Human crypt intestinal epithelial cells are capable of lipid production, apolipoprotein synthesis, and lipoprotein assembly

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Abstract **The recent availability of spontaneously proliferating, non-transformed human crypt intestinal epithelial cells (HIEC) affords an opportunity to investigate lipid metabolism in undifferentiated enterocytes. The major purpose of this study was to explore the capability of undifferentiated crypt cells to synthesize, assemble, and secrete lipids and apolipoproteins. HIEC were cultured in medium with 5% fetal bovine serum for 5 to 21 d. The cells were clearly able to incorporate [^{14}C]oleic acid (dpm/mg protein) into triglycerides (128,279 ± 16,988), phospholipids (30,278 ± 2,107), and cholesteryl esters (2,180 ± 207). Although improvement in lipid secretion was noted with prolongation of cell culture periods, low efficiency of lipid export (10.3 ± 2.2% of intracellular content) characterized the HIEC. All phospholipid classes were elaborated, with phosphatidylcholine accounting for 79.3 ± 1.3% of cellular phospholipids. Chylomicrons were the dominant (46.4%) lipoproteins secreted, followed by high, low, and very low density lipoproteins (HDL, LDL, and VLDL) comprising 22.5, 20.2, and 10.8% of the total, respectively. HIEC elaborated most of the major apolipoprotein (apo) classes (A-I, A-IV, B-100, C, and E), but were less efficient in producing apoB-48. In contrast to the production of apoA-I and C as early as 5 days after confluence, apoA-I and A-IV were maximally expressed at 11 d. Culture media accumulated much more apoB-100 than apoB-48 (B-48/B-100 ratio 0.21 ± 0.03), reflecting limited apoB mRNA editing. HIEC demonstrated both endogenous cholesterol synthesis and LDL receptor expression. Cholesterol synthesis was sensitive to 25-hydroxycholesterol and mevinolin, but unresponsive to LDL treatment, suggesting independent regulation pathways. In contrast, LDL inhibited receptor activity. The present findings provide the first solid evidence that immature HIEC are capable of key fat absorptive functions of well-differentiated enterocytes. The intracellular mechanisms required for lipid and apolipoprotein synthesis as well as for lipoprotein assembly are already present in intestinal crypt cells. These cells also retain the capacity for sterol enzyme and receptor expression. However, certain limitations, especially apoB-48 production and lipoprotein secretion as well as unresponsiveness of cholesterol synthesis to LDL, may be ascribed to the lack of differentiation.**—Levy, E., J-F. Beaulieu, E. Delvin, E. Seidman, W. Yotov, J-R. Basque, and D. Ménard. **Human crypt intestinal epithelial cells are capable of lipid production, apolipoprotein synthesis, and lipoprotein assembly.** ***J. Lipid Res.*** **2000.** 41: **12–22.**

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The last two decades have witnessed a rapid expansion in the understanding of the processes of fat digestion and absorption (for review, see 1–5). After the enzymatic hydrolysis of fatty acid esters and dispersion of lipolytic products into an absorbable form, it is now recognized that the subsequent cellular events are essential for the transport of dietary lipids into the circulation. The formation of chylomicrons within the enterocyte is a multi-step process that includes the uptake of lipolytic products, re-esterification and translocation of cellular lipid pools, synthesis and post-translational modification of various apolipoproteins, and the assembly of lipid and apolipoprotein moieties (1–5). Biochemical, molecular and cell biological studies of different congenital lipid transport disorders have disclosed the crucial role of apoB-48 and microsomal transfer protein (MTP) in the packaging of alimentary lipids into triglyceride-rich lipoproteins (6–9). Other apolipoproteins, including A-I and A-IV, reside on intestinal lipo-

Abbreviations: HIEC, human crypt intestinal epithelial cells; HDL, LDL, and VLDL, high, low, and very low density lipoproteins; MTP, microsomal transfer protein; PL, phospholipid; MG, monoglyceride; DG, diglyceride; TG, triglyceride; FFA, free fatty acid; CE, cholesteryl ester; FCS, fetal calf serum; TLC, thin-layer chromatography; LPDS, lipoprotein-deficient serum; CM, chylomicron; FC, free cholesterol.

