Trafficking of exogenous fatty acids within Caco-2 cells

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Abstract Dietary fatty acids (FAs) crossing the apical plasma membrane of small intestinal enterocytes are targeted to different metabolic pathways than serum FAs crossing the basolateral membrane. This apparent compartmentalization of FA metabolism in enterocytes was further investigated using a model human enterocyte-like intestinal cell line. [^{3}H]Oleic acid bound to bovine serum albumin (BSA) was added to the apical or basolateral surfaces of confluent monolayers of Caco-2 cells growing on uncoated polycarbonate filters. In other experiments, [^{3}H]oleic acid incorporated into micelles with taurocholate (± 2-monoacylglycerol) was added apically. Caco-2 cells absorbed oleic acid bound to BSA from both the apical and basolateral surfaces at the same rate. Oleic acid in micellar solution was absorbed more efficiently than oleic acid bound to BSA. Regardless of its site or mode of presentation, the majority of the incorporated oleic acid was found in triglycerides. Only a small fraction was subjected to β-oxidation or esterification into phospholipids. Most of the incorporated oleic acid was still retained intracellularly at 24 h. The polarity of triglyceride secretion was influenced by the experimental conditions. Triglyceride secretion was not significantly polarized when oleic acid-BSA was presented apically. However, the ratio of basolateral to apical secretion at 24 h was 9:1 for oleic acid-BSA presented basolaterally. For oleic acid in taurocholate micelles there was a trend toward polarity of secretion to the apical media (apical to basolateral ratio = 2:1). The inclusion of 2-monoacylglycerol in oleic acid-taurocholate micelles did not augment triglyceride synthesis or secretion. These differences indicate that compartmentation of FA metabolism in Caco-2 cells is influenced by the site of FA presentation. Northern and Western blot hybridization studies indicated that the liver fatty acid-binding protein but not the intestinal fatty acid-binding protein gene is expressed in these cells. The absence of this latter 15 kDa protein indicates that it is not required by Caco-2 cells for the synthesis of triglycerides or for the polarized export of triglyceride. These studies indicate that the Caco-2 cell line will be a useful model system for studying the polarization of FA trafficking/metabolism in enterocytes and defining the role of intracellular fatty acid binding proteins in these processes.—**Levin, M. S., V. D. Talkad, J. I. Gordon, and W. F. Stenson.** Trafficking of exogenous fatty acids within Caco-2 cells. *J. Lipid Res.* 1992. **33:** 9–19.

Supplementary key words fatty acid metabolism • metabolic regulation • macromolecular trafficking • enterocytes

Small intestinal epithelium contains morphologically and functionally polarized absorptive cells (enterocytes) that play a central role in lipid metabolism. They are responsible for assimilation of luminal fatty acids (FAs) and lipids. In addition, they support the de novo synthesis of FAs, lipids, and cholesterol and are a major site of production of extracellular lipid transport proteins, particularly apolipoproteins A-I, A-IV, and B-48. The enterocyte is exposed to dietary FAs at its apical surface and to plasma-derived endogenous FAs at its basolateral surface. The enterocyte can absorb and metabolize FAs from both sources (1, 2).

Luminal FAs are absorbed as free FAs or esterified to 2-monoacylglycerol via two independent pathways (reviewed in ref. 3). After acylation in the rough endoplasmic reticulum (4), the majority of FAs are incorporated into triglycerides via the monoacylglycerol pathway (involving transfer to 2-monoacylglycerol and the resulting diacylglycerol) or via the phosphatidic acid pathway (sequential acylation of endogenous glycerol-3-phosphate to form 1,2-diacylglycerol and triglyceride).

The metabolic fate of plasma-derived FA has been studied in vivo by Gangl and Ockner (1). Double-isotope experiments showed that FAs absorbed from the intestinal lumen are preferentially incorporated into triglycerides, whereas those absorbed simultaneously from the bloodstream are more likely to undergo β-oxidation or incorporation into phospholipids. These data support the hypothesis that plasma-derived FAs are destined to serve the structural and energy needs of the enterocyte whereas luminal-derived FAs serve the energy needs of the whole organism (1).

Abbreviations: FA, fatty acid; I-FABP, intestinal fatty acid-binding protein; L-FABP, liver fatty acid-binding protein.

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A great deal is known about the luminal events accompanying lipid absorption and about the products of intestinal lipid metabolism. However, little is known about how intracellular FA metabolism is orchestrated in space within enterocytes and time (i.e., during their rapid differentiation associated with migration from the crypt to the apical extrusion zone at the villus tip). The differential utilization of the monoacylglycerol and the phosphatidic acid pathways, the differences in the metabolism of luminal- and plasma-derived FAs, and the assembly and polarized secretion of triglycerides and phospholipids in lipoprotein particles all indicate that lipid metabolism is highly compartmentalized in the enterocyte. In the present studies the Caco-2 cell line was used as a model system for elucidating the details of intracellular FA trafficking.

The Caco-2 cell line was established from a human colonic adenocarcinoma (5). It expresses many phenotypic features of adult differentiated small intestinal enterocytes as well as of 15-week fetal colonocytes (reviewed in refs. 6 and 7). Previous analyses of lipid metabolism in Caco-2 cells have suggested that they are a good model for studying transcellular trafficking and intracellular compartmentalization of intestinal lipid metabolism. For example, the lipid metabolic capabilities of Caco-2 cells include: *i*) synthesis and secretion of triglyceride-rich lipoprotein particles (8–11); *ii*) metabolism of 15-hydroxyeicosatetraenoic and arachidonic acids (12); and *iii*) production of acyl-CoA:cholesterol acyltransferase (11, 13) and a number of apolipoproteins (14–16).

The goal of the present study was to use the Caco-2 cell line as a model system to study the effect of the site of oleic acid presentation (apical versus basolateral) and the mode of oleic acid presentation (bound to serum albumin or in taurocholate micelles with or without 2-monoacylglycerol) on its subsequent metabolic processing. Additional studies were done to determine whether Caco-2 cells express the cytosolic fatty acid-binding proteins (FABP) that are abundantly expressed in enterocytes and that have been proposed to form a multicomponent intracellular transport system that delivers various lipid ligands to the sites of their metabolic processing (17).

EXPERIMENTAL PROCEDURES

Materials

Penicillin, streptomycin, nonessential amino acids, L-glutamine, and Dulbecco's minimum essential medium (DMEM) were purchased from Mediatech (Herndon, VA). Gentamycin was from Gibco BRL (Gaithersburg, MD). Fetal bovine serum was obtained from Cell Culture Laboratories (Cleveland, OH). Fatty acid free BSA was from Calbiochem (La Jolla, CA). [9,10(n)-^{3}H]Oleic acid (sp act 5 Ci/mmol) was purchased from Amersham (Arlington Heights, IL). All other chemicals and solvents were from Sigma (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

Cell culture

Caco-2 cells (a gift from Dr. Jeffrey Field, Department of Medicine, University of Iowa, Iowa City, IA) were grown in DMEM supplemented with 20% heat-inactivated fetal bovine serum, glucose (4.5 g/l), glutamine (4mM), nonessential amino acids (0.1 mM), penicillin (50 U/ml), streptomycin (50 μg/ml) and gentamycin (15 μg/ml), at 37°C in a humidified atmosphere containing 5% CO_2. Cells (1×10^6) at passage 37 or 38 were plated on polycarbonate membrane inserts (24 mm, 3.0 μM pore size, Costar, Cambridge, MA) and grown to 12 days post-confluence. Media were changed daily. Transepithelial resistance was determined with an epithelial voltohmmeter using dual "chopstick" electrodes (World Precision Instruments, New Haven, CT).

