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Effects of saturated, mono-, and polyunsaturated fatty acids on the secretion of apo B containing lipoproteins by Caco-2 cells

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Abstract

We studied the effects of addition of physiological concentrations (0.5 mM) of fatty acids i.e., palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic acid (18:2) on lipoprotein secretion by polarized Caco-2 cells. With saturated fatty acids, secreted lipoproteins were at IDL/LDL density, $1.009 < d < 1.068$ g/ml. The numbers of secreted lipoproteins, expressed as secreted apolipoprotein (apo) B, were comparable to control without fatty acid (palmitic acid, 551 $\pm$ 185; stearic acid, 629 $\pm$ 304 and control, 504 $\pm$ 176 ng apo B/4.7 cm^2 filter). With unsaturated fatty acids, apo B containing lipoproteins were secreted at chylomicron/VLDL density ($d < 1.006$ g/ml). Oleic acid caused a two-fold higher secretion of apo B than control (1058 $\pm$ 87 vs. 504 $\pm$ 176 ng/4.7 cm^2 filter, $P < 0.001$). The increase in apo B secretion was attributed to a specific increase in apo B-48. Unsaturated fatty acid caused a two-fold higher secretion of triglyceride than saturated fatty acids but incorporation of newly synthesized lipid into the secreted lipoproteins, measured by incorporation of a fatty acid marker, was 10- to 20-fold higher, indicating preferential translocation of unsaturated triglycerides into lipoproteins. Mixtures rich in either polyunsaturated, monounsaturated, or saturated fatty acids, resembling nutritional fat and oils, were capable of a two-fold stimulation of secretion of apo B containing triglyceride-rich lipoproteins. The triglyceride/apo B ratio in the basolateral medium was higher with the monounsaturated 'olive oil' mixture (12 250 $\pm$ 2000 mol/mol) than with the polyunsaturated 'corn oil' mixture (7830 $\pm$ 2480 mol/mol) and incorporation of newly synthesized lipid into the secreted lipoproteins was 1.5-fold higher as well. In conclusion, unsaturated fatty acids were most potent in stimulating the secretion of apo B by specifically increasing apo B-48 secretion. Unsaturated triglycerides, that contain mainly oleic acid, were more

Abbreviations: apo B, apolipoprotein B; BSA, bovine serum albumin; *d*, density; DMEM, Dulbecco's modified Eagles minimum essential medium; EACA, epsilon aminocaproic acid; ELISA, enzyme linked immuno sorbent assay; FCS, fetal calf serum; HDL, high density lipoprotein; IBAS, interactive image analysis system; 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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efficiently incorporated into lipoproteins than saturated triglycerides, suggesting preferential translocation by microsomal triglyceride transfer protein.

Keywords: Intestine; Apolipoprotein B; Caco-2; Fatty acid; Lipoprotein; Chylomicron

1. Introduction

Elevated concentrations of plasma cholesterol and triglycerides have been associated with an increased risk of premature atherosclerosis [1–5]. Postprandial lipemia is determined by contribution of chylomicrons carrying triglyceride from dietary sources, and VLDL with liver-derived triglycerides. Plasma triglyceride is in the fasting state mainly carried by VLDL, but humans are in the postprandial state during the larger part of the day. Triglycerides present in chylomicrons may therefore, despite their short half-life, contribute substantially to risk on atherosclerosis. In fact, atherosclerosis has been proposed to be a postprandial phenomenon [6]. It is known that various dietary fatty acids have specific effects on plasma triglyceride levels [7–9]. These effects may reflect interference of various fatty acids with the intestinal chylomicron assembly. Triglyceride-rich lipoproteins can be synthesized in both enterocytes and hepatocytes. In enterocytes, apolipoprotein B (apo B) is translocated across the membrane of the endoplasmic reticulum during translation, associates with lipid, and is subsequently secreted as the structural protein of lipoproteins into the mesenteric lymph. In the proposed mechanism for regulation of lipoprotein synthesis, apo B is synthesized in ample amounts in the cell and regulation occurs at the level of protein degradation. In case of incomplete protein translocation or improper assembly of the particle, apo B will be intracellularly degraded at the cytoplasmic side of the endoplasmic reticulum membrane [10–12]. In this process, microsomal triglyceride transfer protein is the obligatory protein for co-translational transfer of triglycerides into the lipoprotein particle [13]. Nascent chylomicrons are very rapidly metabolized into remnant particles by the action of lipoprotein lipase. Exchange of apolipoproteins to and from other lipoproteins occurs simultaneously. Therefore, intestinal explants and cell lines rather than whole organisms are used to study the synthesis of chylomicrons. The Caco-2 cell line cultured on permeable support has been used in the past few years as a model to study the effects of different fatty acids on intestinal lipoprotein synthesis. Caco-2 cells, a human cell line derived from a colon carcinoma, differentiate in culture into enterocytes [14]. Monolayers grown on permeable supports act as polarized permeability barriers between two compartments and show functional properties of transporting epithelia. Caco-2 cells grown on filters to a confluent monolayer have well-developed microvilli and express a variety of cell surface markers that are also found in enterocytes of the intestinal villus. They exhibit well-developed tight junctions forming a monolayer that is unpenetrable for macromolecules [15], thus providing an isolated model of the human intestinal cell. Caco-2 cells synthesize both apo B-48 and apo B-100 and these apolipoproteins are secreted as structural protein of lipoproteins [16]. Under basal conditions, apo B-48 secretion is 50–70%, and apo B-100 is 30–50% of total apo B secretion. Furthermore, Caco-2 cells synthesize the apoproteins A-I, A-IV, C-III, and E [17,18].

