) were 1600 ± 190, 300 ± 90, and 820 ± 180 dpm in these incubations. Thus, 3 μM BMS-200150 in the medium caused a 71% inhibition of triglyceride secretion combined with 49% inhibition of phospholipid secretion into the basolateral medium and these data are consistent with the percentages of inhibition of total triglycerides and phospholipids measured as mass (nmol) presented in Table 2. On the other hand, incubations with low (0.1 mM) supply of palmitic acid without BMS-200150 resulted in similar inhibition of triglyceride secretion, i.e., 70% of control, but much stronger inhibition of phospholipid secretion, i.e., 81% of control, than seen with 3 μM BMS-200150, suggesting that the secretion of newly synthesized phospholipids is specifically reduced when limited amounts of fatty acids are available for cellular lipid synthesis. We verified that the triglycerides and phospholipids detected in the two density fractions are genuine lipoproteins by showing the presence of immunoreactive apoB in both frac-

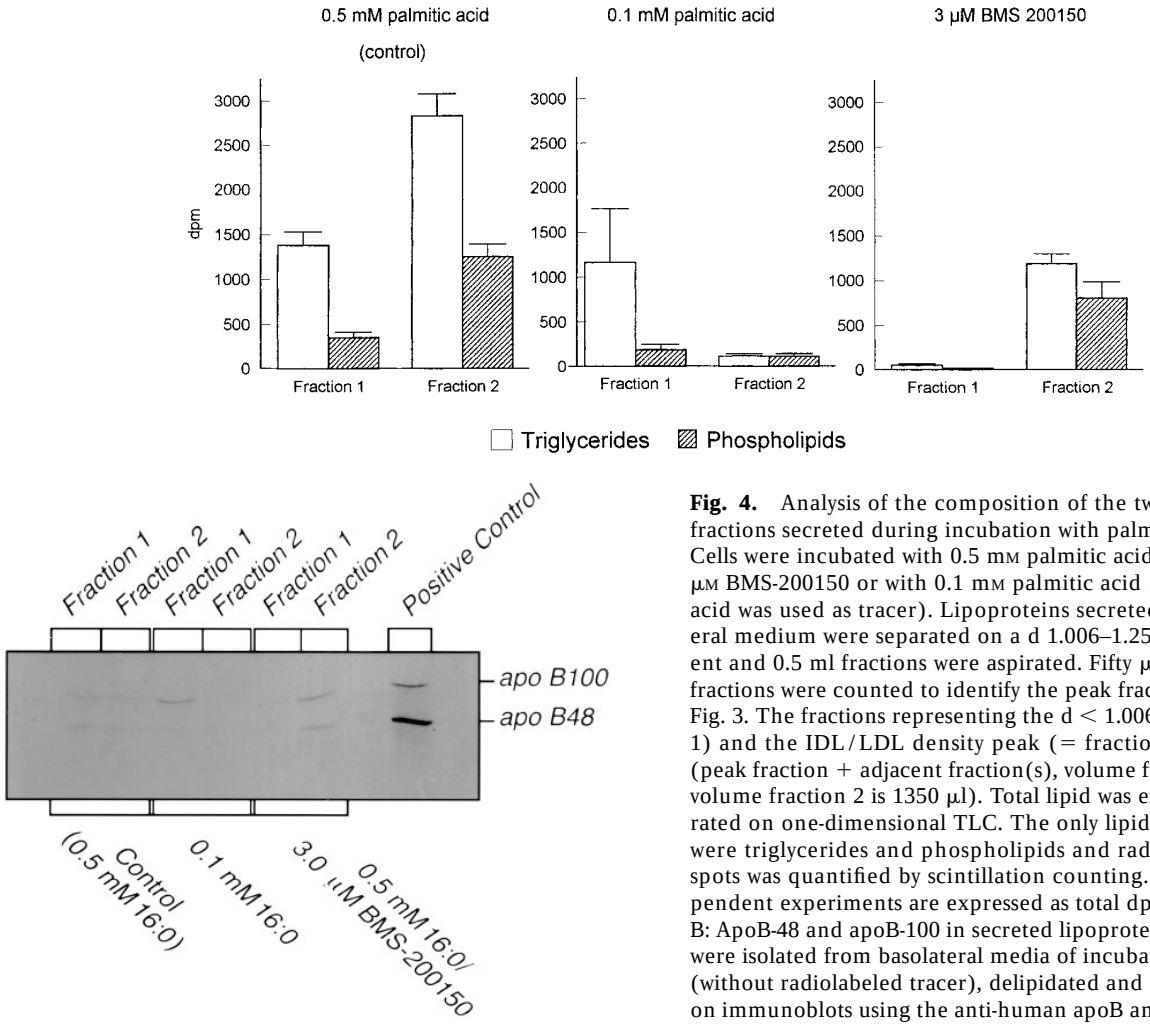

Fig. 4. Analysis of the composition of the two distinct density fractions secreted during incubation with palmitic acid. Panel A: Cells were incubated with 0.5 mM palmitic acid with or without 3 μM BMS-200150 or with 0.1 mM palmitic acid (30 kBq[^{14}C]oleic acid was used as tracer). Lipoproteins secreted into the basolateral medium were separated on a d 1.006–1.250 g/ml KBr gradient and 0.5 ml fractions were aspirated. Fifty μl aliquots of these fractions were counted to identify the peak fractions described in Fig. 3. The fractions representing the d < 1.006 g/ml (= fraction 1) and the IDL/LDL density peak (= fraction 2) were pooled (peak fraction + adjacent fraction(s), volume fraction 1 is 950 μl, volume fraction 2 is 1350 μl). Total lipid was extracted and separated on one-dimensional TLC. The only lipid classes detectable were triglycerides and phospholipids and radioactivity in these spots was quantified by scintillation counting. Data of two independent experiments are expressed as total dpm/fraction. Panel B: ApoB-48 and apoB-100 in secreted lipoproteins. The two peaks were isolated from basolateral media of incubations as in panel A (without radiolabeled tracer), delipidated and apoB was detected on immunoblots using the anti-human apoB antibody 2D8.