Preparation of radiolabeled FA media

The sodium salt of [^{3}H]oleic acid was prepared using a method adapted from Ellsworth, Erickson, and Cooper (18): 1 ml of a 10 mM stock solution of oleic acid prepared in chloroform and 1×10^9 cpm [^{3}H]oleic acid (5 Ci/mmo) were pipetted into a sterile glass screw-cap test tube. The solvent was evaporated to dryness under a stream of N_2. The residue was dissolved in 1 ml of 100% ethanol and the pH was adjusted to 8.0 with 1 N NaOH. After evaporating the ethanol under N_2, the residue of radiolabeled sodium oleate was dissolved in 20 ml of sterile serum-free DMEM containing either 0.15 mM bovine serum albumin (BSA) or 8 mM taurocholate and the pH was adjusted to 7.0–7.4. [^{3}H]Oleic acid was bound to FA-free BSA by incubating the sodium salt of [^{3}H]oleic acid (0.5 mM) in serum-free media containing 0.15 mM FA-free BSA for 60 min at 37°C. The oleic acid to BSA ratio was 3.3 to 1 (mol:mol). A 3.3:1 ratio was chosen because of previous data suggesting that this concentration of oleic acid maximized secretion of newly synthesized triglyceride from Caco-2 cells (10). [^{3}H]Oleic acid–sodium taurocholate micelles were prepared by mixing the sodium salt of [^{3}H]oleic acid (0.5 mM) with media containing 8 mM sodium taurocholate and incubating the solution at 37°C for 1 h. [^{3}H]Oleic acid–taurocholate micelles containing 0.25 mM 2-monopalmitoyl glycerol were prepared by incubating radiolabeled sodium oleate (0.5 mM) and 0.25 mM 2-monopalmitoyl glycerol in serum-free media containing 8 mM taurocholate for 1 h at 37°C.

Incubation of cells with [^{3}H]oleic acid

Caco-2 cells, grown on polycarbonate supports, were incubated with [^{3}H]oleic acid bound to BSA or [^{3}H]oleic acid–taurocholate micelles at 37°C in an atmosphere of 95% air/5% CO_2. For apical labeling, the apical media was replaced with 1.5 ml serum-free media containing [^{3}H]oleic acid–BSA or [^{3}H]oleic acid–taurocholate micelles and the basolateral media was replaced with 2.6 ml of serum-free media (plus or minus 0.15 mM BSA). In all cases the oleic acid concentration in media was 0.5 mM. For basolateral labeling, 1.5 ml of serum-free media (plus or minus 8 mM taurocholate) was added apically and 2.6 ml of media containing [^{3}H]oleic acid–BSA was added basolaterally. Cells were incubated for 15 min, 2 h, 6 h, or 24 h. At the end of the incubation, the apical and basolateral media were removed and centrifuged at 1000 *g* at 4°C for 10 min to remove cellular debris. Cell monolayers were washed three times with phosphate-buffered saline (PBS) containing 4 mg/ml BSA. Cells were scraped off the inserts, suspended in PBS, and disrupted using a Vibra-Cell model VC-500 sonicator (Sonics and Materials Inc., Danbury, CT) equipped with a microtip (a continuous pulse was applied for 30 sec with the output at 10 watts). Media and cell homogenates were stored at –70°C for subsequent lipid analyses. The distribution of radioactivity between the apical and basolateral media, cell wash, and the cell homogenate was determined by scintillation counting. All experiments were performed with at least three filters.

Lipid extraction

Lipids were extracted from the media and cells according to the method of Bligh and Dyer (19). Briefly, 1 ml of media or cell homogenate was mixed with 3.75 ml of methanol–chloroform 2:1 (v/v) and shaken intermittently for 30 min at room temperature. The contents were spun at 10,000 *g* for 15 min to remove protein and the supernatant was saved. The protein pellet was resuspended in 1 ml of PBS and re-extracted with 3.75 ml of methanol–chloroform 2:1. The combined extracts were diluted with 2.5 ml of both chloroform and water, acidified to pH 3.0–4.0 with 1 N HCl, and centrifuged. The chloroform layer was removed and saved. The aqueous layer was again extracted with 2.5 ml chloroform. The combined chloroform extracts were evaporated to dryness under a stream of N_2. The residue was dissolved in 1 ml chloroform and stored at –20°C.

Thin-layer chromatography of radioactive lipids

To determine the incorporation of [^{3}H]oleic acid into radioactive lipids, an aliquot (~50,000–100,000 cpm) of media or cell lipid extract, along with lipid standards (arachidonic acid, triolein, diolein, and cholesteryl oleate) was spotted on a thin-layer chromatography plate (20 cm × 20 cm, Silicagel G-25, Brinkmann Instruments, Inc., Westbury, NY) which was subsequently developed in hexane–ethyl ether–acetic acid 80:20:1 (14). Spots corresponding to FA, diolein, and triolein, and cholesteryl oleate were visualized by exposing the plate to I_2 vapor.

Phospholipid analysis of cell lipid extract was carried out by two-dimensional thin-layer chromatography using the solvent system of Butler, Lichtenberger, and Hills (20). Cell lipid extracts (~50,000 cpm) along with phospholipid standards [phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidic acid (PA), lysophosphatidylcholine (LPC), and sphingomyelin (SM)] were spotted on a thin-layer chromatography plate and developed first with chloroform–methanol–acetic acid–water 50:25:8:4 (lst dimension) and then with the same solvents in a ratio of 50:7.5:8:2 (2nd dimension). Phospholipid spots were visualized by spraying the plate with molybdenum blue. For quantification, the area corresponding to each lipid spot was scraped into 10 ml Budget-Solve (Research Products International, Mt. Prospect, IL) and the amount of incorporated radiolabel was determined by scintillation counting.

β-Oxidation of [^{3}H]oleic acid

Ketone bodies in the media were determined according to the method of Leung and Peters (21). Free FAs were bound to added BSA (final concentration 0.15 mM in a final volume of 600 µl) by incubating the solution at 37°C for 1 h. An equal volume of 0.6 M perchloric acid was added to precipitate albumin-bound FA and the mixture was incubated on ice for 1 h. After centrifugation at 10,000 *g* for 10 min, ketone bodies present in the cleared supernatant were quantified by counting an aliquot in 10 ml Budget-Solve.