In the present study, we investigated the effects of isolated fatty acids (palmitic-, stearic-, oleic-, and linoleic acid) on secretion of lipoproteins and apo B by Caco-2 cells. This is an extension of our previous experiments concerning the metabolism of palmitic- and linoleic acid in Caco-2 cells [19]. In addition, we studied the effects of fatty acids, present in physiological mixtures resembling human dietary fat, on the composition and density of triglyceride-rich particles secreted by Caco-2 cells.

2. Materials and methods

[1-^{14}C]Oleic acid (1.82 GBq/mmol) was purchased from New England Nuclear (Boston, MA). Palmitic acid, stearic acid, oleic acid, and linoleic

acid (99% pure by gas chromatography) and essentially fatty acid free BSA were obtained from Sigma (St Louis, MO). Isopropanol (HPLC grade) was purchased from Riedel de Haehn (Seelze, Germany).

2.1. Cell culture

Caco-2 cells [14] of intermediate passages (p69–p92) were grown in DMEM supplemented with 20% heat-inactivated fetal calf serum (FCS), 1% non-essential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco Brl, Grand Island, NY). Cells were subcultured weekly as described [20]. For experiments, cells were cultured on microporous membranes of 0.45 μm pore size and 24.5 mm diameter (Transwell COL™, Costar, Cambridge, MA) as described in detail [19]. Experiments were performed on days 14–18 after plating when monolayers were 5–7 days confluent.

2.2. Experimental design

In this study we compared the effects of different isolated fatty acids (palmitic acid, 16:0; stearic acid, 18:0; oleic acid, 18:1; linoleic acid, 18:2) on triglyceride, apolipoprotein B (apo B), and lipoprotein secretion by Caco-2 cells. We also studied the effects of combinations of these fatty acids as they are present in western human diets. By varying the relative contribution of saturated and unsaturated fatty acid species (16:0, 18:1, 18:2), we composed mixtures of fatty acids resembling western type diet fat, olive oil, corn oil, and cream fat, respectively (Table 1). Fatty acids were complexed to fatty acid free BSA in serum free DMEM as described [19–21]. Total final concentrations of fatty acids and BSA were 0.5 mM and 0.5%, respectively in all incubations. Fatty acid/BSA ratio is 7.5:1 (mol/mol). When used, [1-^{14}C]oleic acid was added to the fatty acid stock solution.

Table 1
Composition of fatty acid mixtures

Fatty acid	'Western type diet fat'	'Olive oil'	'Corn oil'	'Cream fat'
Palmitic acid (16:0)	41%	11%	11%	61%
Oleic acid (18:1)	40%	71%	26%	31%
Linoleic acid (18:2)	19%	18%	63%	8%

Relative contributions of various fatty acids to the mixtures used for incubation of Caco-2 cells. Compositions are based on data from literature [48,52].

2.3. Incubation of cells with fatty acids

Cells were rinsed three times with serum free DMEM before adding 1.5 ml of 0.5 mM fatty acid-BSA complexes to the apical side of the cells. Three ml of serum free DMEM without fatty acid or BSA was added to the basolateral compartment. Cells were incubated with fatty acid for 24 h at 37°C. We showed in previous experiments [19] that both saturated and unsaturated fatty acids are absorbed efficiently and in equal amounts (80–85% per 24 h) from the apical medium during the incubation.

At the end of the incubation, apical and basolateral media were collected and protease inhibitors (EDTA and EACA, final concentration 2 mM) were added. Media were centrifuged at 3000 rev./min for 10 min to remove cell debris. Cells on filters were rinsed with cold 0.9% NaCl. Filters were cut out of their holders and sonicated to solubilize cells. Sonication was optimized at 30 min in an ultrasound waterbath, with less than 1% of cellular phospholipid remaining on the filter. Total cellular phosphate and protein were determined according to the methods of Rouser et al. [22] and Lowry et al. [23], respectively. Cholesterol and triglyceride (TG) were determined in the sonicated cells using enzymatic colorimetry (Boehringer Mannheim, Germany). Lipids in medium were first extracted from 1–1.5 ml medium according to the method of Bligh and Dyer [24]. The chloroform phase was dried under N_2 and lipids were redissolved in 100 μl isopropanol (HPLC grade). Triglyceride was determined using the same enzymatic reaction. Cholesterol in media was below detection limits of the kit (< 10 nmol/filter).