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protein particles and display important functions. They contribute toward the structural stabilization of lipoprotein particles, interact with cell-surface receptors, and function as powerful activators of plasma enzyme activity (10, 11).

The crypt–villus axis represents the functional unit of the absorptive intestinal mucosa (12–15). It is generally accepted that the ability to elaborate apolipoproteins and lipoproteins is restricted to mature villus enterocytes (16–19). As also deduced from various studies conducted on laboratory animals, it has been assumed that only mature villus enterocytes exhibit full brush border hydrolases (20). The latter have, therefore, been used as specific markers for the investigation of enterocyte differentiation, a concept that is now questioned (21) given the delineation of two classes of brush border markers in the human gut: one specific to differentiated enterocytes (maltase–glucoamylase) and the other expressed in both undifferentiated crypt cells and differentiated villus cells (sucrase–isomaltase, aminopeptidase N and dipeptidyl peptidase IV (22, 23). Due to the lack of adequate experimental models, relatively little information is available about lipid biogenesis and metabolism as well as apolipoprotein and lipoprotein synthesis in human intestinal crypt cells. Our recent analysis of apolipoproteins A-I, A-IV, B-48, and B-100 along the crypt–villus axis of the human jejunum revealed the expression of these molecules in both crypt and villus cells (24). However, the issue as to whether human crypt cells are actually able to synthesize apolipoproteins, elaborate various lipoprotein species, and participate in lipid metabolism and transport remains open. As a first step toward answering this question, we chose to focus on a novel human intestinal crypt cell model in order to investigate their potential for human lipid transport and metabolism.

METHODS

Cell culture

Normal human intestinal epithelial cells were generated with the use of the dissociating enzyme thermolysin as previously described (25). Cell populations were expanded and then kept frozen (in DMEM containing DMSO and FCS) at passage 2 or 3, in liquid nitrogen. For the following studies, cell aliquots were cultured at 37°C in high glucose DMEM containing 5% FBS, 10 mm HEPES, 4 mm glutamine, 50 U/ml penicillin, 50 μg/ml streptomycin, 5 μg/ml epidermal growth factor, and 100 μl/ml insulin–transferrine–selenium, all from Gibco/BRL. Confluence was reached by day 13. Media were replaced every 2 days. The experiments were carried out either during the exponential growth phase (5 d), at confluence (13 d), or 8 d after confluence (21 d).

Indirect immunofluorescence

Cells were grown on 8-well Lab-Tek slides (Nunc Inter-Med, Rocklide, Denmark) using Dulbecco's modified Eagle's medium (DMEM; Gibco/BRL) supplemented with 5% fetal bovine serum (FBS; Cellect Gold, ICN/Flow, Costa Mesa, CA) until confluence. Cells were washed twice with incomplete DMEM and fixed in ethanol (10 min, −20°C) before immunostaining (24). The primary antibodies were apo [A-IV, 1:100; apoA-I, 1:200; apoB-100 (4G3), 1:100 and apoB-48/apoB-100 (2D8), 1:200] in PBS containing 10% Blotto. Secondary antibodies were either fluorescein-conjugated goat anti-mouse IgG or fluorescein-conjugated goat anti-sheep IgG (Boehringer Mannheim), both at a final dilution of 1:25 in PBS containing 10% Blotto. In all cases, no fluorescent staining was observed when the primary antibodies were replaced by the appropriate nonimmune mouse or sheep control sera.