One product of β-oxidation of ^{3}H-labeled fatty acids is [^{3}H]acetate which in enterocytes serves as a substrate for the synthesis of new fatty acids, primarily [^{3}H]palmitate. After incubating Caco-2 cells with [^{3}H]oleic acid, cellular lipids were extracted as above. Cellular triglycerides and phospholipids were isolated by thin-layer chromatography and subjected to base methanolysis (12). The released fatty acids were then dried under N_2, redissolved in chloroform, and separated by argentation thin-layer chromatography using chloroform–methanol 99:1 (v/v), to resolve methyl esters containing 0–3 double bonds. Bands representing different classes of fatty acid methyl esters were visualized as dark brown spots by spraying the plate with 50% H_2SO_4 and heating on a hot plate. The area cor-

responding to palmitic acid was scraped into 10 ml Budget-Solve and counted in a liquid scintillation counter as described (12).

Northern and RNA blot hybridization

Total RNA was purified from differentiated (10 or 18 days postconfluent) and undifferentiated (6 days postplating, 95% confluent) Caco-2 cells using the guanidinium isothiocyanate method (22). Northern and RNA dot blots were prepared, probed, and washed as described in Levin et al. (23, 24). Human liver (L-) FABP cDNA (25) and a BamHI/HindIII fragment encompassing exon I of the human intestinal (I-) FABP gene (26) were labeled with α[^{32}P]d-ATP using the random primer method (27) and used as hybridization probes. Autoradiographs were scanned by laser densitometry and the data were analyzed as described (23, 24).

Western blots

Eighteen day postconfluent Caco-2 monolayers were washed three times with ice-cold PBS. They were harvested by scraping into a small volume of PBS. The cells were centrifuged (1000 *g*, 10 min) and the pellets were resuspended in lysis buffer [Tris-HCl (2 mM, pH 7.1), mannitol (50 mM), benzamidine (17.5 μg/ml), aprotinin 10 μg/ml), phenylmethanesulfonyl fluoride (1 mM), pepstatin (1 μg/ml)]. Homogenates were prepared from these cells as well as from adult (250 g) Sprague-Dawley rat small intestine and liver. After reduction, denaturation, and electrophoresis through 12.5% polyacrylamide gels containing sodium dodecyl sulfate (0.1%, ref. 28), Western blots were prepared and processed as described (29). Polyclonal rabbit anti-rat I-FABP and L-FABP sera which cross-react with human I-FABP and L-FABP, respectively (ref. 30; and Levin, M. S. and J. I. Gordon unpublished observations) were used to probe the blots.

Statistical analysis

The Student's *t*-test for unpaired observations was used for calculation of *P* values.

RESULTS

Uptake of oleic acid from BSA-oleic acid complexes: effect of apical versus basolateral presentation

Oleic acid (0.5 mM) bound to BSA was presented to either the apical or basolateral surface of confluent Caco-2 cell monolayers. Control polycarbonate filters without cells allowed free passage of the oleic acid–BSA complexes whereas confluent cell monolayers did not; after 6-h incubations with [^{3}H]oleic acid–BSA, less than 0.1% of the radiolabel was recovered from the side opposite the side of presentation.[2] **Fig. 1** shows that the amount of intracellular radiolabel that accumulated within cells at 15 min, 2 h, and 24 h was similar whether the oleic acid–BSA complex was added to the basolateral or the apical media. At 6 h, uptake of oleic acid from the apical side was significantly ($P < 0.05$) greater than from the basolateral side (**Table 1**), although the difference was only 1.4-fold. Under the conditions of the experiment, uptake of oleic acid was 86–136 nmol/filter at 6 h and 310–370 nmol/filter at 24 h.

Uptake of oleic acid complexes to BSA or taurocholate micelles

Oleic acid incorporated into micelles with taurocholate or with taurocholate plus 2-monoacylglycerol was delivered to the apical surface of confluent mono-

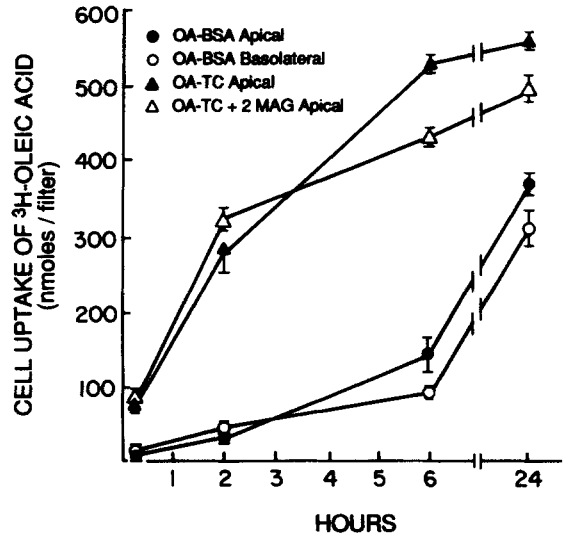

Fig. 1. Uptake of [^{3}H]oleic acid by Caco-2 cells. Caco-2 cells (12 days postconfluent) grown on Costar inserts were incubated with 0.5 mM [^{3}H]oleic acid bound to BSA (OA-BSA) added to the apical media or to the basolateral media or with [^{3}H]oleic acid–taurocholate (OA-TC) micelles or with [^{3}H]oleic acid–taurocholate–monoacylglycerol micelles (OA-TC-MAG) added to the apical media. Apical and basolateral additions were made in 1.5 and 2.6 mls of serum-free media, respectively. Incubations were at 37°C for 0.25, 2, 6, or 24 h. At the end of the incubation, the apical and basolateral media were separately collected and centrifuged at 1000 *g* for 10 min to remove cell debris. The cells were washed three times with PBS containing 4 mg/ml BSA, scraped off the inserts, and suspended in Tris-buffered saline. After a brief sonication, an aliquot of cell homogenate was taken for counting and the counts per minute were converted to the equivalent nanomoles of [^{3}H]oleic acid based on the specific activity of the [^{3}H]oleic acid stock solution. For each incubation time a single aliquot from each of three inserts was used.

[2] At least 95% of the added radioactivity was recovered at the 0.25, 2, and 6 h time points. At 24 h, total recoveries were at least 83% for [^{3}H]oleic acid–BSA presented apically and 94% when it was presented basolaterally.

TABLE 1. Distribution of [^{3}H]oleic acid in media and cells

Mode of Presentation	Site of Presentation	Distribution of Label into		
		Apical Media	Basolateral Media	Cells
			nmol	
OA-BSA[a]	Apical	558.3 ± 37.1	24.4 ± 12.4	136.1 ± 13.2
OA-BSA	Basolateral	3.7 ± 0.2	1171.1 ± 51.7	86.2 ± 4.7

Cell monolayers were incubated as indicated for 6 h with 0.5 mM oleic acid–0.15 mM bovine serum albumin. Values are means ± SEM (n = 3).