tions (Fig. 4, bottom). The amount of apoB in the density fractions paralleled the concentration of triglycerides. In the control incubations, apoB was present in both fractions (fraction 1; chylomicron/VLDL density, fraction 2; IDL/LDL density). With low cellular lipid supply, apoB was low in both fractions, most strongly reduced in fraction 2. With 3 μM BMS-200150, apoB was hardly detectable in fraction 1 but clearly present in fraction 2.

Effects of BMS-200150 on morphology Caco-2 cells

Morphology of the Caco-2 cells was examined by electron microscopy. An absence of MTP activity in intestinal cells (as in α-betalipoproteinemia) is known to be associated with the presence of intercellular lipid droplets (7). **Figure 5** shows the morphology of the control Caco-2 cells after incubation with 0.5 mM oleic acid (Fig. 5, panel A) or with 0.5 mM palmitic acid (Fig. 5, panel B). Triglyceride secretion in incubations with 0–2–6 μM BMS-200150 was 52.3 ± 2.4, 24.6 ± 1.2, or 12.2 ± 6.8 nmol/filter, respectively, with 0.5 mM oleic acid, and 39.2 ± 2.0, 20.8 ± 0.3, or 20.5 ± 2.4 nmol/filter with 0.5 mM palmitic acid. No accumulation of lipid or lipid droplets was seen with either fatty acid with any of the concentrations of BMS-200150 used.

We tried to induce the "a-betalipoproteinemia" phenotype in the cells by incubating with 8 μM BMS-200150 in the presence of a high concentration (1.0 mM) of oleic acid. Triglyceride secretion with 1.0 mM oleic acid was 94.2 ± 6.0 nmol/filter and this was reduced to 15.3 ± 10.0 nmol/filter with the MTP inhibitor; at the same time triglycerides increased from 194 ± 12 nmol/filter to 236 ± 15 nmol/filter. These conditions did not induce any morphologic changes in the cells (Fig. 5, panel C). Interestingly, in agreement with the observed morphology and our present data on total triglycerides after incubations with BMS-200150, total (cellular plus basolateral) triglycerides was *lower* in the incubations with 8 μM BMS-200150 than with 1.0 mM oleic acid alone (245 ± 17 vs. 292 ± 16 nmol/filter, respectively, $P = 0.025$).