2.4. Density gradient ultracentrifugation of secreted lipoproteins

For determination of lipoprotein density, cells were incubated with isolated or combined fatty

acids using [^{14}C]oleic acid (7.4 kBq/filter) as tracer. At the end of the incubation, lipoproteins in the basolateral media were separated on a KBr density gradient [19,25]. In short, 3 ml basolateral medium were adjusted with KBr to *d* 1.250 g/ml and overlaid with 2.8 ml of *d* 1.063, *d* 1.019, and *d* 1.006 g/ml, respectively. Gradients were centrifuged for 24 h at 32 000 rev./min in a Beckman SW 41 rotor at 21°C. Fractions of 0.5 ml were aspirated, mixed with 3 ml Ultima Gold (Packard, Meriden, CT, USA) and counted in a Packard 1900 CA Tri Carb liquid scintillation counter.

2.5. Apolipoprotein B determination

Total apolipoprotein B (apo B) secreted into the basolateral medium was determined using a sandwich ELISA for apo B as described by Ordovas et al. [26] with modifications. Sheep polyclonal anti-human-apo B antibody (Boehringer Mannheim) was used as capturing antibody at a concentration of 800 ng/well. The same antibody was purified over a LDL coupled Sepharose 4B column (Pharmacia, Uppsala, Sweden). The affinity-purified antibody was coupled to horseradish peroxidase and used as detecting antibody. We used Precinorm L 0–10 000 ng/ml (Boehringer Mannheim, Germany) as a standard. No cross-reactivity occurred with HDL, apo A-I, albumin, or lipoprotein free serum. Intra- and interassay coefficients of variation were 5.4% and 9.3%, respectively.

For determination of the relative increase of apo B-48 and apo B-100 as compared to the control, 500 μl of basolateral medium were delipidated, using $CHCl_3$:methanol:diethylether (27:27:46 v/v), and dissolved in 25 μl sample buffer according to Laemmli [27]. Proteins were separated on 4–15% SDS PAGE using the PhastSystem configuration (Pharmacia, Uppsala, Sweden). Following silverstaining, gels were analyzed with the IBAS image analysis system (Zeiss Kontron, Eching, Germany) as described [28]. The integrated optical density (i.o.d.) was measured and expressed as arbitrary units (a.u.). It is a semi-quantitative method for determination of changes in individual apolipoproteins.

2.6. Statistical analysis

All values are expressed as mean $\pm$ standard deviation (S.D.) unless stated otherwise. Mean differences between groups were calculated by unpaired students *t*-tests. Statistical significance was defined as $P < 0.05$ (two-tailed).

3. Results

All experiments in the present study are performed with 0.5 mM final fatty acid concentration. This is a physiological concentration of fatty acid in the intestinal lumen, which is able to stimulate triglyceride synthesis without changing cell morphology. We have shown in earlier experiments [19] that low concentrations of fatty acid (0.1 mM) are not sufficient to induce substantial triglyceride synthesis necessary for lipoprotein secretion, whereas high concentrations (1.0 mM) of saturated fatty acids may cause adverse morphological changes of the cells. All fatty acids used were efficiently absorbed from the fatty acid-BSA complexes at the apical side of the monolayer. At the end of the 24 h incubations, no differences were found in either total cellular cholesterol (159 $\pm$ 24 nmol/filter) or protein (1406 $\pm$ 156 μg/filter). Cellular phosphate was not significantly different, although cells incubated with saturated fatty acids tended to have slightly more intracellular phospholipid than cells incubated with unsaturated fatty acids. With palmitic- and stearic acid, phospholipid levels were 1720 $\pm$ 250 and 1790 $\pm$ 190 nmol/filter, respectively compared to 1580 $\pm$ 300 and 1470 $\pm$ 100 nmol/filter with oleic- and linoleic acid.

3.1. Isolated fatty acids

3.1.1. Triglyceride in cells and medium

The total amount of intracellular triglyceride in the Caco-2 cells at the end of the incubation was determined (Fig. 1. upper panel). From low to high, it ranked BSA < palmitic acid = stearic acid < oleic acid = linoleic acid. Differences reached significance with palmitic-, oleic-, and linoleic acid, compared to BSA ($P < 0.05$). The amount of triglyceride that is secreted to the

