Increased production of apolipoprotein B and its lipoproteins by oleic acid in Caco-2 cells

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Abstract The production of lipids, apolipoproteins (apo), and lipoproteins induced by oleic acid has been examined in Caco-2 cells. The rates of accumulation in the control medium of 15-day-old Caco-2 cells of triglycerides, unesterified cholesterol, and cholesteryl esters were 102 ± 8, 73 ± 5, and 11 ± 1 ng/mg cell protein/h, respectively; the accumulation rates for apolipoproteins A-I, B, C-III, and E were 111 ± 9, 53 ± 4, 13 ± 1, and 63 ± 4 ng/mg cell protein/h, respectively. Whereas apolipoproteins A-IV and C-II were detected by immunoblotting, apoA-II was absent in most culture media. In contrast to an early production of apolipoproteins A-I and E occurring 2 days after plating, the apoB expression appeared to be differentiation-dependent and was not measurable in the medium until the sixth day post-confluency. In the control medium, very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL), and lipid-poor very high density lipoproteins (VHDL) accounted for 12%, 46%, 18%, and 24% of the total lipid and apolipoprotein contents, respectively. The triglyceride-rich VLDL contained mainly apoE (75%) and apoB (23%), while the protein moeity of LDL was composed of apoB (59%), apoE (20%), apoA-I (15%), and apoC-III (6%). The cholesterol-rich HDL contained mainly apoA-I (69%) and apoE (27%). In the control medium, major portions of apolipoproteins B and C-III (93–97%) were present in LDL, whereas the main parts of apoA-I (92%) and apoE (76%) were associated with HDL and VHDL. Oleate increased the production of triglycerides 10-fold, cholesteryl esters 7-fold, and apoB 2- to 4-fold. There was also a moderate increase (39%) in the production of apoC-III but no significant changes in those of apolipoproteins A-I and E. These increases were reflected mainly in a 55-fold elevation in the concentration of VLDL, and a 2-fold increase in the level of LDL; there were no significant changes in HDL and VHDL. VLDL contained the major parts of total neutral lipids (74–86%), apoB (65%), apoC-III (81%) and apoE (58%). In the presence of oleate, the VLDL, LDL, HDL, and VHDL accounted for 76%, 15%, 3%, and 6% of the total lipoproteins, respectively. Oleate had no major effect on the composition of apolipoproteins in LDL, but altered the composition of VLDL by producing particles that had significantly higher relative contents of apoA-I (14%), apoB (51%), and apoC-III (7%), and a lower content of apoE (28%) in comparison with those of control VLDL. These observations indicate that in Caco-2 cells, oleate causes a coordinated induction in the production of triglycerides and apoB.—**Dashti, N., E. A. Smith, and P. Alaupovic.** Increased production of apolipoprotein B and its lipoproteins by oleic acid in Caco-2 cells. *J. Lipid Res.* 1990. **31:** 113–123.

Supplementary key words triglyceride synthesis • apoB synthesis • fatty acids • VLDL • intestine

The biosynthesis, assembly, and secretion of plasma lipoproteins occur both in the liver and intestine. Although numerous in vivo and in vitro studies have been conducted on the regulation of hepatic and intestinal lipoprotein production using various animal species, the lack of adequate experimental models has hindered similar studies in humans. In recent years, two human-derived cell lines, HepG2 and Caco-2 representing human liver and intestine, respectively, have been shown to be the most convenient and accessible models for studying human lipoprotein metabolism. The human hepatoblastoma-derived cell line HepG2 has successfully been used for establishing the regulatory role of lipids (1–4) and hormones (5–8) in the synthesis and secretion of apolipoproteins. The human colonic adenocarcinoma Caco-2 cells undergo in vitro differentiation and express structural characteristics and functional properties (9) typical of the small intestinal enterocytes. The synthesis and secretion of human apolipoproteins A-I, B, E, and C peptides by Caco-2 cells have been demonstrated (10, 11).

Intestinal lipoprotein production is markedly influenced by the nutritional state of the animal. In vivo studies have shown that, on a fat-free diet, rat intestine contributes 10–20% to plasma VLDL (12) and that the intestinal chylomicrons and VLDL secretion rates are increased proportionately with the amount of fat in the diet (13). The effect of dietary fat on the concentrations of intestinal apolipoproteins A-I and B is, however, controversial. In the rat, the apoA-I content of both whole mesenteric lymph (14, 15) and corresponding d < 1.006 g/ml lipoproteins (15) was increased by lipid infusion. In addition, the apoA-I content of intestinal mucosa from both the rat (16) and human (17) has been shown to increase upon fat feeding. On the other hand, the apoA-I content of rat enterocytes was

Abbreviations: apo, apolipoprotein; d, density; FBS, fetal bovine serum; DMEM, Dulbecco's Modified Eagle's Medium; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; VHDL, very high density lipoprotein; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate.

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reported to decrease shortly after fat feeding (18). Whereas lipid infusion was shown to stimulate apoB secretion into whole lymph and d < 1.006 g/ml lipoproteins in the intact rat (15) and intestinal mucosa (16), fat feeding was reported to decrease the apoB content of human jejunal mucosa (19). Similar controversy exists with regard to fatty acid-induced changes in apoB secretion by Caco-2 cells. Studies by Traber, Kayden, and Rindler (11) have shown that prior incubation of Caco-2 cells with lipoprotein-deficient serum decreased the secretion of apoB and addition of oleate to the medium restored the level of apoB to that observed in control cultures. On the other hand, Hughes, Ordovas, and Schaefer (20) have reported the lack of regulation in the synthesis of apoB by Caco-2 cells in response to fatty acid addition.