Incorporation of [^{14}C]oleic acid into lipids

[^{14}C]oleic acid (Amersham, Oakville, Ontario, Canada) was first complexed to bovine serum albumin (4:1, mol:mol, ratio) (26). After various periods of culture (5,13,21 d), monolayers were washed twice with MEM (serum free) and the [^{14}C]oleic acid substrate (equivalent to 1.0 μmol/ml) containing 2–2.5 μCi/well (sp act of 53.9 mCi/mmol), was added to the medium. Cells were incubated for 18 h at 37°C, in order to determine lipid synthesis and secretion. At the end of this incubation period, the cells were washed and scraped off with a rubber policeman into maleate buffer (pH 6.0), and sonicated. Another aliquot was taken for lipid extraction after the addition of unlabeled carriers [phospholipids (PL), monoglycerides (MG), diglycerides (DG), triglycerides (TG), free fatty acids (FFA), and cholesteryl ester (CE)], using standard methods (26).The various lipid classes synthesized from [^{14}C]oleic acid were separated by thin-layer chromatography (TLC) using the nonpolar solvent mixture: hexane–ether–acetic acid 80:20:3 (vol/vol/vol). A polar solvent, chloroform–methanol–water–acetic acid, 65:25:4:1 (vol/vol/vol/vol), containing small amounts of phospholipid standards was used to separate phospholipid classes. The area corresponding to each lipid was scratched off the TLC plates. The silica powder was then placed in a scintillation vial with Ready Safe (Beckman Instruments, Fullerton, CA), and radioactivity was measured by scintillation counting (Beckman #LS5000 TD, Ontario, Canada). Cell protein was quantified by the method of Lowry et al. (27), and results were expressed as dpm/mg cell protein. Lipids secreted into the medium were analyzed and quantified as above, after centrifugation (2000 rpm, 30 min, 4°C) to remove cell debris.

Lipid carrier

Blood was drawn 1.5 h after the oral intake of a fat meal, and postprandial plasma was prepared to serve as a carrier for the lipoproteins synthesized by HIEC, as described previously (26). The triglyceride-enriched plasma was incubated at 56°C for 1 h to inactivate enzymatic activity.

Isolation of lipoproteins

For the determination of secreted lipoproteins, HIEC were incubated with lipid substrate (as above), for a culture period prolonged to 20 h, so as to detect an appreciable amount of lipoproteins secreted into the medium. The medium was supplemented with anti-proteases [phenylmethyl-sulfonyl fluoride (PMFS), pepstatin, EDTA, aminocaproic acid, chloramphenicol, leupeptin, glutathione, benzamidine, dithiothreitol (DDT), sodium azide, trasylol], all at a final concentration of 1 mm. A plasma lipid carrier was added (2:0.6 vol/vol). Lipoproteins were then isolated by sequential ultracentrifugation using a TL-100 ultracentrifuge (Beckman, Montréal, Québec, Canada), as per our established method (26). Briefly, very low density lipoproteins (VLDL) ($d < 1.006$ g/ml) and low density lipoprotein (LDL) ($d < 1.063$ g/ml) were separated by centrifugation (100,000 *g*) for 2.26 h with a TLA 100.4 rotor at 4°C. The high density fraction (HDL) was obtained by adjusting the LDL infranatant to density < 1.21 g/ml, and centrifuging for 6.5 h at 100,000 *g*. Each lipoprotein fraction was exhaustively dialyzed against 0.15 m NaCl, 0.001 m EDTA, pH 7.0, at 4°C for 24 h.

Electron microscopy of HIEC lipoproteins

Isolated lipoproteins were negatively stained with 1% phosphotungstic acid (pH 7.2) as described previously (28). The diameter of 200–300 particles of each fraction was then determined.