[a]OA-BSA, oleic acid–bovine serum albumin.

layers. Oleic acid–taurocholate micelles were not applied to the basolateral surface of the Caco-2 cells because they did not readily traverse the polycarbonate filters: only 0.7% of the recovered radiolabel was found in the apical media 24 h after addition of ^{3}H-labeled fatty acid in taurocholate micelles to the basolateral media of control filters. Addition of 2-monoacylglycerol to the taurocholate micelles did not augment oleic acid uptake (Fig. 1). Presentation of oleic acid in taurocholate micelles (± 2-monoacylglycerol) resulted in more rapid cellular uptake of this 18:1 FA compared to when it was presented bound to BSA. Uptake of oleic acid presented in taurocholate micelles exceeded the uptake of oleic acid from oleic acid–BSA by 10-fold at 0.25 h, 8-fold at 2 h, and 4-fold at 6 h (Fig. 1). At 24 h less marked or no differences in the steady-state levels of intracellular [^{3}H]oleic acid were observed in different experiments; this is probably attributable to relatively greater substrate depletion in the micellar conditions at 24 h. For example, for the experiment used to generate Fig. 1, the concentration of oleic acid was reduced to a greater extent in taurocholate micelles (~0.1 mM at 24 h) than in the BSA complexes (~0.3 mM at 24 h).[3]

Cellular distribution of radioactivity as a function of site and mode of presentation of exogenous oleic acid

Caco-2 cells incorporated the majority of the absorbed [^{3}H]oleic acid into triglycerides at all time points, regardless of the site or mode of presentation (**Fig. 2**). Incorporation into phospholipids, diglycerides, and cholesteryl esters was also observed. For conditions in which the cells were exposed to oleic acid–BSA complexes for at least 6 h, the relative incorporation of radioactivity into lipids was as follows: triglycerides >> phospholipids > free FA > diglycerides > cholesteryl ester. These relationships were observed when the oleic acid–BSA complexes were presented to the apical or basolateral surface (see Fig. 2, cholesteryl esters are not included in Fig. 2 because they comprised less than 2% of total cellular radioactive lipids under all study conditions). The distribution of radioactivity among the various phospholipid subclasses was the same for oleic acid–BSA complexes added to either the apical or basolateral surface: PC >> PE > PI > PS > SM = LPC (**Table 2**).

Modest but reproducible differences were seen in the distribution of radiolabel into cellular lipids when presentation of [^{3}H]oleic acid bound to BSA was compared with presentation of [^{3}H]oleic acid in taurocholate micelles. In particular, the proportion of intracellular radiolabel found in diglycerides was greater for oleic acid presented as apical taurocholate micelles (11%) than as apical oleic acid–BSA complexes (2%; compare Fig. 2 panels A and C). Control experiments indicated that the differences in accumulation of ^{3}H-labeled diglycerides were not due to independent metabolic effects of the added BSA or taurocholate. For example, when oleic acid–BSA was presented basolaterally, the addition of apical taurocholate did not change the amount or proportion of cellular diglyceride. Conversely, the addition of basolateral BSA did not decrease the amount of ^{3}H-labeled diglyceride accumulation from apical oleic acid–taurocholate. There were also differences in the distribution of radiolabel among phospholipid classes. Three times as much PE was synthesized and thus a greater proportion of the radiolabel recovered in the phospholipid fraction was found in PE (47% vs. 27%) and a somewhat smaller proportion was found in PC (39% vs. 63%) after incubation for 24 h with apical oleic acid–taurocholate and apical oleic acid–BSA, respectively. The distribution of radioactivity among the other phospholipid subclasses was similar to that seen when cells were incubated with apical oleic acid–BSA (Table 2).

Synthesis of triglycerides through the monoacylglycerol pathway requires monoacylglycerol and fatty acid as substrates. Addition of 2-monoacylglycerol to

[3]The concentration of oleic acid remaining in the apical media was estimated by subtracting the amount of radiolabeled oleic acid that was cell associated at 24 h from the amount that was added to the apical media at the start of the incubation period.

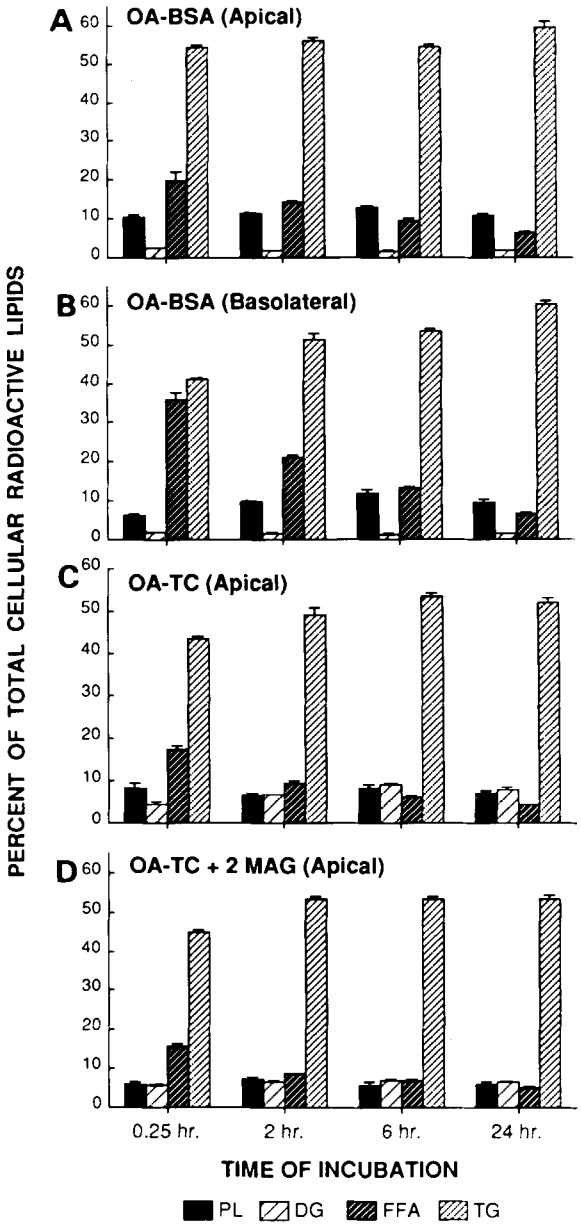

Fig. 2. Incorporation of [^{3}H]oleic acid into cellular lipids. Caco-2 cells grown on Costar inserts were incubated at 37°C for 0.25, 2, 6, and 24 h with [^{3}H]oleic acid–BSA complexes (panel A: apical; panel B: basolateral) or with apical [^{3}H]oleic acid–taurocholate micelles (panel C) or with apical [^{3}H]oleic acid–taurocholate–monoacylglycerol micelles (panel D) as described in Fig. 1. After the incubation the media was removed, the cells were washed with PBS containing 4 mg/ml BSA, and scraped off the inserts. The cells were suspended in Tris-buffered saline and disrupted by sonication. Lipids from the cell homogenates were extracted by the method of Bligh and Dyer (21) and analyzed by thin-layer chromatography. The mean ± SEM of the data obtained from three filters, at each condition, are presented.