The purpose of the present study was to determine the rates of accumulation of neutral lipids, apolipoproteins, and lipoprotein density classes in the medium of Caco-2 cells, and to assess the effect of oleic acid on these processes with special emphasis on the production of apoB and its lipoproteins. Preliminary results have been already reported in an abstract form (21).

EXPERIMENTAL PROCEDURES

Materials

Fetal bovine serum, trypsin, L-glutamine, nonessential amino acids, and all culture media were purchased from Grand Island Biological Company (Grand Island, NY). Oleic acid and kanamycin sulfate were obtained from Sigma Chemical Co. (St. Louis, MO). Fatty acid-free bovine serum albumin was purchased from Miles Laboratories (Elkhart, IN). All reagents used for the slab gel electrophoresis were obtained from Bio-Rad Laboratories (Richmond, CA).

Cell culture

The human colonic adenocarcinoma cell line Caco-2 was obtained from American Type Culture Collection (Rockville, MD). The cells were maintained in 25-cm^2 tissue culture flasks in 5 ml of Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM glutamine, 1% nonessential amino acids, kanamycin sulfate (50 μg/ml), and 10% fetal bovine serum (FBS) in a 5% CO_2, 95% air atmosphere at 37°C. The cells were split at a 1:4 ratio every 4 days when they were 80% confluent and medium was changed 48 h after plating. For each experiment, cells were seeded in 12–15 dishes (100 mm diameter) in 12 ml of supplemented DMEM. The first medium change was 48 h after plating and then daily after cells reached confluency. All experiments were conducted with 10–12 days post-confluence cells unless otherwise stated. At the start of each experiment, the maintenance medium was removed, monolayers were washed twice with phosphate-buffered saline (PBS), and 12 ml of serum-free DMEM was added to each dish. In studies where the effect of fatty acid was to be assessed, medium was supplemented with various concentrations of oleate bound to 1.5% bovine serum albumin. At the end of each incubation period, the conditioned medum was removed and centrifuged at 2000 rpm for 30 min at 4°C to remove small amounts of cells and debris. To prevent oxidative and proteolytic damage, preservatives added to conditioned medium were EDTA (1 mg/ml), glutathione (0.2 mg/ml), ϵ-amino caproic acid (1.3 mg/ml), penicillin G (500 units/ml), and chloramphenicol (0.02 mg/ml). The monolayers were washed twice with PBS, scraped off the plate after addition of 2 × 2 ml of PBS, and sonicated. Aliquots of cell suspension were analyzed for cellular neutral lipids and proteins.

Isolation of lipoproteins. VLDL (d < 1.006 g/ml), LDL (d 1.006–1.063 g/ml), HDL (d 1.063–1.21 g/ml), and VHDL (d > 1.21 g/ml) were isolated by sequential preparative ultracentrifugation (22). The isolated lipoproteins were dialyzed against PBS containing preservatives described above. The conditioned medium and lipoprotein density classes were concentrated approximately 10- to 15-fold by polyvinylpyrrolidone (PVP) and sucrose placed outside the dialysis bag (M_r 5000 mol wt cut-off). Concentrations of lipoprotein density classes were expressed as the sum of measured neutral lipids and apolipoproteins of each density class.

Determination of neutral lipids and apolipoproteins. The concentrations of triglycerides and free and esterified cholesterol were determined by gas–liquid chromatography (23). The concentrations of apolipoproteins A-I, A-II, B, C-III, and E were measured by electroimmunoassays developed in this laboratory as previously described (24–27). Cell protein was measured by the method of Lowry et al. (28) and ranged from 14 to 18 mg per dish in 15-day-old cultures.

Polyacrylamide gel electrophoresis and immunoblotting. Electrophoresis of 10 μl of concentration (10- to 15-fold) culture medium was carried out in an SDS containing 12% polyacrylamide slab gel using the Laemmli buffer system (29). The proteins were transferred to Nitroplus 2000 nitrocellulose by electroblotting using the Bio-Rad minitransblot electrophoretic transfer cell. The membranes were immunoblotted with polyclonal antibodies to apoA-IV and apoC peptides as described previously (30).

RESULTS

Rates of neutral lipid and apolipoprotein production as a function of fetal bovine serum concentration and duration of culture

The term production, used herein, refers to combined processes of synthesis and secretion and is measured by the rate of accumulation of lipids and apolipoproteins in the culture medium. To determine the optimal concentration of

FBS for production of lipoproteins, Caco-2 cells were plated and grown in DMEM containing either 10% or 20% FBS for 15 days. This study showed that increasing concentration of serum from 10 to 20% had no effect on the rates of accumulation of neutral lipids and apolipoproteins (data not presented). Therefore, in all subsequent experiments, cells were grown in medium containing 10% FBS.

The next series of experiments were conducted to establish the duration of culture necessary for the maximum rate of lipoprotein accumulation in the medium. Caco-2 cells were plated and grown in DMEM containing 10% FBS for 23 days. As shown in **Fig. 1**, apolipoproteins A-I and E were present in the medium 3 days after plating (the time of the first measurement), reaching the maximum level in 15-day-old cultures and declining thereafter. ApoB was not measurable in medium during the first 11 days of culture; it was present in considerable amount after 13 days and reached its maximum level in culture medium approximately 15 days after plating (Fig. 1). Similarly to apoA-I and apoE, the accumulation of apoB in medium was declining in cultures older than 18 days (Fig. 1). Small amounts of triglycerides and cholesterol were detected in the medium 3 days after plating, reaching the maximum level on the 15th day and declining thereafter (data not shown). Based on these results, Caco-2 cells were cultured and maintained in DMEM containing 10% FBS and all experiments were conducted with 14- to 15-day-old cells unless otherwise stated.