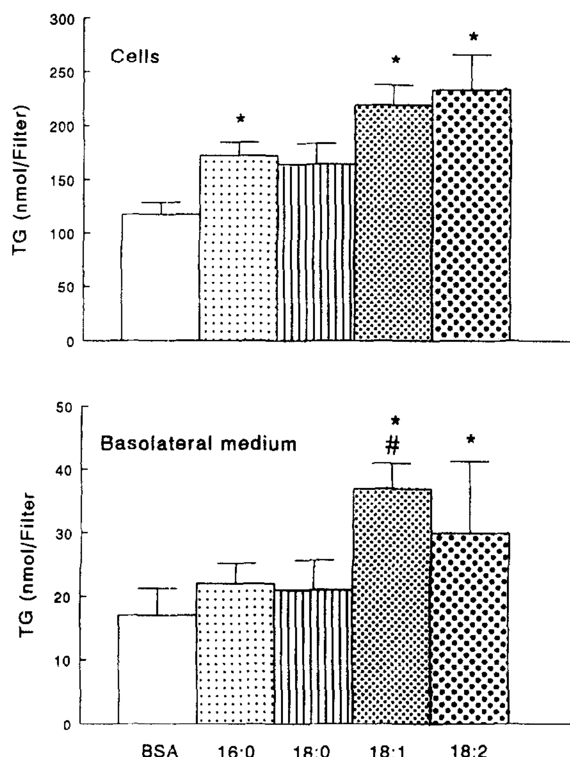

Fig. 1. Cellular and secreted triglyceride during 24 h incubation with 0.5 mM isolated fatty acids. Upper panel: Cellular triglyceride was higher than control □ (0.5% BSA) with 0.5 mM palmitic acid (16:0, $P < 0.01$), 0.5 mM oleic acid (18:1, $P < 0.001$), and 0.5 mM linoleic acid (18:2, $P < 0.01$). Stearic acid did not differ from control. Lower panel: Secretion of triglyceride to the basolateral medium was higher than control with 0.5 mM oleic acid ($P < 0.005$) and 0.5 mM linoleic acid ($P < 0.05$). Secreted triglyceride was also higher with 0.5 mM oleic acid than 0.5 mM palmitic acid ($P < 0.05$).

basolateral medium during incubation with 0.5 mM fatty acid is a parameter for the capacity of Caco-2 cells to secrete lipoproteins. Essentially the same differences as seen intracellularly were found in secretion of triglyceride into the basolateral medium. Oleic- and linoleic acid, but not palmitic- and stearic acid, increased triglyceride secretion compared to BSA (Fig. 1, lower panel). Secreted triglyceride was about 15% of cellular triglycerides with all fatty acids used. Cellular and secreted triglycerides were 117 ± 30 nmol/filter and 17 ± 10 nmol/filter respectively with 0.5% BSA (control), 219 ± 53 and 37 ± 11 with 0.5 mM oleic acid, and 233 ± 88 and 30 ± 11 with 0.5 mM linoleic acid.

3.1.2. Total apo B, apo B-48, and apo B-100 in basolateral medium

The amount of apo B in the medium directly reflects the number of lipoproteins secreted since one apo B molecule is present per secreted particle [29]. Basal apo B secretion, measured using an ELISA, was 504 ± 176 ng/filter. Palmitic and stearic acid did not increase apo B secretion (551 ± 185 and 629 ± 304 ng apo B/filter, respectively). Basolateral apo B was 2-fold increased with oleic acid (1058 ± 87 ng/filter, $P < 0.001$ vs. BSA) and 1.6-fold with linoleic acid (824 ± 204 ng/filter, $P = 0.06$). Human intestinal cells secrete mainly apo B-48 but are also capable of apo B-100 synthesis and secretion [30]. We estimated the percentage increase in secreted apo B-48 or B-100, determined on SDS PAGE, in various incubations (Fig. 2). Apo B-48 changed in a similar manner to total apo B. None of the various fatty acids affected the basal level of apo B-100 secretion. Oleic acid (0.5 mM) significantly increased total apo B secretion (210 ± 4%) as well as apo B-48 secretion (161 ± 10%) but did not affect the level of apo B-100 secretion (Fig. 2).

3.1.3. Density of secreted lipoproteins

Lipoproteins secreted into the basolateral medium were separated on a discontinuous KBr density gradient. A distinct difference was seen in the density of lipoproteins secreted during incubation with saturated or with unsaturated fatty acids. In all experiments, 7.4 KBq [1-^{14}C]oleic acid was added as a tracer to the apical medium. We verified non-selectivity of the fatty acid label used in our experiments. When either [^{14}C]linoleic acid or [^{14}C]palmitic acid were used as a marker in incubations with 0.5 mM palmitic acid, no differences were found in incorporation of label into cellular or secreted lipid. The same was seen in experiments with 0.5 mM linoleic acid. Because of this non-selectivity, we used [^{14}C]oleic acid as a marker in all incubations. During incubations with unsaturated fatty acid, lipoproteins with $d < 1.006$ g/ml, i.e., VLDL/Chylomicron density, were secreted. In contrast, during incubation with saturated fatty acids lipoproteins of $1.009 < d < 1.068$ g/ml i.e., at IDL/LDL density were secreted (Fig. 3). The incorporation of newly synthesized