[^{3}H]oleic acid in apical taurocholate micelles did not increase incorporation of [^{3}H]oleic acid into triglyceride and, in fact, had no effect on the distribution of intracellular radiolabel (Fig. 2, panels C and D).

Oxidative metabolism of exogenous oleic acid as a function of the site and mode of its presentation

Accumulation of ^{3}H-labeled ketone bodies in the media is a useful indicator of the extent to which ^{3}H-labeled fatty acids undergo β-oxidation. After 24 h incubation of Caco-2 cells with [^{3}H]oleic acid, accumulation of ketone bodies in the media ranged from 20 to 37 nm per filter. This corresponds to only 2–7% of the total radiolabel recovered regardless of the mode of oleic acid presentation. For all four conditions (i.e., oleic acid–BSA apically or basolaterally; oleic acid–taurocholate apically; oleic acid–taurocholate–monoacylglycerol apically), the amount of ketone body production paralleled the accumulation of radioactivity within cells (compare Fig. 1 and **Fig. 3**). The initial rate of ketone body formation (nmol/filter per h) and the amount accumulated were much greater when oleic acid was presented in taurocholate micelles than when bound to BSA. Thus, the mode of presentation may influence the degree of β-oxidation. However, it is also possible that the rate and quantity of ketone body production simply reflect cellular oleic acid levels (see Fig. 1).

The site of presentation of BSA–oleic acid had no detectable influence on the degree of β-oxidation at 0.25 or 4 h. However, at 6 and 24 h, ketone bodies in the media accounted for a somewhat greater proportion of recovered radiolabel after apical as opposed to basolateral presentation (6 h 1.6% vs. 0.65%, $P < 0.001$; 24 h, 4.4% vs. 1.7%, $P < 0.002$).

In addition to ketone body formation, β-oxidation of ^{3}H-labeled fatty acids can lead to the formation of [^{3}H]acetate which enterocytes use to synthesize new ^{3}H-labeled fatty acids, particularly [^{3}H]palmitic acid. To assess the synthesis of [^{3}H]palmitic acid, cellular phospholipids and triglycerides were saponified and the released fatty acids were separated, based on their degree of saturation, by argentation thin-layer chromatography. The amount of radiolabel recovered from palmitic acid ranged from 0.6% of the recovered label (basolateral oleic acid–BSA) to 3.0% (apical oleic acid–taurocholate; data not shown). Thus, when combined with ketone body production, these data indicate that 2.3–8% of the [^{3}H]oleic acid undergoes β-oxidation under these experimental conditions. Other oxidative products such as CO_2 and Kreb cycle intermediates would be expected to increase these estimates somewhat.

Triglyceride secretion

The secretion of newly synthesized lipids into the apical and basolateral media was analyzed. Most of the incorporated oleic acid was retained intracellularly: by 24 h only 5–35% of the radiolabel found in trigly-

TABLE 2. Phospolipid composition of Caco-2 cells

Mode and Site of Presentation	Radiolabel in					
	LPC	SM	PC	PS	PI	PE
	nmol					
OA-BSA Apical	0.22 ± 0.06	0.23 ± 0.04	16.77 ± 1.9	1.63 ± 0.17	2.35 ± 0.38	5.62 ± 0.62
OA-BSA Basolateral	0.25 ± 0.12	0.14 ± 0.02	13.68 ± 2.2	1.17 ± 0.17	1.88 ± 0.19	3.85 ± 0.59
OA-TC Apical	0.28 ± 0.06	0.53 ± 0.15	14.65 ± 4.8	2.62 ± 0.74	2.05 ± 0.44	17.57 ± 4.0
OA-TC+MAG Apical	0.18 ± 0.01	0.35 ± 0.03	10.32 ± 1.3	1.59 ± 0.28	1.54 ± 0.09	11.87 ± 4.0

Values reported here are mean ± SEM (n = 3 filters). Abbreviations: LPC, lysophospatidylcholine; SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; OA-BSA, oleic acid–bovine serum albumin; OA-TC, oleic acid–taurocholate; 2MAG, 2-monoacylglycerol.

cerides was present in the media regardless of the site or mode of oleic acid presentation (see Fig. 2 and **Table 3**).

Under most conditions, apical presentation resulted in nearly equal secretion to the basolateral and apical sides with a trend towards apical secretion (Table 3). Basolateral presentation of oleic acid–BSA resulted in highly polarized secretion with 85–90% of secreted TG going basolaterally (Table 3). These data indicate that triglyceride synthesis and/or secretion is compartmentalized differently depending on the site of delivery of the exogenous FA. Control experiments were done to determine whether the mode of delivery influenced the polarity of triglyceride secretion independent of the side of presentation of exogenous oleic acid. Caco-2 monolayers were incubated with [^{3}H]oleic acid bound to BSA presented apically in the presence or absence of basolateral BSA. In both cases, secretion of ^{3}H-labeled triglycerides into the apical and basolateral media was approximately equal (Table 3). Thus, BSA did not independently change the magnitude or the polarity of triglyceride secretion.

The mode of presentation of oleic acid had a minor effect on the polarity of triglyceride secretion (Table 3). When oleic acid was presented apically in taurocholate micelles (± 2-monoacylglycerol) the trend was towards greater apical secretion (Table 3) after 24 h when compared to results obtained with apical oleic acid–BSA. To determine whether the presence of taurocholate in the media influenced the polarity of triglyceride, we presented [^{3}H]oleic acid bound to BSA basolaterally in the presence or absence of apical taurocholate. In both cases, secretion of ^{3}H-labeled triglycerides was still highly polarized in favor of the basolateral surface, indicating that taurocholate did not independently affect the polarity of triglyceride secretion

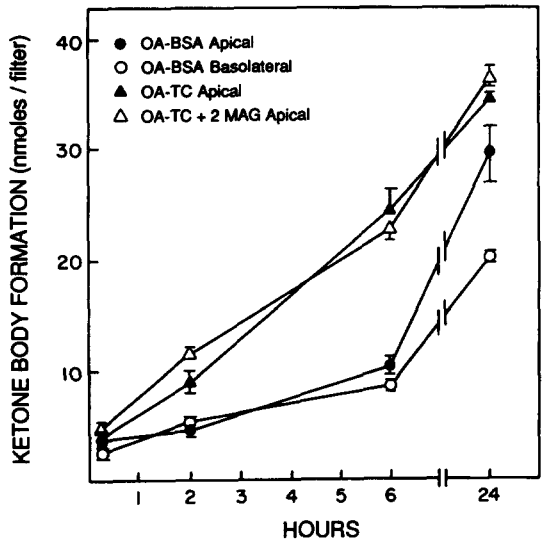

Fig. 3. Metabolism of [^{3}H]oleic acid to ketone bodies. [^{3}H]Oleic acid–BSA and [^{3}H]oleic acid–taurocholate micelles and [^{3}H]oleic acid–taurocholate–monoacylglycerol micelles were incubated with 12-day postconfluent Caco-2 cells for 0.25, 2, 6, or 24 h at 37°C as described in Fig. 1. The apical and basolateral media were collected and centrifuged to remove cell debris. Ketone bodies in the media were determined according to the method of Leung and Peter (23) as described in Methods. Each data point presents the mean ± SEM for three inserts.