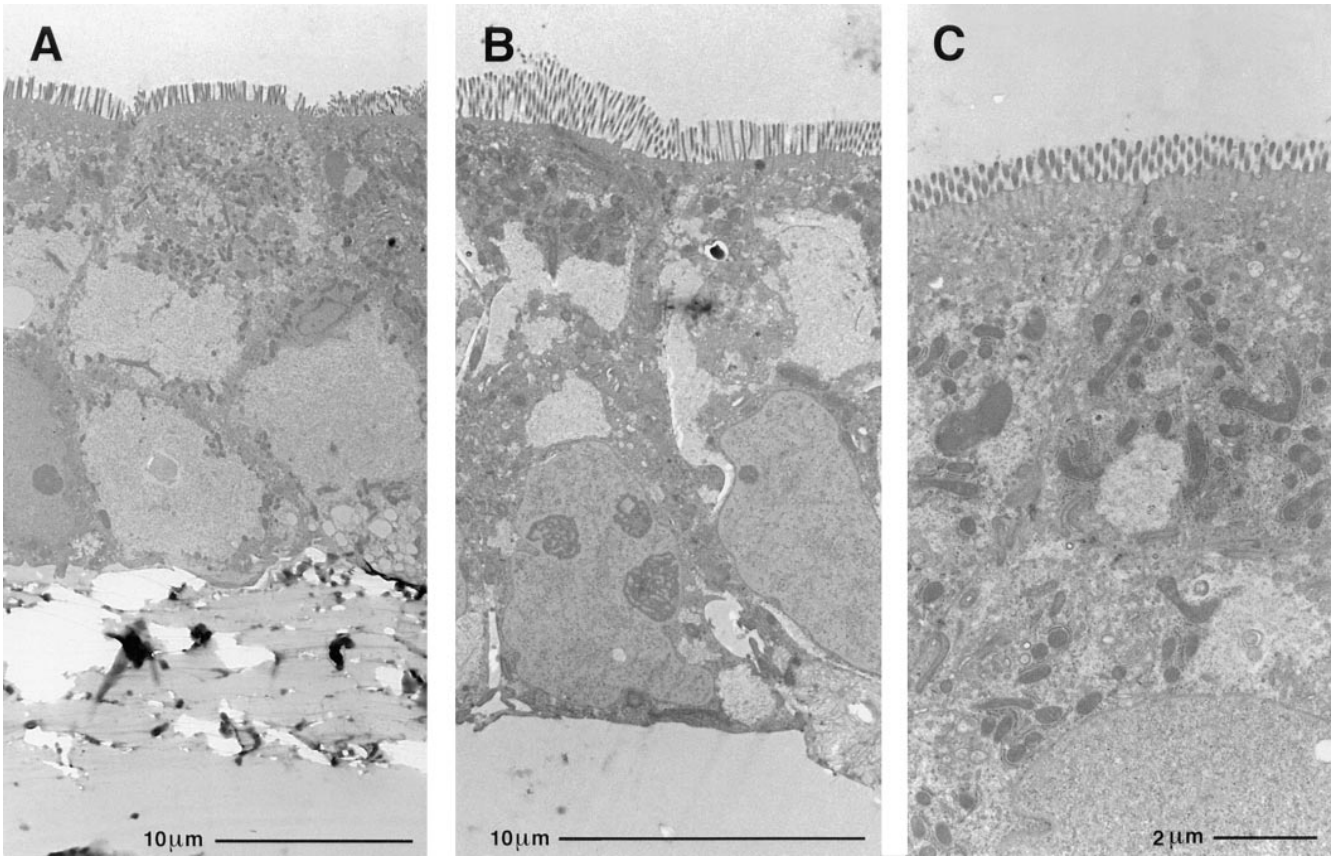

Fig. 5. Morphology of Caco-2 cells after inhibition of MTP activity. Electron micrographs of cells incubated with 0.5 mM oleic acid (panel A) or 0.5 mM palmitic acid (panel B). The 50–75% reduction in triglyceride secretion with 6 μM BMS-200150 did not result in any morphological change in the cells in the presence of oleic acid. Also, incubation with 1.0 mM oleic acid and 8 μM BMS-200150 (85% inhibition of basolateral triglyceride secretion) did not result in any change in cell morphology (panel C).

DISCUSSION

The effects of inhibition of MTP activity with BMS-200150 were studied in a model of the human enterocyte, i.e., Caco-2 cells cultured as polarized monolayers on microporous membranes. BMS-200150 inhibited the basolateral secretion of triglycerides from Caco-2 cells with an IC_{50} of 2 μM, which is similar to the IC_{50} of 1.8 μM BMS-200150 reported for inhibition of apoB secretion in HepG2 cells (15). Inhibition of MTP activity in Caco-2 cells resulted in a decrease in the secretion of triglycerides and phospholipids from the cells, with a mildly inhibiting effect on the secretion of apoB (specifically apoB-100, not apoB-48). Three μM BMS-200150 resulted in a decrease in both the triglycerides/apoB ratio and the triglycerides/phospholipids ratio in the lipoproteins secreted into basolateral medium. By electron microscopy, no intracellular accumulation of lipid droplets was seen in the presence of 8 μM BMS-200150 and 1.0 mM oleic acid.