Agarose column chromatography

Confluent HIEC were cultured in serum-free, methionine-free DMEM containing [^{14}C]oleate and [^{3}H]leucine for 20 h. At the end of the incubation period, the conditioned media was adjusted to density 1.21 g/ml with KBr and ultracentrifuged at 4°C as detailed above. The top fraction of $d < 1.21$ g/ml was concentrated, dialyzed, and applied to a 1.0 × 70 cm column of Bio-Gel A-5m (Bio-Rad) (26). The latter was previously calibrated with small amounts of human lipoprotein fractions (CM, $d < 0.96$ g/ml; VLDL, $d < 1.006$ g/ml; LDL, $d < 1.063$ g/ml; HDL, $d < 1.21$ g/ml) and eluted with 150 mm NaCl, 10 mm Tris-HCl, pH 8.0. Fractions of 1.0 ml were collected.

De novo apolipoprotein synthesis

To study newly synthesized apolipoproteins, HIEC were incubated with [^{35}S]methionine (50 mCi/mmol, Amersham) (29). In order to induce lipoprotein and apolipoprotein synthesis, cells were incubated with unlabeled oleic acid (0.8 mm) 20 h prior to [^{35}S]methionine incubation. Cells were then rinsed (three times) with serum-free, methionine-free MEM and incubated with unlabeled oleic acid and [^{35}S]methionine (100 μCi/ml, Amersham). After incubation for 1 h at 37°C, the cells were scraped off the dish into a cell lysis buffer (Tris 10 mm, NaCl 150 mm, EDTA 5 mm, SDS 0.1%, Triton 1%, Na deoxycholate 0.5%). The medium and cell lysates were supplemented with the antiprotease mixture as described above. The cell lysates from two dishes were pooled for assays of apolipoprotein synthesis.

Immunoprecipitation of apolipoproteins A-I and B

After supplementing the medium and the cell lysate with unlabeled methionine (0.1 mm), immunoprecipitation was performed using polyclonal anti-human apolipoproteins (Boehringer Mannheim Biochemicals, Mannheim, Germany) at 4°C overnight (29). Samples were then washed with Nonidet (0.05%), and centrifuged. The immunoprecipitate was resuspended in sample buffer (1.2% SDS, 12% glycerol, 60 mm Tris, pH 7.3, 1.2% β-mercaptoethanol, 0.003% bromophenol blue), and loaded on a 3% polyacrylamide stacking gel. Radioactive molecular weight standards were purchased from Amersham. Gels were sectioned into 2-mm slices and counted after an overnight incubation with 1 ml of BTS-450 (Beckman) and 10 ml of Ready Safe scintillation fluid (Beckman). Results are expressed as % of total [^{35}S] methionine-labeled protein/mg cell protein.

Cholesterol synthesis assay

Cells were seeded in MEM media with 5% FBS and grown to subconfluent to confluent growth (80% of the plate was covered after day 5). Cells were then rinsed with isotonic PBS (pH 7.4) and switched to a lipoprotein-deficient serum (LPDS)-based medium. After 24 h, the media were replaced with media containing 5 μCi/ml of [2-^{14}C]acetate (51 mCi/mmol NEN) with the following treatments: *i*) FBS; *ii*) LPDS, the basal media for all subsequent additions (each of which contained 10% LPDS); *iii*) LDL (50 μg protein/ml); *iv*) 25-hydroxycholesterol (25 μm); *v*) mevinolin at a 1 μm concentration; and *vi*) mevinolin + LDL. After a 24-h incubation period, the medium was removed and monolayers were harvested in 1 ml 0.1 N NaOH and pooled with their respective media to be saponified according to Brown, Faust, and Goldstein (30). After thin-layer chromatography, newly synthesized [^{14}C]cholesterol was isolated and quantified.

LDL isolation and LPDS preparation

Low density lipoprotein (LDL) ($1.020 < d < 1.063$ g/ml) were isolated by sequential ultracentrifugation as reported previously (31). The LDL fraction was washed by its equilibrium density. Lipoprotein-deficient serum (LPDS) was prepared by ultracentrifugation at d 1.25 g/ml (31). LDL and LPDS were dialyzed exhaustively against 0.15 mmol/l NaCl, 0.001 mmol/l EDTA, pH 7.0 at 4°C.