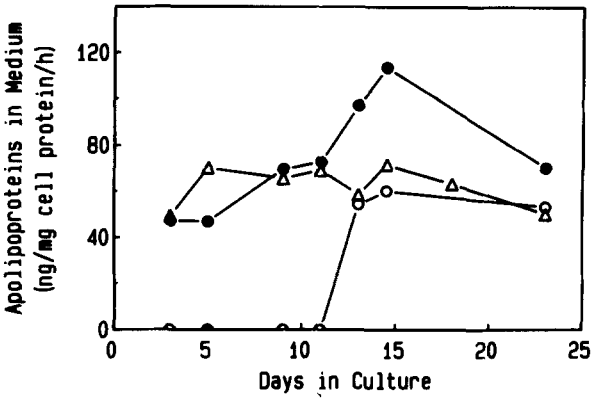

Fig. 1. Accumulation of apolipoproteins A-I, B, and E in the medium during the growth in culture of Caco-2 cells. At each time point, maintenance medium was removed, monolayers were rinsed with PBS and incubated in serum-free DMEM. The rates of accumulation of apoA-I (●), apoB (○), and apoE (△) in the culture medium were determined after a 17-h incubation. Values are mean of three experiments.

Effects of oleate on the rates of neutral lipid and apolipoprotein accumulation in the culture medium

After 15 days in culture, the maintenance medium was removed, monolayers were washed with PBS, and the serum-free DMEM (control medium) was added. The concentrations of triglycerides, cholesterol, and apolipoproteins in the culture medium were determined after an incubation period of 15–17 h. In the control medium, triglycerides accounted for 54%, unesterified cholesterol for 40%, and cholesteryl esters for 6% of the total neutral lipid content (**Table 1**). Approximately 87% of total cholesterol in the medium was in the unesterified form and the remaining 13% was in the form of cholesteryl esters (Table 1).

Apolipoprotein A-I was the major apolipoprotein that accumulated in the culture medium followed, in the order of decreasing concentrations, by apolipoproteins E, B, C-III, and A-II (**Table 2**). Although small amounts of apoA-II were detected in some culture media, in most experiments the concentration of this apolipoprotein, if present, was too small to be detected by either electroimmunoassay or immunoblotting. In addition, the presence in the culture

TABLE 1. Effect of oleate on the accumulation of neutral lipids in the culture medium and Caco-2 cells

	Triglycerides	Unesterified Cholesterol	Cholesterol Esters	Total Cholesterol
		ng/mg cell protein per h		
Medium[a]				
Control	101.9 ± 8.2	73.4 ± 5.4	11.0 ± 0.8	80.0 ± 5.8
Oleic acid	1053.8 ± 157.2[c]	124.1 ± 10.4[c]	73.4 ± 11.8[c]	167.8 ± 15.7[c]
Cells[b]				
Control	4369.4 ± 445.0	1449.4 ± 26.7	451.1 ± 4.4	1718.9 ± 24.4
Oleic acid	9158.9 ± 2.8[d]	1395.6 ± 23.3	581.7 ± 21.7[e]	1742.2 ± 36.1

Cells were cultured in DMEM containing 10% FBS for 15 days. The maintenance medium was removed, cells were washed three times with PBS, and DMEM with or without 0.8 mM oleate-1.5% bovine serum albumin was added. The concentrations of triglycerides and cholesterol in the medium and cells were measured after a 15–19 h incubation. Statistical analysis was performed by Student's *t*-test.

[a]Values are mean ± SEM of 11 experiments.

[b]Values are mean ± SEM of triplicate dishes.

[c]The difference between the control and oleate-supplemented system was significant at $P < 0.001$.

[d]The difference between the control and oleate-supplemented system was significant at $P < 0.005$.

[e]The difference between the control and oleate-supplemented system was significant at $P < 0.02$.

TABLE 2. Effect of oleate on the accumulation of apolipoproteins in the culture medium of Caco-2 cells

Medium	ApoA-I	ApoA-II	ApoB	ApoC-III	ApoE
			ng/mg cell protein per h		
DMEM	110.6 ± 9.3	2.4 ± 1.3	52.8 ± 3.9	12.7 ± 1.0	63.2 ± 4.3
DMEM + oleate	135.6 ± 13.5	2.8 ± 1.9	171.8 ± 15.6	17.6 ± 1.3	76.4 ± 5.8
% Increase	22.6	16.7	225	39	21
P^a	NS[b]	NS[b]	< 0.001	< 0.01	NS[b]

Cells were grown under the experimental conditions described in Table 1. Values are mean ± SEM of 11 experiments.
[a]Determined by Student's *t*-test.
[b]NS, not significant ($P > 0.05$).

medium of apoA-IV and apoC-II was demonstrated by immunoblotting (**Fig. 2**). Under the present experimental conditions, apoC-I was not detected in the culture medium.

To determine the optimal concentration of fatty acid, cells were incubated in the presence of 0.1–1.2 mM oleic acid bound to bovine serum albumin (1.5% final concentration). As shown in **Fig. 3**, there was a dose-dependent increase in the accumulation of neutral lipids in the culture medium. Bovine serum albumin alone (indicated as 0.0 mM oleate) had no significant effect on the production of lipids (Fig. 3). Triglycerides were the major neutral lipids affected by oleate followed by cholesteryl esters; the production of unesterified cholesterol was less sensitive to oleate addition (Fig. 3).