The study was designed to test the two models describing the functional role of MTP in the first and in possible further steps of the lipidation of apoB during intracellular lipoprotein assembly which are described in the introduction of this paper. When MTP activity is partially inhibited with BMS-200150, fewer lipoproteins are secreted by Caco-2 cells. These lipoproteins are less triglyceride-rich and more dense than those secreted in control incubations without the inhibitor. These results clearly indicate that, at least in the intestinal Caco-2 cells, MTP is not only involved in the first step of lipoprotein assembly, i.e., the intracellular rescue of apoB, but also in the further lipidation of the primordial lipoprotein along the secretory pathway. MTP can thus be a candidate determinant of the density of lipoproteins that are secreted from the cells.

A recent paper by Haghpassand, Wilder, and Moberly (27) describes the effects of a different MTP inhibitor, i.e., a bromine derivate of metaqualone CP-10447, on lipoprotein secretion by HepG2 and Caco-2 cells. Their data on Caco-2 cells parallel ours, despite the observation that CP-10447 is 30 times less potent in inhibiting MTP activity in Caco-2 cells (IC_{50} = 150 μM) than in HepG2 cells (IC_{50} = 5 μM). Inhibition of MTP activity has the largest effect on triglyceride secretion, with mild inhibition of apoB-100 secretion, and little or no effect on secretion of apoB-48. No differences were observed in cellular triglycerides or phospholipids with or without MTP inhibitor in the present study and by Haghpassand et al. (27). Similar results obtained with two different MTP inhibitors, BMS-200150 and CP-10447, strongly support the view that the described effects are the result of specific inhibition of MTP and not side-effects of one of the compounds used. Haghpassand et al. (27) suggest that phospholipid secretion may not be affected during MTP inhibition, but we show that 3 μM of BMS-200150 caused, in addition to a 70% inhibition of triglyceride secretion, also a 50% inhibition secretion of phospholipids (measured as phospholipid mass; Table 2, or as newly synthesized phospholipids using [^{14}C]oleic acid as tracer). Additionally, our study demonstrated that the substrate used to stimulate cellular lipid synthesis (i.e., saturated or unsaturated fatty acid) may interact with the effect of MTP on lipoprotein assembly.

A 70% reduction in the core contents (i.e., triglycerides) of a lipoprotein particle would correspond with a 50% reduction in its surface (i.e., phospholipids). Reduction in number of secreted lipoproteins would cause an equal percent decrease in triglyceride and phospholipid secretion. The approximate 70% inhibition of triglyceride secretion induced by 3 μM BMS-200150, combined with an approximate 50% inhibition of phospholipid secretion probably represents a reduction in size of secreted lipoproteins and not their number. We can, at present, not explain the decrease in total triglycerides (cellular plus basolateral) in the system that we observed in the incubations with BMS-200150. It has been reported (27) that triglyceride synthesis did not change during MTP inhibition and that the mass of intracellular triglycerides did not significantly increase. The latter observation was explained by the low triglyceride secretion efficiency of the cells used in the experiments (retention of triglycerides in the cells would be too small to cause a significant increase in cellular triglycerides), but the authors did not show a calculation of the total mass of triglycerides in the incubation system. We observed a lower total triglyceride mass, both after MTP inhibition during incubation with oleic acid (strong stimulation of cellular lipid synthesis and secretion) and with palmitic acid present (mild stimulation of lipid synthesis) with BMS-200150 concentrations up to 8 μM. This phenomenon therefore appeared to be the specific consequence of inhibition of cellular MTP activity. Uptake of fatty acids from the apical medium is not changed during MTP inhibition (unpublished observations) and the present data thus imply that triglyceride synthesis in Caco-2 cells may be affected during MTP inhibition. Further experiments are needed to study the cellular triglyceride synthesis and the fate of the absorbed fatty acids during inhibition of MTP activity with BMS-200150 in Caco-2 cells.

The effects of inhibition of MTP activity in the presence of oleic acid are very straight-forward. When lipoprotein secretion by Caco-2 cells is stimulated with 0.5 mM oleic acid, the larger part of secreted lipoproteins are at $d < 1.006$ g/ml. Both the inhibition of MTP ac-

tivity (with 3 μM BMS-200150) and the general reduction of cellular lipid availability (by adding 0.1 mM instead of 0.5 mM fatty acid in the apical medium) resulted in the secretion of fewer lipoproteins at d < 1.006 g/ml.