LDL labeling and LDL receptor activity

^{125}I labeling of LDL was performed by the iodine monochloride method (31). About 98% of the ^{125}I radioactivity was precipitable by incubation with 10% TCA. The labeled tracer was sterilized by membrane filtration (0.45 μm Amicon filter) prior to incubation with the cells. The determination of HIEC binding and internalization were carried out as described previously (31).

RESULTS

Characterization of HIEC

Before investigating the ability of HIEC to synthesize and metabolize lipids and lipoproteins, it was critical that the nature of our intestinal model be ascertained. In the first step, the epithelial origin and nature of HIEC were confirmed by detecting the expression of the specific intestinal keratins 8, 18, and 21, at all the experimental periods by means of indirect immunofluorescent staining and Western blot analysis (results not shown). Using similar techniques, we were then able to demonstrate the presence of MIM-1/39 antigen specific for crypt cells at the time points studied (results not shown). In addition, these cells kept growing in culture and never displayed morphological differential features, including brush border microvilli, tight junctions, or dome formation. Finally, sucrase–isomaltase, a well-known intestinal brush border differentiation marker, was consistently absent from HIEC (results not shown). These data show that, under all experimental conditions, HIEC retained intestinal crypt cell proliferative markers, while being devoid of specific differentiation features characteristic of mature villus enterocytes.

Incorporation of [^{14}C]oleic acid and secretion of labeled lipids

HIEC were incubated for 18 h with [^{14}C]oleic acid in order to evaluate their capacity to synthesize and secrete newly formed lipids. De novo cellular lipid esterification is presented in **Fig. 1A**. The major esterified lipids were triglycerides (TG), phospholipids (PL), and cholesteryl esters (CE), which collectively represented more than 98% of total synthesis (**Table 1**). As reflected by the pattern of total lipids, the intracellular content of these major lipid classes tended to decrease slightly in parallel with culture period (Fig. 1A).

Lipid secretion into the culture medium is shown in Fig. 1B. Again, TG, PL, and CE were the predominant (80%) lipid classes (Fig. 1B). Their absolute level increased in the medium, and this increase may in part account for the observed decrease in the cellular content. The remainder of the lipids secreted was composed of