The effect of increasing concentrations of oleate on the accumulation of apolipoproteins in Caco-2 cell culture medium is shown in **Fig. 4**. There was a small increase of 9–15% in the production of apolipoproteins when medium was supplemented with 1.5% bovine serum albumin and in the absence of oleate (Fig. 4). The concentrations of apolipoproteins A-I and E in the medium were not altered significantly by oleate, while apoC-III levels showed a moderate increase of 38% at 0.2 mM oleate without any further change (Fig. 4). On the other hand, oleate caused a dose-dependent increase in the apoB production paralleled by similar changes in triglyceride accumulation at low concentrations of oleic acid. However, at concentrations higher than 0.4 mM oleic acid, the increase in triglyceride accumulation (Fig. 3) exceeded that of apoB (Fig. 4). This difference was not related to the possible effect of oleate on molecular species of apoB, because apoB-100 was the major form of this apolipoprotein in both systems (data not shown). Since apoB concentration reached maximum at 0.8 mM fatty acid (Fig. 4), all subsequent experiments were performed in the presence or absence of 0.8 mM oleate bound to 1.5% albumin.

In a series of 11 experiments, the concentrations of triglycerides, cholesteryl esters, and to a lesser extent, unesterified cholesterol were significantly elevated by oleate (Table 1). Triglycerides were the major neutral lipids produced in the presence of oleate accounting for 84% of the total neutral lipid content of the medium (Table 1), which was significantly higher ($P < 0.001$) than that in control medium. In oleate-supplemented medium, the contribution of unesterified cholesterol to the total neutral lipids was only 10%, a value significantly lower ($P < 0.001$) than that in the control system (Table 1). Although oleate had no effect on the percent composition of cholesteryl esters (6% of the total neutral lipids), it caused a marked increase in their contribution (37%) to the total cholesterol mass as compared to the 13% observed in the control system (Table 1). The rate of triglyceride accumulation in Caco-2 cells were increased significantly by oleate (Table 1). There was also a 29% increase in the cholesteryl esters content of oleate-treated cells, while the total cholesterol was not affected by the addition of fatty acid.

The rate of apoB accumulation in the culture medium was increased two- to fourfold by oleate (Table 2). There was also a moderate increase in the apoC-III accumulation

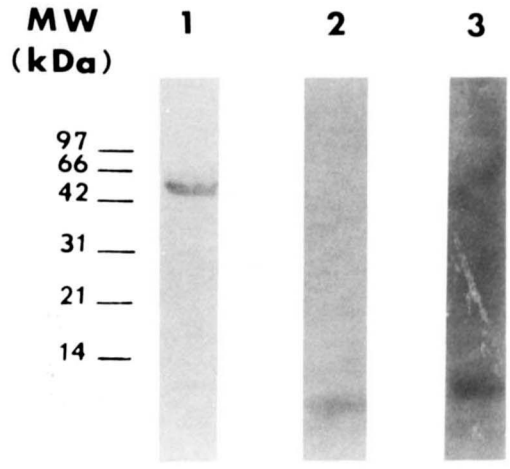

Fig. 2. Analysis of Caco-2 cell culture medium by electrophoresis and immunoblotting. After 15 days in culture, maintenance medium was removed, monolayers were rinsed with PBS and incubated in serum-free DMEM for 17 h. The concentrated medium was analyzed by 12% polyacrylamide gel slab electrophoresis in the presence of SDS. The proteins were transferred to nitrocellulose and immunoblotted with polyclonal antisera to apoA-IV (lane 1), apoC-II (lane 2), and apoC-III (lane 3).

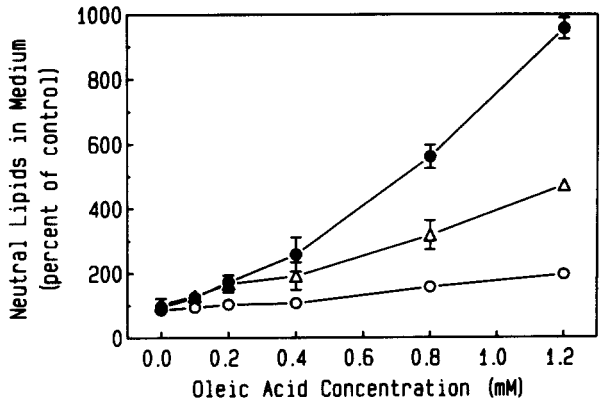

Fig. 3. Effect of increasing concentration of oleic acid on the accumulation of neutral lipids in Caco-2 cell culture medium. After 15 days in culture, maintenance medium was removed, cells were washed with PBS, and DMEM containing 1.5% bovine serum albumin with and without oleic acid was added. The effect of increasing concentration of oleic acid on the rates of accumulation of triglycerides (●), cholesteryl esters (△), and unesterified cholesterol (○) after a 17-h incubation was determined. Values are mean ± SEM of triplicate plates. The absence of error bars indicate that these values were too small to be detected by the plotter. The 100% values for triglycerides, unesterified cholesterol, and cholesteryl esters were 162.3, 90.2, and 18.2 ng/mg cell protein per h, respectively.

in response to oleate, while small changes in those of apolipoproteins A-I, A-II, and E were not significant (Table 2). Because of the specific stimulation of apoB net accumulation, the weight percent apolipoprotein composition in oleate-supplemented medium was considerably different from that of the control system. As a major apolipoprotein in the control medium, apoA-I accounted for 45% of the total apolipoprotein (Table 2). In oleate-supplemented medium, however, apoB was the major apolipoprotein, representing 42% of the total apolipoprotein content which was significantly higher ($P < 0.001$) than that observed in the control system (Table 2). The percent compositions of apoC-III (4.6%) and apoE (20%) were similar to those observed in the control medium (Table 2). Thus, when data were expressed on a relative molar basis, the ratios of apolipoproteins A-I:B:C-III:E were 39:1:13.3:18.5 in the control and 14.7:1:5.6:6.8 in the oleate-supplemented culture media, demonstrating a preferential stimulation of apoB production by oleic acid. The results of the present study do not rule out the possibility that the accumulated lipids and apolipoproteins in the medium might reflect postsecretory modification of secreted lipoproteins during the 15–19 h incubation. Although the catabolism of nascent lipoproteins was not examined directly, a time study (data not shown) demonstrated that the concentrations of triglycerides and apoA-I in the medium increased linearly with time up to 20 h in the presence or absence of oleate. A constant rate of lipid and apolipoprotein accumulation for several hours is indicative of constant rates of both synthesis and secretion and, possibly, degradation of lipoproteins. These results also indicate that oleate had little or no effect on the degradation or postsecretory modification of lipoproteins.