Characterization of the FABP phenotype of Caco-2 cells

Studies suggesting a role for the FABPs in modulating enterocytic FA metabolism prompted us to determine the FABP "phenotype" of the Caco-2 cell line. Northern and RNA dot blots as well as Western blots revealed that these cells express L-FABP: the relative amounts of L-FABP mRNA in 18-day postconfluent cells was 15 times that in preconfluent cells (data not shown). No I-FABP mRNA (26) or protein was detectable in pre- or postconfluent cells (data not shown).

DISCUSSION

In this study, Caco-2 cells were used as a model system to analyze the trafficking of exogenous oleic acid

TABLE 3. Intracellular and secreted triglycerides

Conditions				Secreted Triglyceride	
Apical	Basolateral	N	Intracellular Triglyceride	Apical+ Basolateral media	Apical: Basolateral Ratio
			nmol	*nmol*	
OA-BSA		6	147.6 ± 9.0	14.5 ± 3.0	1.11 ± 0.26
OA-BSA	BSA	3	102.2 ± 1.7	8.5 ± 0.5	1.31 ± 0.02
	OA-BSA	6	98.6 ± 25.7	20.5 ± 2.0	0.11 ± 0.03
TC	OA-BSA	3	35.5 ± 1.8	19.0 ± 1.1	0.14 ± 0.03
OA-TC		12	155.1 ± 20.7	17.9 ± 2.6	1.91 ± 0.64
OA-TC-MAG		3	227.3 ± 5.0	13.0 ± 0.5	2.70 ± 0.15

Postconfluent Caco-2 cell monolayers were incubated on polycarbonate filters (24 mm, 3-μm pore size) for 24 h in the presence of the indicated additions. Values represent means ± SEM. Abbreviations: OA-BSA, [^{3}H]oleic acid–bovine serum albumin; TC, taurocholate; OA-TC, [^{3}H]oleic acid–taurocholate micelles; 2MAG, 2-monoacylglycerol.

in enterocytes. Caco-2 cells were grown on filter inserts that allowed independent manipulation of the apical and basolateral media bathing monolayers. This system was used to examine the effects of varying the site (e.g., apical versus basolateral) or mode (e.g., bound to BSA or incorporated into taurocholate micelles with or without 2-monoacylglycerol) of oleic acid presentation on metabolic processing and export of lipids in 12-day postconfluent Caco-2 monolayers. By this time, the majority of the cells have well-developed microvilli, express a variety of cell surface markers found on differentiated villus enterocytes, and exhibit well-developed tight junctions (31, 32). Uptake studies comparing the absorption of oleic acid from oleic acid–BSA complexes presented apically or basolaterally indicate that, like small intestinal enterocytes, postconfluent Caco-2 cells absorb FAs from their basolateral as well as their apical surfaces (33). The magnitude of oleic acid absorption was essentially identical from the apical and the basolateral surfaces.

The majority of luminal fatty acids absorbed by small intestinal enterocytes in vivo are solubilized in mixed micelles. Incubation of Caco-2 monolayers with oleic acid in taurocholate micelles led to dramatically enhanced oleic acid absorption compared to incubation with oleic acid–BSA complexes for incubation times ranging from 15 min to at least 6 h. This was not attributable to an independent metabolic effect of taurocholate as demonstrated by comparing oleic acid absorption by monolayers presented with basolateral oleic acid–BSA in the presence or absence of apical taurocholate. The presence of taurocholate did not augment oleic acid uptake (data not shown).

The cellular distribution of radioactivity after absorption of [^{3}H]oleic acid by Caco-2 cells was qualitatively similar to that of small intestinal enterocytes (1). The majority of the radiolabel was recovered in cellular triglycerides whether [^{3}H]oleic acid was presented apically or basolaterally. In previous studies, incorporation into triglycerides was found to be the major metabolic fate of both exogenous palmitate (13, 33) and oleic acid (8, 34). Although the rate of FA uptake was essentially the same for apical and basolateral presentation, the rate of fatty acid esterification into triglycerides was greater for apical presentation (Fig. 2A and B). A minor increase in the rate of esterification into phospholipids was also noted. These observations are consistent with the hypothesis that FAs absorbed from the apical surface enter into different metabolic pools than FAs absorbed basolaterally. The mode of presentation also affected the intracellular metabolism of oleic acid. For example, the amounts of radioactivity recovered in intracellular diglyceride and PE were significantly greater when [^{3}H]oleic acid was presented in taurocholate micelles.

Intestinal enterocytes synthesize triglycerides by the monoacylglycerol or the phosphatidic acid pathways. Enzymes of the monoacylglycerol pathway are associated with the smooth endoplasmic reticulum and account for approximately 70% of triglyceride synthesis under physiologic conditions (reviewed in ref. 3). The phosphatidic acid pathway is associated with the rough endoplasmic reticulum, predominates during fasting, and uses luminal as well as plasma-derived substrates obtained from glucose metabolism and endogenous FAs (reviewed in ref. 3).

We examined how changes in the site or mode of presentation of FAs to morphologically polarized, postconfluent Caco-2 cells affect: *i*) the relative utilization of the monoacylglycerol and phosphatidic acid pathways for triglyceride synthesis, *ii*) the proportion of radiolabeled FA shunted to oxidative metabolism and, *iii*) the secretion of newly synthesized triglycerides.

2-Monoacylglycerol was added to oleic acid–taurocholate micelles to provide Caco-2 cells with sub-

strate for the monoacylglycerol pathway. However, the distribution of intracellular radioactivity was not affected and no stimulation of oleic acid incorporation into triglyceride was detected. This suggests that the phosphatidic acid pathway predominates in Caco-2 cells, although this conclusion is predicated on two assumptions: *i*) that sufficient 2-monoacylglycerol was absorbed to significantly augment triglyceride synthesis, and *ii*) that stimulation of the 2-monoacylglycerol pathway does not proportionately inhibit the phosphatidic acid pathway. Studies by Trotter and Storch (35) provide a possible explanation for our observation that the phosphatidic acid pathway seems predominant. They have shown that in Caco-2 homogenates, glycerol-3-phosphate acyltransferase activity was comparable to that found in rat jejunum, whereas monoacylglycerol acyltransferase activity was low or absent.