When cellular triglyceride synthesis and lipoprotein secretion are stimulated with 0.5 mM palmitic acid, the secreted lipoproteins are present in two distinct density fractions i.e., chylomicron/VLDL and IDL/LDL, a characterized feature in Caco-2 cells (12). The results of either inhibition of MTP activity or reduced apical fatty acid supply on lipoprotein secretion and composition during incubation with palmitic acid are more complex than with oleic acid. Reduction of triglyceride secretion by inhibition of MTP activity with BMS-200150 resulted in the complete disappearance of chylomicron/VLDL density lipoproteins and a shift of the IDL/LDL density peak toward higher density (d 1.02 g/ml to d 1.03 g/ml). A completely different result was obtained with a general reduction of cellular lipid availability with 0.1 mM apical palmitic acid. Those incubations caused an increase of lipoproteins at chylomicron/VLDL into the medium and a reduction of lipoproteins at IDL/LDL density. The synthesis and secretion of the lipoproteins in the two density classes thus seems to be regulated in different ways.

Based on the results in this study and in an earlier publication (13), we hypothesize that the two classes that are synthesized by Caco-2 cells result from differences in the fatty acid moiety of the triglycerides in the cells. Lipoproteins at chylomicron/VLDL density will carry mainly 'unsaturated' triglycerides (i.e., triglycerides with mainly unsaturated fatty acid esterified to their glycerol backbone), with relatively high triglycerides/phospholipids ratio. Lipoproteins at IDL/LDL density will carry more 'saturated' triglycerides, and have a relatively low triglycerides/phospholipids ratio. Our hypothesis is based on the observations that *a*) substitution of palmitic acid with oleic acid (13), which causes an increase of the ratio of unsaturated/saturated fatty acids in the cells, resulted in disappearance of the IDL/LDL density fraction with a concomitant increase in chylomicron/VLDL secretion, *b*) reduction of apical palmitic acid (0.1 mM vs. 0.5 mM in controls, also causing an increase in cellular unsaturated/saturated fatty acid ratio) caused a decrease in the IDL/LDL density fraction, and a relative (not absolute as less total lipid was available) increase in secretion of chylomicron/VLDL, and *c*) inhibition of triglyceride secretion with BMS-200150 specifically decreased the secretion of chylomicron/VLDL (almost to zero) with much less effect on secretion of lipoprotein of IDL/LDL density. This might be the result of retention of saturated fatty acid in the cells, and thus a decrease in the cellular unsaturated/saturated fatty acid ratio, potentially caused by inhibition of MTP with BMS-200150.

Both apoB-48 and B-100 are present in the chylomicron/VLDL as well as in the IDL/LDL density fractions. The effects of inhibition of MTP activity in Caco-2 cells (Fig. 4, bottom) are therefore not likely to be the result of differences in the assembly of B-48 and B-100 containing particles.

BMS-200150 specifically inhibited the secretion of the large, light chylomicrons. In these experiments, the lipoproteins secreted at IDL/LDL density particles became even more dense. Thus, the lower activity of MTP in the cells in combination with the presence of a specific (saturated) fatty acid, resulted in major changes in the density of the secreted lipoproteins. Naturally occurring differences or fluctuations in MTP activity in intestinal (and perhaps also hepatic) cells may thus lead to the secretion of lipoproteins differing in size and density and this can have potentially important physiological implications for the metabolism of these particles in vivo (28, 29). It is known that human enterocytes secrete lipoproteins of variable size and lipid core. The present data suggest that fluctuations in MTP activity in the enterocyte (as they may occur in vivo) can modulate the secretion of chylomicrons of different sizes. Cells with low MTP activity would secrete a smaller number of lipoproteins, which would be more dense as a result of lower triglyceride and higher phospholipid content per particle. These effects of MTP expression levels can be additional to the proposed effects of dietary fat or cholesterol on the levels of MTP mRNA in (hepatic) cells (20, 30). Additionally, substrate specificity of MTP towards more saturated or unsaturated triglycerides may result in the secretion of chylomicrons of different sizes. The present results suggest that differences in physiological MTP activity in vivo in the enterocyte may explain differences in sensitivity for different dietary fats. Such a mechanism bears potential relevance to the human dietary response with regard to plasma lipid levels.

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