Effects of oleate on the concentrations of lipoprotein density classes

In the control medium, LDL were the major lipoproteins followed, in the order of decreasing concentrations, by VHDL, HDL, and VLDL (**Table 3**). Thus, in the absence of oleate, VLDL, LDL, HDL, and VHDL accounted for 12%, 46%, 18%, and 24% of the total lipoproteins, respectively (Table 3). The addition of oleic acid to the culture medium stimulated the accumulation in the medium of lipoproteins with d < 1.006 g/ml by 55-fold and LDL by 2-fold (Table 3). The slight to moderate increases in the HDL and VHDL concentrations were not statistically significant (Table 3). Thus, in the presence of oleate, VLDL, LDL, HDL, and VHDL accounted for 76%, 15%, 3%, and 6% of the total lipoproteins, respectively (Table 3).

Effects of oleate on the neutral lipid and apolipoprotein composition of lipoprotein density classes

In the control medium, triglycerides were the major neutral lipids in both VLDL and LDL, whereas unesterified cholesterol was the main neutral lipid of HDL (**Table 4**). ApoE was the major apolipoprotein of VLDL, while apoB was the predominant apolipoprotein of LDL (Table 4). ApoA-I was the main apolipoprotein of both HDL and VHDL followed by apoE as the next most abundant apolipoprotein (Table 4). In the control system, apoC-III was detected only in lipoproteins with d < 1.063 g/ml (Table 4).

The addition of oleate to the culture medium not only increased the total concentration of VLDL (Table 3), but also altered their neutral lipid and apolipoprotein composition. The VLDL particles contained significantly lower percentages of unesterified cholesterol and apoE and higher

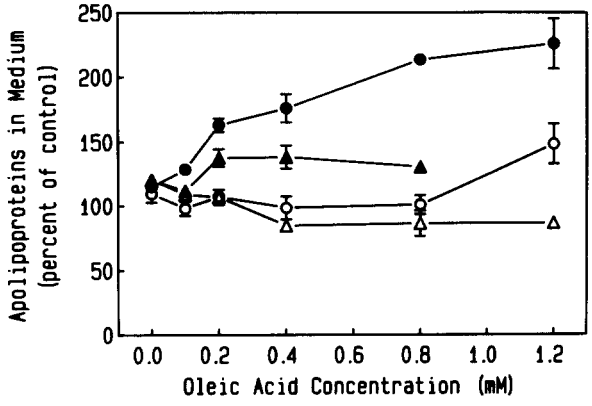

Fig. 4. Effect of increasing concentration of oleaic acid on the accumulation of apolipoproteins in Caco-2 culture medium. Cells were grown under experimental conditions described in the legend to Fig. 3. The effect of increasing concentration of oleic acid bound to bovine serum albumin (1.5% final concentration) on the rates of accumulation of apoA-I (△), apoB (●), apoC-III (▲), and apoE (○) after a 17-h incubation was determined. Values are mean ± SEM of triplicate plates. The absence of error bars indicate that these values were too small to be detected by the plotter.

TABLE 3. Effect of oleate on the concentration and distribution of liopoprotein density classes

Lipoprotein Density Class	Concentration		Distribution	
	Control	Oleate	Control	Oleate
g/ml	*ng/mg cell protein per h*		*%*	
d < 1.006	21.3 ± 6.1	1168.9 ± 220.9[a]	11.7 ± 2.9	76.1 ± 5.0[a]
1.006 < d < 1.063	86.4 ± 14.4	199.3 ± 27.4[b]	45.9 ± 3.9	15.2 ± 4.3[a]
1.063 < d < 1.21	32.2 ± 4.2	40.3 ± 1.1	17.7 ± 2.0	2.9 ± 0.4[a]
d > 1.21	47.6 ± 11.0	78.8 ± 9.4	24.7 ± 3.8	5.8 ± 1.0[c]

Cells were grown under the experimental conditions described in Table 1. Values are mean ± SEM of five experiments. The concentration of lipoproteins represents the sum of all measured neutral lipids and apolipoproteins in each density class.

[a]The difference between the control and oleate-supplemented medium was significant at $P < 0.001$.
[b]The difference between the control and oleate-supplemented medium was significant at $P < 0.01$.
[c]The difference between the control and oleate-supplemented medium was significant at $P < 0.005$.

percentages of triglycerides and apolipoproteins A-I, B, and C-III when compared to control medium (Table 4). ApoB was the major (51% of the total) apolipoprotein component of VLDL produced by oleate-treated cells (Table 4). Oleate caused a 2.5-fold increase in the absolute concentration of triglyceride in LDL without affecting their cholesterol content and thus producing particles with a significantly higher percentage of triglycerides and a lower percent content of unesterified cholesterol (Table 4). The addition of oleate did not alter the apolipoprotein composition of LDL (Table 4). Although the HDL particles produced by oleate-treated cells were characterized by a higher percentage of triglycerides and cholesteryl esters and a lower percentage of unesterified cholesterol, these changes were not statistically significant (Table 4). Except for a slightly decreased relative content of apoE in HDL, there were no other significant changes in the apolipoprotein composition of HDL and VHDL (Table 4).