Only a minor portion (< 10%) of the absorbed oleic acid underwent β-oxidation as determined by ketone body formation and the incorporation of [^{3}H]acetate into newly synthesized palmitate. This does not indicate a general defect in the ability of Caco-2 cells to β-oxidize FAs. In an earlier study, we compared Caco-2 metabolism of apically presented arachidonic acid and 15-hydroxy-5,8,11,13-eicosatetraenoic acid (HETE), a hydroxylated metabolite of arachidonic acid (12). Most (55%) of the absorbed HETE underwent several cycles of β-oxidation. The major product of β-oxidation of [^{3}H]15-HETE in Caco-2 cells is [^{3}H]acetate which is incorporated into newly synthesized [^{3}H]palmitate. In contrast, this was a relatively minor metabolic fate for [^{3}H]arachidonic acid (12) as it was for [^{3}H]oleic acid in the present study. [^{3}H]Arachidonic acid, like [^{3}H]oleic acid, is largely esterified into complex lipids with little (~0.3%) undergoing β-oxidation.

In vivo, triglycerides and phospholipids, regardless of the source of FA and the metabolic pathways through which they are synthesized, are packaged with apolipoproteins into lipoprotein particles (primarily chylomicrons and very low density lipoproteins) which are exported through a polarized pathway through the basolateral surface of enterocytes. Caco-2 cells also secrete triglyceride in VLDL and chylomicrons (8–10). Traber, Kayden, and Rindler (8) have shown that triglyceride secretion is polarized in Caco-2 cells. We have extended these studies to look at the effect of the site and mode of presentation on triglyceride secretion. The site of presentation had a dramatic effect on the polarity of triglyceride secretion. The vast majority of secreted triglyceride was secreted into the basolateral media when oleic acid was absorbed basolaterally from BSA–oleic acid. When BSA–oleic acid was presented apically, however, secretion was nearly equal into the apical and the basolateral media. The polarity of triglyceride secretion was not due to an independent effect of BSA since the addition of BSA to the basolateral media did not enhance basolateral secretion when oleic acid was presented apically. Apical presentation of oleic acid in taurocholate micelles resulted in a slight bias toward more apical secretion. Control experiments indicated that taurocholate alone did not affect the polarity of triglyceride secretion.

The above studies demonstrate that Caco-2 cells differentially process exogenous oleic acid in a manner that is dependent on both the site and mode of presentation. In contradistinction to the studies of Gangl and Ockner using rats (1), preferential shunting of basolateral (equivalent to plasma-derived) oleic acid to phospholipids and β-oxidation was not observed. Differences in experimental methods and intrinsic differences in the two model systems may account for these findings. Variables such as the actual concentration of oleic acid to which the cells are exposed can be readily controlled in vitro, whereas in vivo the amount actually delivered to individual enterocytes by the intraduodenal and intravenous routes of administration used by Gangl and Ockner cannot be controlled. Another feature of the in vitro system is the ability to control exogenous factors which may otherwise influence enterocyte FA metabolism. For example, FA uptake and metabolism are influenced by luminal factors such as bile salts and phospholipids, by humoral substances such as insulin, and by the nutritional status of the animal (36). Although postconfluent Caco-2 cells share several phenotypic features with distal small intestinal enterocytes, it is clear that they are even more closely related to first trimester fetal colonocytes (reviewed in refs. 6 and 7). Enterocytes at this stage of gestation are not exposed to luminal nutrients. Thus, cellular metabolism may not be compartmentalized to the same extent as later in development, perhaps accounting for differences in the metabolic handling of apically presented versus basolaterally presented oleic acid between experiments using Caco-2 cells and whole animals.

Differences in the cytosolic FA binding protein content of Caco-2 cells and adult rat enterocytes may also be important variables that affect their metabolic processing of exogenous FA. Small intestinal enterocytes express I-FABP and L-FABP in large amounts (37, 38). Although the physiological functions of intestinal L-FABP and I-FABP are unknown, it has been postulated (reviewed in ref. 39) that they are involved in the uptake, intracellular processing, and metabolic compartmentation of FAs. Based on in vitro ^{13}C NMR studies of the ligand binding specificities and stoichiometries of purified *E. coli*-derived mammalian FABPs, Cistola et al. (17) have proposed that L-FABP

transports intestinal long-chain FAs as well as monoacylglycerols and/or lysophospholipids to sites of metabolic processing whereas I-FABP binds and transports FAs.

Although Caco-2 cells do not express I-FABP, they are capable of esterifying FAs into triglycerides. This indicates that I-FABP is not required by Caco-2 cells to absorb and metabolize dietary or plasma-derived long chain FAs via the phosphatidic acid pathway. The absence of I-FABP may account for the observation that FAs acquired through the basolateral membrane are not preferentially β-oxidized or incorporated into phospholipids as they are in vivo. This is being directly tested by producing human I-FABP in Caco-2 cells using a suitably constructed expression vector.

This work was supported by grants from the National Institutes of Health (DK43029, DK33165, and DK30292), and the Lucille P. Markey Charitable Trust. M.S.L. is a Future Leader Awardee of the International Life Sciences Institute-Nutrition Foundation.

Manuscript received 14 January 1991 and in revised form 28 October 1991.