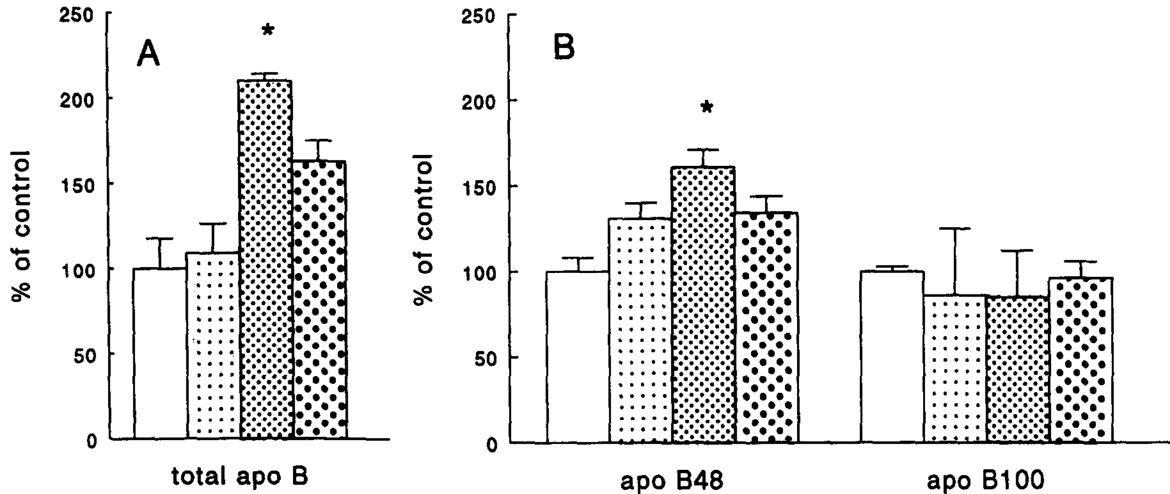

Fig. 2. Secretion of total apo B, apo B-48 and apo B-100 into the basolateral medium during 24 h incubation with 0.5 mM isolated fatty acids. Data are expressed as % of control (0.5% BSA). $n = 4$ with BSA □, palmitic acid (16:0) ⁙ , and linoleic acid (18:2) , and $n = 5$ with oleic acid (18:1) ▒ . Total apo B in medium was determined by ELISA (panel A). The relative increase of apo B-48 and apo B-100 compared to BSA was determined by measuring optical density using interactive image analysis, following separation of the proteins on SDS-PAGE and silverstaining (panel B). With 0.5% BSA, total apo B was 504 ± 176 ng/filter apo B-48 was 0.293 ± 0.046 (a.u.), and apo B-100 0.416 ± 0.024 (a.u.). With oleic acid, both total apo B and apo B-48 were significantly higher than control ($P < 0.05$). Apo B-100 did not differ from control in either fatty acid incubation.

lipid into $d < 1.006$ g/ml was higher with oleic acid (22 700 ± 1800 dpm) than with linoleic acid (10 100 ± 300 dpm, $p < 0.01$) and both were also higher than BSA with ^{14}C oleic acid marker, as control (3100 ± 1100 dpm). With saturated fatty acid we found differences in incorporation of newly synthesized lipid in lipoproteins at $d = 1.020$ g/ml, i.e., the peak value of [^{14}C]oleic acid incorporation in these incubations, between palmitic acid (2150 ± 60 dpm) and stearic acid (1060 ± 40 dpm, $P < 0.02$), with control being 1500 ± 60 dpm.

3.2. Mixtures of dietary fatty acids resembling fat and oils in the human diet

When cells were incubated for 24 h with 0.5 mM of fatty acid mixture resembling the human diet (defined in Table 1), no differences were found in the intracellular amount of triglycerides at the end of the incubation ('western type diet fat', 228 ± 30; 'olive oil', 220 ± 30; 'corn oil', 210 ± 25; and 'cream fat', 189 ± 21 nmol triglyceride/ filter).

3.2.1. Density of secreted lipoproteins

In all incubations lipoproteins at chylomicron/ VLDL density ($d < 1.006$ g/ml) were secreted. A typical example is given in Fig. 4. Even when saturated fatty acids were abundantly present in the apical medium, i.e., with 'cream fat' mixture (palmitic acid is 61% of total fatty acid), lipoproteins at $d < 1.006$ g/ml were secreted. This finding is in contrast with the results of experiments with isolated saturated fatty acids (Fig. 3).

3.2.2. Composition and amount of secreted lipoproteins

Total basolateral apo B secretion was increased 2-fold compared to basal (504 ± 176 ng/filter), but did not differ between the groups ('western type diet fat', 1112 ± 159; 'olive oil', 1152 ± 159; 'corn oil', 1091 ± 238 and 'cream fat', 1084 ± 230 ng apo B/filter). This implies secretion of a constant number of lipoprotein particles in the basolateral medium under these experimental conditions. The composition of the lipoprotein particles was substantially different between the different fatty acid mixture incubations, despite

their similar density on a KBr gradient. Three parameters were used to characterize the composition of secreted lipoproteins — total basolateral secreted triglyceride, incorporation of [^{14}C]oleic acid into lipoproteins, and the triglyceride:apo B ratio in medium. The major differences were found between the incubations with 'corn oil' mixture (63% is polyunsaturated fatty acid), and incubations with 'olive oil' mixture, (71% is monounsaturated fatty acid). Both the triglyceride:apo B ratio and the incorporation of newly synthesized lipids into lipoproteins were significantly higher with 'olive oil' mixture than with 'corn oil' mixture (12 250 ± 2000 vs. 7830 ± 2480, $P < 0.05$ and 19 900 ± 360 dpm vs. 13 900 ± 2700 dpm, $P < 0.04$, respectively, Table 2). Both parameters indicate that the lipoproteins secreted during incubation with 'olive oil ' mixture were larger than with 'corn oil' mixture. The differences in total secreted