Effects of oleate on the distribution of neutral lipids and apolipoproteins between lipoprotein density classes

In control medium, the major amounts of triglycerides, cholesterol, apoB, and apoC-III were associated with VLDL and LDL (d < 1.063 g/ml), while the major portions of apoA-I and apoE were present in the d > 1.063 g/ml fraction (**Table 5**). Analysis of lipoprotein density classes showed that 24% triglycerides and cholesteryl esters, 13% of unesterified cholesterol, 3–5% of apolipoproteins

TABLE 4. Effect of oleate on the neutral lipid and apolipoprotein composition of lipoprotein density classes produced by Caco-2 cells

Medium	Neutral lipids			Apolipoproteins			
	Triglyceride	Unesterified Cholesterol	Cholesteryl Esters	ApoA-I	ApoB	ApoC-III	ApoE
	%			*%*			
DMEM							
d < 1.006 g/ml	71.1 ± 2.1	19.9 ± 3.2	9.1 ± 2.4	1.2 ± 1.2	22.6 ± 14.2	1.6 ± 1.6	74.7 ± 15.5
d 1.006–1.063 g/ml	67.2 ± 2.9	25.5 ± 2.8	7.2 ± 0.3	15.3 ± 3.6	59.1 ± 7.0	6.1 ± 1.6	19.5 ± 4.5
d 1.063–1.21 g/ml	21.8 ± 5.0	72.0 ± 7.7	6.3 ± 3.0	68.6 ± 4.7	4.6 ± 4.6	0	26.9 ± 0.2
d > 1.21 g/ml	ND	100.0 ± 0.0	ND	63.8 ± 3.4	0	0	36.3 ± 3.4
DMEM + oleate							
d < 1.006 g/ml	87.3 ± 1.5[a]	9.1 ± 1.6[b]	3.6 ± 0.5	13.7 ± 2.3[d]	51.2 ± 2.1[e]	6.6 ± 0.6[b]	28.6 ± 3.0[e]
d 1.006–1.063 g/ml	81.4 ± 2.0[c]	11.9 ± 1.5[c]	6.7 ± 0.8	15.8 ± 3.1	64.7 ± 2.5	3.7 ± 0.9	15.8 ± 0.8
d 1.063–1.21 g/ml	31.3 ± 2.8	55.2 ± 3.1	13.5 ± 0.8	73.2 ± 9.6	6.9 ± 5.3	2.1 ± 2.1	17.9 ± 2.7[f]
d > 1.21 g/ml	ND	100.0 ± 0.0	ND	76.2 ± 9.3	0	0	23.3 ± 8.9

Experimental conditions are described in Table 1. Neutral lipid and apolipoprotein compositions of lipoprotein density classes produced by Caco-2 cells were determined after a 15–19 h incubation in DMEM with or without 0.8 mM oleate–1.5% bovine serum albumin. Values are mean ± SEM of four experiments; ND, not detectable.

[a]The difference between the control and oleate-supplemented medium was significant at $P < 0.001$.
[b]The difference between the control and oleate-supplemented medium was significant at $P < 0.025$.
[c]The difference between the control and oleate-supplemented medium was significant at $P < 0.01$.
[d]The difference between the control and oleate-supplemented medium was significant at $P < 0.005$.
[e]The difference between the control and oleate-supplemented medium was significant at $P < 0.05$.
[f]The difference between the control and oleate-supplemented medium was significant at $P < 0.02$.

TABLE 5. Effect of oleate on the neutral lipid and apolipoprotein distribution between lipoprotein density classes produced by Caco-2 cells

Medium	Neutral lipids			Apolipoproteins			
	Triglyceride	Unesterified Cholesterol	Cholesteryl Esters	ApoA-I	ApoB	ApoC-III	ApoE
	%			%			
DMEM							
d < 1.006 g/ml	24.2 ± 6.0	13.0 ± 3.7	22.7 ± 5.8	0.1 ± 0.1	4.5 ± 3.1	3.2 ± 3.2	7.6 ± 3.3
d 1.006–1.063 g/ml	72.6 ± 5.5	56.0 ± 2.8	68.8 ± 5.3	7.5 ± 1.3	92.9 ± 3.4	96.8 ± 3.2	16.4 ± 3.4
d 1.063–1.21 g/ml	3.2 ± 0.5	23.8 ± 2.2	8.5 ± 2.3	32.4 ± 4.3	2.6 ± 1.6	0	24.0 ± 3.7
d > 1.21 g/ml	0	7.2 ± 3.1	0	60.0 ± 4.2	0	0	52.0 ± 5.1
DMEM + oleate							
d < 1.006 g/ml	86.1 ± 4.4[a]	73.8 ± 5.8[a]	75.2 ± 4.9[a]	22.3 ± 3.4[a]	65.3 ± 6.0[a]	81.0 ± 6.6[a]	57.9 ± 6.8[a]
d 1.006–1.063 g/ml	13.4 ± 4.3[a]	15.4 ± 4.0[a]	20.4 ± 4.3[a]	8.6 ± 1.3	33.1 ± 5.7[a]	13.4 ± 2.8[a]	13.0 ± 2.6
d 1.063–1.21 g/ml	0.5 ± 0.1[b]	6.8 ± 1.0[a]	4.4 ± 0.8	16.8 ± 1.3[c]	1.6 ± 0.8	3.1 ± 3.1	6.8 ± 1.4[b]
d > 1.21 g/ml	0	4.1 ± 1.2	0	52.3 ± 3.3	0	0	22.3 ± 6.7[d]

Experimental conditions are described in Table 3. Values are mean ± SEM of four experiments.
[a]The difference between the control and oleate-supplemented medium was significant at $P < 0.001$.
[b]The difference between the control and oleate-supplemented medium was significant at $P < 0.005$.
[c]The difference between the control and oleate-supplemented medium was significant at $P < 0.02$.
[d]The difference between the control and oleate-supplemented medium was significant at $P < 0.01$.