REFERENCES

1. Gangl, A., and R. K. Ockner. 1975. Intestinal metabolism of plasma free fatty acids, intracellular compartmentation and mechanism of control. *J. Clin. Invest.* **55:** 803–813.
2. Shiau, Y., D. A. Popper, M. Reed, C. Umstetter, D. Capuzzi, and G. M. Levine. 1985. Intestinal triglycerides are derived from both endogenous and exogenous sources. *Am. J. Physiol.* **248:** G164–G169.
3. Shiau, Y. F. 1987. Lipid digestion and absorption. *In* Physiology of the Gastrointestinal Tract. Second Edition. Leonard R. Johnson, editor. Raven Press, New York. 1527–1556.
4. Senior, J. R., and K. J. Isselbacher. 1960. Activation of long-chain fatty acids by rat-gut mucosa. *Biochim. Biophys. Acta.* **44:** 399–400.
5. Fogh, J., J. M. Fogh, and T. Orfeo. 1977. One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice. *J. Natl. Cancer Inst.* **59:** 221–226.
6. Rousset, M. 1986. The human colon carcinoma cell lines HT-29 and Caco-2: two in vitro models for the study of intestinal differentiation. *Biochimie.* **68:** 1035–1040.
7. Neutra, M., and D. Louvard. 1989. Differentiation of intestinal cells in vitro. *In* Modern Cell Biology. Vol 8. Functional Epithelial Cells in Culture. K. S. Matlin and J. D. Valentich, editors, Alan R. Liss, Inc., New York. 363–398.
8. Traber, M. G., H. J. Kayden, and M. J. Rindler. 1987. Polarized secretion of newly synthesized lipoproteins by the Caco-2 human intestinal cell line. *J. Lipid Res.* **28:** 1350–1363.
9. Hughes, T. E., W. V. Sasak, J. M. Ordovas, T. M. Forte, S. Lamon-Fava, and E. J. Schaefer. 1987. A novel cell line (Caco-2) for the study of intestinal lipoprotein synthesis. *J. Biol. Chem.* **262:** 3762–3767.
10. Field, F. J., E. Albright, and S. N. Mathur. 1988. Regulation of triglyceride-rich lipoprotein secretion by fatty acids in Caco-2 cells. *J. Lipid Res.* **29:** 1427–1437.
11. Field, F. J., E. Albright, and S. N. Mathur. 1987. Regulation of cholesterol esterification by micellar cholesterol in Caco-2 cells. *J. Lipid Res.* **28:** 1057–1066.
12. Riehl, T. E., N. M. Bass, and W. F. Stenson. 1990. Metabolism of 15-hydroxyeicosatetraenoic acid by Caco-2 cells. *J. Lipid Res.* **31:** 773–780.
13. Murthy, S., E. Albright, S. N. Mathur, and F. J. Field. 1988. Modification of Caco-2 cell membrane fatty acid composition by eicosapentaenoic acid and palmitic acid: effect on cholesterol metabolism. *J. Lipid Res.* **29:** 773–780.
14. Dashti, N., E. A. Smith, and P. Alaupovic. 1990. Increased production of apolipoprotein B and its lipoproteins by oleic acid in Caco-2 cells. *J. Lipid Res.* **31:** 113–123.
15. Hughes, T. E., J. M. Ordovas, and E. J. Schaefer. 1988. Regulation of intestinal apolipoprotein B synthesis and secretion by Caco-2 cells. *J. Biol. Chem.* **263:** 3425–3431.
16. Moberly, J. B., T. G. Cole, D. H. Alpers, and G. Schonfeld. 1990. Oleic acid stimulation of apolipoprotein B secretion from HepG2 and Caco-2 cells occurs post-transcriptionally. *Biochim. Biophys. Acta.* **1042:** 70–80.
17. Cistola, D. P., J. C. Sacchettini, L. J. Banaszak, M. T. Walsh, and J. I. Gordon. 1989. Fatty acid interactions with rat intestinal and liver fatty acid-binding proteins expressed in *Escherichia coli:* a comparative 13C NMR study. *J. Biol. Chem.* **264:** 2700–2710.
18. Ellsworth, J. L., S. K. Erickson, and A. Cooper. 1986. Very low and low density lipoprotein synthesis and secretion by the human hepatoma cell line Hep-G2: effects of free fatty acid. *J. Lipid Res.* **27:** 858–874.
19. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37:** 911–917.
20. Butler, R. D., L. M. Lichtenberger, and B. A. Hills. 1983. Distribution of surfactants in the canine gastrointestinal tract and their ability to lubricate. *Am. J. Physiol.* **244:** G645–G651.
21. Leung, N. W. Y., and T. J. Peters. 1986. Palmitic acid oxidation and incorporation into triglyceride by needle liver biopsy specimens from control subjects and patients with alcoholic fatty liver disease. *Clin. Sci.* **71:** 253–260.
22. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry.* **18:** 5294–5299.
23. Levin, M. S., E. Li, D. E. Ong, and J. I. Gordon. 1987. Comparison of the tissue-specific expression and developmental regulation of two closely linked rodent genes encoding cytosolic retinol binding proteins. *J. Biol. Chem.* **262:** 7118–7124.
24. Levin, M. S., A. J. Pitt, A. L. Schwartz, P. A. Edwards, and J. I. Gordon. 1989. Developmental changes in the expression of genes involved in cholesterol biosynthesis and lipid transport in human and rat fetal and neonatal livers. *Biochim. Biophys. Acta.* **1003:** 293–300.
25. Lowe, J. B., M. S. Boguski, D. A. Sweetser, N. A. Elshourbagy, J. M. Taylor, and J. I. Gordon. 1985. Human liver fatty acid binding protein: isolation of a full length

cDNA and comparative sequence analyses of orthologous and paralogous proteins. *J. Biol. Chem.* **260:** 3413–3417.
26. Sweetser, D. A., E. H. Birkenmeier, I. J. Klisak, S. Zollman, R. S. Sparkes, T. Mohandas, A. J. Lusis, and J. I. Gordon. 1987. The human and rodent intestinal fatty acid binding protein genes: a comparative analysis of their structure, expression, and linkage relationships. *J. Biol. Chem.* **262:** 16060–16071.
27. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132:** 6–13.
28. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
29. Levin, M. S., B. Locke, N. C. Yang, E. Li, and J. I. Gordon. 1988. Comparison of the ligand binding properties of two homologous rat (apo)cellular retinol-binding proteins expressed in *Escherichia coli*. *J. Biol. Chem.* **263:** 17715–17723.
30. Carrol, S. L., K. A. Roth, and J. I. Gordon. 1990. Liver fatty acid-binding protein: a marker for studying cellular differentiation in gut epithelial neoplasms. *Gastroenterology.* **99:** 1727–1735.
31. Pinto, M., S. Robine-Leon, M. D. Appay, M. Kedinger, N. Riadou, E. Dussaulx, B. Lacroix, P. Simon-Assmann, K. Haffen, J. Fogh, and A. Zweibaum. 1983. Enterocyte-like differentiation and polarization of the human colon carcinoma cell line Caco-2 in culture. *Biol. Cell* **47:** 323–330.
32. Hidalgo, I. J., T. J. Raub, and R. T. Borchardt. 1989. Characterization of the human colon carcinoma cell line (Caco-2) as a model system for intestinal epithelial permeability. *Gastroenterology.* **96:** 736–749.
33. Trotter, P. J., and J. Storch. 1991. Fatty acid uptake and metabolism in a human intestinal cell line (Caco-2): comparison of apical and basolateral incubation. *J. Lipid Res.* **32:** 293–304.
34. Murthy, S., E. Albright, S. N. Mathur, and F. J. Field. 1990. Effect of eicosapentaenoic acid on triacylglycerol transport in Caco-2 cells. *Biochim. Biophys. Acta.* **1045:** 147–155.
35. Trotter, P. J., and J. Storch. 1991. Fatty acid esterification during differentiation of Caco-2 intestinal cells. *FASEB J.* **5:** A1640.
36. Shiau, Y. F., and P. G. Holtzapple. 1980. Effect of insulin on in vitro intestinal fatty acid esterification in the rat. *Am. J. Physiol.* **238:** E364–E370.
37. Haq, R. U., L. Christodoulides, B. Ketterer, and F. Shrago. 1982. Characterization and purification of fatty acid-binding protein in rat and human adipose tissue. *Biochim. Biophys. Acta.* **713:** 193–198.
38. Bass, N. M., J. A. Manning, R. K. Ockner, J. I. Gordon, S. Seetharam, and D. H. Alpers. 1985. Regulation of the biosynthesis of two distinct fatty acid-binding proteins in rat liver and intestine: influences of sex difference and of clofibrate. *J. Biol. Chem.* **260:** 1432–1436.
39. Sweetser, D. A., R. O. Heuckeroth, and J. I. Gordon. 1987. The metabolic significance of mammalian fatty-acid-binding proteins: abundant proteins in search of a function. *Annu. Rev. Nutr.* **7:** 337–359.