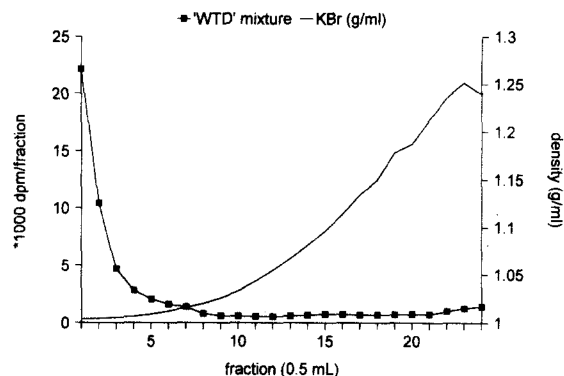

Fig. 4. Characteristics of secreted lipoproteins in density gradient. Density of lipoproteins secreted during incubations with 0.5 mM dietary fatty acids in mixtures resembling fat from human diet was $d < 1.006$ in all incubations. A representative example is given in this figure–data are from four independent experiments in which Caco-2 cells were incubated with fatty acids resembling the western type diet (Table 1) and secreted lipoproteins are of $d < 1.006$ g/ml (VLDL/chylomicron density).

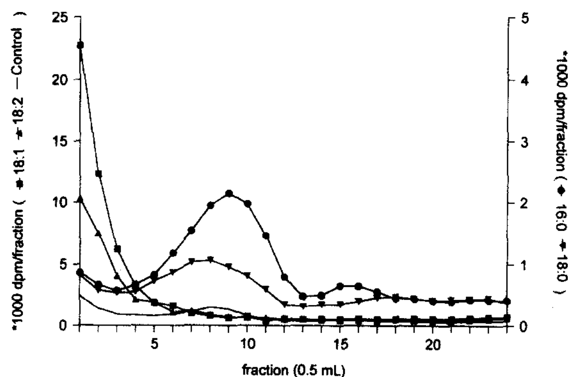

Fig. 3. Characteristics of secreted lipoproteins in density gradient analysis. Lipoproteins were recovered from the basolateral media in two independent experiments when cells were incubated with BSA (Control), 0.5 mM 16:0, 0.5 mM 18:0, 0.5 mM 18:1, or 0.5 mM 18:2. Lipoproteins were separated on a 1.006–1.250 g/ml KBr density gradient and [1-^{14}C]oleic acid was used as tracer. Unsaturated fatty acids (18:1, 18:2) caused secretion of $d < 1.006$ lipoproteins that showed significant differences in incorporation of newly synthesized lipids (fraction 1 is 22 700 ± 1800 dpm with 18:1 vs. 10 100 ± 300 dpm with 18:2, $P < 0.01$). Saturated fatty acids (16:0, 18:0) caused secretion of more dense lipoproteins ($1.009 < d < 1.067$) and incorporation of newly synthesized lipids into secreted lipoproteins was lower with 18:0 than with 16:0 (fraction 9 is 2160 ± 60 dpm with 16:0 vs. 1080 ± 40 dpm with 18:0, $P < 0.02$). Note the different Y-axes with oleic acid, linoleic acid and control or with palmitic acid, stearic acid.

triglycerides with 'olive oil' mixture and 'corn oil' mixture (35.2 ± 3.4 vs. 26.3 ± 10.3 nmol triglyceride/filter), did not reach significance ($P = 0.1$). The compositional parameters did not differ significantly between any of the other dietary fatty acid mixtures used in the incubations. With 'western type diet fat' mixture, secreted triglyceride, incorporation of newly synthesized lipids and the triglyceride:apo B ratio were 29.3 ± 7.8 nmol triglyceride/filter, 20 400 ± 4900 dpm, and 9600 ± 1900, respectively, and with the 'cream fat' mixture, these data were 28.5 ± 4.1 nmol triglyceride/filter, 19 980 ± 600 dpm, and 9400 ± 5000, respectively.

4. Discussion

Although the Caco-2 cell line has its specific limitations [31–33], it is evident from the results of other authors [34–37] and from our group [19] that Caco-2 cells are capable of polarized secretion of lipoproteins at VLDL/chylomicron density, preferentially to the basolateral side of the monolayer, after stimulation with fatty acids. In the present study, we found different effects of various fatty acids, i.e., palmitic-, stearic-, oleic-, and linoleic acid on lipoprotein secretion by

Table 2
Analysis of lipoproteins in basolateral media following incubation with 'olive oil' and corn oil' mixtures

Incubation	TG secreted into basolateral medium nmol/filter ± S.D.(%)	Incorporation of [1-^{14}C]oleic acid into d < 1.006 dpm g/ml ± S.D.(%)	TG/apo B ratio mol/mol ± S.D.(%)
0.5 mM olive oil	35.2 ± 3.4 (100%)	19 920 ± 360 (100%)	12 250 ± 2000 (100%)
0.5 mM corn oil	26.3 ± 10.3 (75%)	13 920 ± 2700 (70%)*	7830 ± 2480 (64%)*

Characteristics of lipoproteins secreted into basolateral medium during 24 h incubation of Caco-2 cells with 0.5 mM 'olive oil' mixture or 'corn oil' mixture (defined in Table 1). Data on total secreted triglyceride, incorporation of [1-^{14}C]oleic acid, and TG/apo B ratio all indicate that lipoproteins secreted during corn oil incubation are smaller than those secreted during incubation with olive oil. *$P < 0.05$.