B and C-III, and 8% of apoE were associated with VLDL (Table 5). The LDL particles contained 56–73% of the total neutral lipids and 93–97% of total apoB and apoC-III (Table 5). The HDL particles accounted for a small amount (3%) of total triglycerides and apoB, 24% of unesterified cholesterol and apoE, and 32% of apoA-I (Table 5). Whereas apolipoproteins B and C-III were not detected in VHDL, over 50% of apolipoproteins A-I and E were recovered in this lipid-poor fraction (Table 5).

When culture medum was supplemented with oleate, the d < 1.006 g/ml lipoproteins contained the major part of triglycerides (86%), cholesterol (75%), apoB (65%), apoC-III (81%), and apoE (58%); there was also an increase in the apoA-I in this fraction (Table 5). Oleate resulted in the redistribution of apoE from HDL and VHDL to particles with d < 1.006 g/ml (Table 5). While there was a moderate redistribution of apoA-I from HDL to d < 1.006 g/ml lipoproteins subsequent to oleate addition, the major portion (52%) of this apolipoprotein remained in the lipoprotein-deficient fraction (Table 5).

DISCUSSION

The major goal of the present study was to establish the type and properties of lipoprotein particles synthesized and secreted by Caco-2 cells in the presence and absence of oleate.

To select the appropriate experimental conditions, we have first established that the optimal rate of lipoprotein production is achieved when cells are grown in DMEM containing 10% FBS. Since Caco-2 cells undergo spontaneous in vitro differentiation into enterocytes (9), it has been equally important to examine the differentiation-dependent apolipoprotein gene expression. The results have shown that apolipoproteins A-I and E are already present at high concentrations in the culture medium of 3-day-old undifferentiated cells. Whereas the accumulation rate of apoE remained relatively constant for 18 days, the apoA-I concentration reached maximum level after 15 days and declined slowly thereafter. In contrast, apoB was not measurable in the culture medium until the 6th day after cells reached confluency; however, a marked elevation and subsequent maintenance of its rate of accumulation occurred as late as 12 days after plating. The pattern of apoA-I appearance in the culture medium of Caco-2 cells was similar to the expression of apoA-I mRNA in rat fetal small intestine during development (31). However, the high rate of apoE production by Caco-2 cells observed in the present study is in contrast with reports of either undetectable or very low levels of apoE mRNA in the small intestine of adult rat (31) and nonhuman primate (32), and the negligible contribution of rat intestine to the plasma pool of apoE (33). The differentiation-dependent expression of apoB gene in Caco-2 cells is similar to that reported by Demmer et al. (34) who demonstrated that the low levels of apoB mRNA in the rat fetal small intestine persisted almost throughout the entire gestational period and then increased 20-fold during its last 2 days.

The major neutral lipid produced by Caco-2 cells grown in basal culture medium was triglycerides followed by unesterified cholesterol and cholesteryl esters. Similar results have been reported with rat perfused liver (35), cultured rat hepatocytes (36), and our previous studies using HepG2 cells (4). The high percentage of unesterified cholesterol in the Caco-2 cell conditioned medium was similar to that observed in the rat perfused liver system (35), rat cultured hepatocytes (36, 37), and HepG2 cells (4). The low concen-

tration of cholesteryl esters in the culture medium, in comparison to that in human plasma (38), may reflect the low activity of lecithin:cholesterol acyltransferase (LCAT) in Caco-2 cells grown in control medium. The cellular neutral lipid composition in the present study was similar to that reported recently by Field, Albright, and Mathur (39) with the exception of a higher percent content of cholesteryl esters. This variation may be due to differences in the culture medium and the age of the cells used in these two studies.

ApoA-I was the major apolipoprotein in the culture medium followed, in the order of decreasing concentrations, by apolipoproteins E, B, and C-III. Although a small amount of apoA-II was present in some cultures, in most experiments it could not be detected by either electroimmunoassay or immunoblotting. Although apoA-II is synthesized mainly in the liver (40), its presence in the intestine has been reported by the use of both immunochemical techniques (41) and mRNA analysis (42). Further studies are necessary to establish unequivocally the presence of apoA-II in Caco-2 cells. The occurrence of apoA-IV and apoC-II in Caco-2 culture medium is consistent with their reported (43) presence in rat intestinal lymph. The detection of apoA-IV by immunoblotting in the present study is in agreement with that reported by Traber et al. (11) but is at variance with the studies by Hughes et al. (10) demonstrating the lack of apoA-IV secretion or the presence of corresponding mRNA in Caco-2 cells. The lack of apoC-I production by Caco-2 cells observed in the present study is similar to that reported for the rat small intestine (33).

In agreement with our previous study on HepG2 cells (4), the concentration profile of lipoprotein density classes in the control medium of Caco-2 cells was characterized by LDL as the major (46%) and VLDL (12%) and HDL (18%) as the minor lipoproteins; VHDL accounted for a relatively high percentage (25%) of the total lipoproteins. The LDL were found to contain major portions of total neutral lipids and apolipoprotein B and C-III (Table 5). On the other hand, HDL and VHDL contained 80–90% of the total apoA-I and apoE. These findings are in agreement with those reported by Traber et al. (11), but differ considerably from those of Hughes et al. (10) who found HDL (76%) to be the major and VLDL (10%) and LDL (14%) the minor lipoproteins. However, in this latter study, VHDL also represented a large proportion of total lipoproteins. The VLDL and LDL particles produced by Caco-2 cells contained triglycerides as the major, and cholesteryl esters as the minor neutral lipid constituents, a finding similar to those reported for rat intestinal Golgi VLDL (44) and intestinal intracellular LDL (45). In VLDL, apoE was the major and apoB the minor apolipoprotein constituent, whereas in LDL, apoB was the major and apoE the minor protein component. In both density classes, apoB was identified as apoB-100, consistent with findings reported by others (10, 11). The lipid composition of HDL and VHDL was characterized by a very high percentage of unesterified cholesterol; apoA-I accounted for approximately 60–70% and apoE for 30–40% of the protein moiety of these two lipoprotein density classes. A relatively large proportion of apolipoproteins A-I and E (52–60%) occurred in VHDL in accordance with a report by Magun, Mish, and Glickman (45) showing that in fasting rat intestine, 90% of intracellular apoA-I is not associated with lipoproteins. The high concentration of apoA-I and apoE in lipid-poor VHDL observed in this study may represent artifacts caused by dissociation of apolipoproteins from lipoproteins during ultracentrifugation (46). Alternatively, it may suggest that in Caco-2 cells, a large proportion of apolipoproteins A-I and E occur in a lipid-deficient form.

The plasma concentrations of lipoproteins are markedly influenced by the nutritional state of the animal. In the present study total triglyceride and cholesteryl esters increased by 10- and 7-fold, respectively, when the culture medium was supplemented with 0.8 mM oleate. The oleate-mediated increase in triglyceride accumulation is in agreement with that reported recently by Field et al. (39). The observed 2-fold increase in the accumulation of unesterified cholesterol was similar to previous studies from this laboratory using HepG2 cells (4). While there is general agreement that long-chain fatty acids stimulate the synthesis and secretion of hepatic and intestinal lipids, their effects on apolipoprotein secretion remain controversial. It has been reported that, in HepG2 cells, apoB secretion is increased (1, 2), decreased (5), or unchanged (3, 47). Our previous studies using HepG2 cells (4) demonstrated only a moderate increase in the apoB accumulation by oleate. The lack of a coordinated induction of triglycerides and apoB has also been demonstrated in cultured rat hepatocytes (48, 49). In contrast to the lack of regulation of apoB by fatty acids in hepatocytes, the present study has demonstrated a marked elevation in the apoB accumulation in Caco-2 cell culture medium in the presence of oleate; apolipoproteins A-I and E were not significantly affected. Studies from several laboratories have shown decreased (19), increased (16, 50), or unchanged (51) intestinal apoB synthesis and secretion after a fat infusion. Our data, demonstrating clearly and reproducibly the stimulation of apoB production by oleate in Caco-2 cells, are at variance with results reported by Hughes et al. (20) using the same cell line. Whereas these differences may possibly be explained by differences in the culture conditions and methodology used for apoB measurement in these two studies, the exact factors responsible for these variations require further investigation. The finding of unchanged levels of apoA-I in response to oleate addition observed in the present study is in agreement with that reported by Hughes et al. (20), but is at variance with reports showing either an increase (14, 16, 17) or decrease (18) in the concentration of this apolipoprotein under similar nutritional manipulation. In contrast to unaltered levels of apoC-III in rat small intestine after fat feeding (18), our data suggest that, in Caco-2 cells,

fatty acids cause a moderate increase in apoC-III production. Although the effect of oleate on apoA-IV secretion was not investigated in the present study, an elevation in its synthesis in response to dietary fat has been shown to occur in the rat intestine (52).

Although there was a twofold increase in the concentration of LDL, there were only minor changes in the composition of this lipoprotein density class; there were no changes in the concentration and composition of HDL. Similar results were obtained in our previous study (4) using HepG2 cells.

The oleate-induced increases in the levels of neutral lipids and apoB were, thus, reflected primarily in the concentration, distribution, and composition of VLDL. The highly significant increase in the concentration of VLDL (55-fold) was brought about by marked increases in the concentrations of triglycerides (58-fold) and apolipoproteins B (53-fold), C-III (61-fold), and E (15-fold). Consequently, there was also a significant shift in the distribution of neutral lipids and apolipoproteins A-I, B, C-III, and E from higher density classes to VLDL. The main compositional change in VLDL was a significant increase in the relative content of apoB and an equally significant decrease in the percentage of apoE. The elevated concentration of VLDL lipids in response to fatty acid substrate was also observed in experiments with the rat perfused liver (35), HepG2 cells (3, 4), and Caco-2 cells (39). However, a commensurate increase in the concentration of apolipoproteins, especially apoB, was not demonstrated either in rat hepatocytes (48, 49) or HepG2 cells (4). Instead, a redistribution of apolipoproteins B and E from higher density lipoprotein classes to VLDL was found to occur in both the liver (3, 4, 48, 49) and intestine (20, 39). It appears that the oleate-induced changes in the concentration and composition of VLDL particles were brought about by both increased size and number of these lipoprotein particles. This is supported by the observations that *1*) oleate caused a marked redistribution of apolipoproteins A-I and E from VHDL and apolipoproteins B and C-III from LDL to VLDL-like lipoproteins; *2*) these latter particles contained a higher ratio of triglycerides to apoB (9.7 ± 1.3) than the corresponding particles isolated from control medium (5.7 ± 0.3); and *3*) the marked elevation in triglycerides was concurrent with two- to fourfold increase in apoB levels.

The present study has shown the ability of Caco-2 cells to synthesize and secrete all the major neutral lipid and apolipoprotein constituents of human plasma lipoprotein density classes. Furthermore, these data have clearly demonstrated the coordinated induction in the production of triglycerides and apoB by oleate suggesting that, in human-derived intestinal Caco-2 cells, the regulation of VLDL-triglyceride and apoB by fatty acids may be a coordinated process.

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