Caco-2 cells. Unsaturated fatty acids were more potent than saturated fatty acids in stimulating triglyceride synthesis and secretion of lipoproteins at chylomicron/VLDL density. Unsaturated, but not saturated, fatty acids were capable of elevating total apo B secretion compared to control. Saturated fatty acids initiated secretion of lipoproteins at LDL/IDL density. When mixtures of fatty acids were used resembling dietary fat and oils, all secreted lipoproteins were at VLDL/chylomicron density regardless of the composition of the mixture. Differences were found between 'olive oil' mixture (containing predominantly monounsaturated fatty acid) and 'corn oil' mixture (predominantly consisting of polyunsaturated fatty acids). With either of these fatty acid mixtures, lipoproteins at chylomicron/VLDL density were recovered in the basolateral medium but with 'olive oil' mixture lipoproteins had a significantly higher triglyceride:apo B ratio than with 'corn oil' mixture, indicating that the secreted particles were larger.

In an earlier study, we reported that during incubation with 1.0 mM palmitic acid, Caco-2 cells secreted lipoproteins at IDL/LDL density and with 1.0 mM linoleic acid lipoproteins were at chylomicron/VLDL density. After incubation with these concentrations of palmitic acid, cells were laden with intracellular membrane. It was suggested that the differences in lipoprotein density could be the result of changes in cell morphology rather than a direct effect of palmitic acid on lipoprotein assembly. The fatty acid concentrations used in the present study are sufficient to stimulate triglyceride synthesis, but do not affect cell morphology. Our present and earlier observations therefore represent genuine effects of the saturated fatty acids. Several studies with lymph fistula rats yielded comparable results. Lipoproteins that are secreted after intraduodenal infusion with saturated fatty acid are slightly smaller than those secreted following infusion of (poly)unsaturated fatty acids [38,39]. During incubation with isolated fatty acids, total intracellular and secreted triglyceride was lower with saturated fatty acid (± 190 nmol/filter) than with unsaturated fatty acid (± 250 nmol/filter) although equal amounts of fatty acid were absorbed during the incubations. Fatty acids that are not incorporated into triglycerides can be used for synthesis of cholesteryl esters, phospholipids, monoglycerides or diglycerides or can be routed into the β oxidation. Palmitic acid at 1.0 mM but not 0.1 mM is preferentially incorporated into phospholipids [19]. In the present study, intracellular phospholipid was slightly but not significantly, higher in the incubations with saturated fatty acids. Furthermore with stearic acid even less newly synthesized lipid was incorporated into secreted lipoproteins than with palmitic acid. One explanation is that stearic acid is even more insoluble than palmitic acid and may therefore not be incorporated into triglyceride for secretion into lipoproteins, but retained intracellularly in a similar way as we have proposed earlier for palmitic acid [19].

We found additional differences between palmitic- and stearic acid and also between oleic and linoleic acid with respect to the amount of newly synthesized lipid (mainly triglyceride, with

some phospholipid) that is incorporated into the secreted lipoproteins. Both palmitic acid and stearic acid have been reported to have no effect on serum triglyceride [2]. In this study we show that during incubation of Caco-2 cells with palmitic- and stearic acid, secretion of triglyceride is not different from control and two times lower than with oleic acid. Our data on the mass of triglyceride secreted during incubation with various fatty acids, and on the incorporation of newly synthesized lipids into secreted lipoproteins (Figs. 1 lower panel and 3) show that lipids synthesized during incubations with oleic acid are most efficiently incorporated into the secreted lipoproteins. During incubation with oleic acid 10–20 times more newly synthesized lipid was incorporated into the secreted lipoproteins but total mass of secreted triglyceride was only two times higher than with saturated fatty acids. Lipids synthesized during incubation with oleic acid were also more efficiently incorporated into lipoproteins than those synthesized during incubation with linoleic acid since basolateral triglyceride mass was only 1.3 times higher, with a 2.5 increase in incorporation of [^{14}C]oleic acid with oleic acid compared to linoleic acid.

Microsomal triglyceride transfer protein (MTP) is the functional protein in the co-translational transfer of triglyceride, cholesterylesters and phospholipids into the nascent particle [13,40]. MTP is dimerized with protein disulfide isomerase (PDI), and it is postulated that the MTP/PDI complex plays a key role in the rescue of apo B from intracellular degradation; the MTP subunit of the complex by translocating lipid into the nascent protein on the endoplasmic reticulum, the PDI subunit by mediating proper folding of the large hydrophobic apo B molecule during translation [13,41–43]. It has been shown that intestinal expression of the microsomal triglyceride transfer protein/protein disulfide isomerase complex (MTP/PDI) is responsive to dietary fat [44]. Our data suggest specificity of MTP/PDI for translocation of (mono)unsaturated triglyceride species. Lipids synthesized during incubation with monounsaturated fatty acids are efficiently translocated into the forming lipoprotein on the endoplasmic reticulum. When cells are incubated with saturated fatty acids, synthesized lipids will contain mainly saturated fatty acids esterified to their glycerol backbone. In these incubations, the MTP/PDI complex translocates mainly non-labeled triglyceride into the lipoprotein particles. We conclude from these results in Caco-2 cells that during incubations with oleic acid and to a minor extent with linoleic acid, newly synthesized triglycerides are efficiently incorporated into lipoproteins, whereas the triglycerides used for synthesis of lipoproteins during incubation with palmitic- and stearic acid originate mainly from a pre-existing intracellular pool.

Oleic acid increased the number of particles secreted by Caco-2 cells compared to other incubations. The increase in apo B could be completely attributed to an increase in apo B-48, with no changes in apo B-100. This specific increase in apo B-48 containing particles may be the result of increased expression of the apo B mRNA editing enzyme in response to oleic acid [45–47]. Apo B-48 and apo B-100 are both products of a single gene. Full length B-100 mRNA is transcribed and apo B-48 mRNA is produced by posttranslational C to U editing at nucleotide 6666, introducing a stop codon. Increased levels of editing enzyme, a cytidine deaminase, will increase the amount of apo B-100 mRNA that is edited, thus increasing the amount of apo B-48 relative to apo B-100 that is synthesized on the endoplasmic reticulum of the Caco-2 cells.

In the second part of the present study we used mixtures of dietary fatty acids to verify whether data found with isolated fatty acids could be of physiological relevance. An important common effect of all four fatty acid mixtures was the secretion of equal amounts of lipoproteins exclusively at chylomicron/VLDL density. These results were surprising since we expected, based on the effects palmitic acid and stearic acid described above, and the results of studies in rats [38,39], that mixtures rich in saturated fatty acids would initiate secretion of lipoproteins at higher density than the unsaturated mixtures. The observation that all fatty acid mixtures caused secretion of lipoproteins at VLDL/chylomicron density did however support our hypothesis that the MTP/PDI complex preferentially translocates unsatu-

rated triglyceride. When a considerable amount of saturated fatty acid is replaced with (mono)unsaturated fatty acid, as was done in the incubation with 'cream fat' mixture, newly synthesized lipid is efficiently translocated into the nascent lipoprotein particle.

Several studies have been described in literature comparing the effects of diets rich in mono- or polyunsaturated fatty acids on serum lipids in humans [48–50]. Most of these studies report that olive oil increases serum triglyceride to a higher extent than soy bean- or corn oil. The triglyceride:apo B ratio was higher during incubation with 'olive oil' mixture than with the 'corn oil' mixture, indicating that the secreted lipoproteins were larger (Table 2). We feel that data from our experiments with mixtures of dietary fatty acids, although at present inconclusive, support the two-step hypothesis of lipoprotein assembly [51]. The first step of assembly is the rescue of apo B from intracellular degradation, by MTP mediated transfer of neutral lipids to apo B on the endoplasmic reticulum. The second step requires fusion of this nascent apo B particle with large triglyceride-rich particles lacking apo B, which are synthesized on the smooth endoplasmic reticulum. With 'olive oil' and 'corn oil' mixtures, equal intracellular triglyceride and secreted apo B levels were found, while triglyceride content of the secreted lipoproteins was different. This may indicate that the presence of a certain amount of triglyceride is the determinant of the number of apo B molecules that is rescued from intracellular degradation. In the second step of lipoprotein assembly, triglyceride synthesized during incubation with 'olive oil' mixture will be more efficiently translocated into the preformed particle than triglycerides synthesized during incubation with 'corn oil' mixture, resulting in secretion of larger lipoprotein particles.

In conclusion, major differences are found in the effects of saturated and unsaturated fatty acids when present either as pure fatty acids or as physiological mixtures. During incubation with isolated saturated fatty acid, Caco-2 cells secreted lipoproteins at IDL/LDL density but when mixtures of fatty acids were used containing 60% palmitic acid, secreted lipoproteins were at VLDL/chylomicron density. In Caco-2 cells, the presence of unsaturated fatty acids is therefore required to secrete lipoproteins at proper chylomicron/VLDL density. Using (poly)unsaturated fatty acids, effects on lipoprotein synthesis and secretion were maintained when fatty acids were added as pure fatty acids or as physiological mixtures. Both with oleic acid and with 'olive oil' mixture, more newly synthesized lipids are incorporated into the secreted VLDL/chylomicrons than with either linoleic acid or 'corn oil' mixture. We hypothesize that the different effects of the various fatty acids on lipoprotein secretion and density are, at least in part, the result of substrate-specificity of the MTP/PDI complex.

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