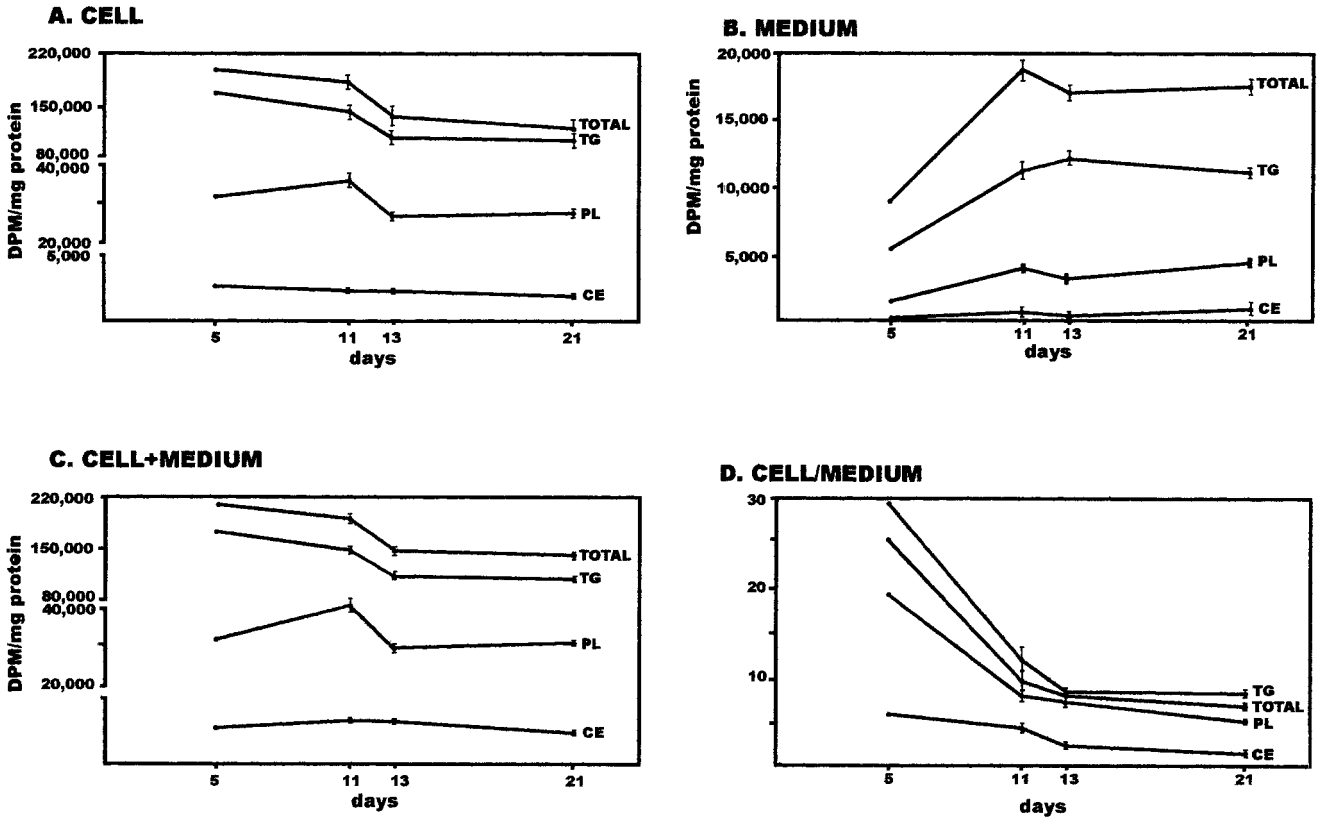

Fig. 1. Incorporation of [^{14}C]oleic acid into cell-associated and secreted lipids. HIEC were cultured for 20 h at different postconfluence periods. Media devoid of cellular debris and homogenized cells were extracted with chloroform–methanol 2:1 (v/v). Lipids were separated by TLC and counted. Data are expressed as means (±SEM) of 3–4 experiments; PL, phospholipids; TG, triglycerides; CE, cholesteryl esters.

monoglycerides (traces), diglycerides, and free cholesterol (Table 1). As anticipated, TG were by far the major lipid class in the medium, indicative of TG-rich lipoprotein secretion. These data suggest some improvement of lipid secretion with regard to culture period. Nevertheless, relatively poor efficiency of HIEC lipid export was observed (Figs. 1B and 1C). Lipid secretion was considerably limited at any time point for the three classes studied, compared with their abundant intracellular synthesis, and as evidenced by the high cell/medium ratio (Fig. 1D).

Lipid composition was also assessed. No significant differences were noted among the culture periods listed in Table 1. However, important variations were observed in lipid distribution between cells and media.

The profile and composition of phospholipids analyzed by thin-layer chromatography are depicted in **Table 2**. The data show the ability of HIEC to elaborate the five main PL types. Individual PL classes were higher in cells than in media, consistent with the absolute amounts of total PL reported above (Fig. 1). Phosphatidylcholine was

TABLE 1. HIEC lipid synthesis and secretion

Sample	Culture Period	% Distribution				
		PL + MG	FC	DG	TG	CE
Cells	5	15.1	0.4	0.8	82.5	1.21
	11	19.7 ± 0.9	0.5 ± 0.1	0.6 ± 0.1	77.9 ± 1.0	1.3 ± 0.1
	13	19.6 ± 0.3	0.4 ± 0.1	0.9 ± 0.1	77.5 ± 0.2	1.6 ± 0.1
	21	21.8 ± 0.1	0.4 ± 0.1	1.0 ± 0.1	75.6 ± 0.1	1.3 ± 0.1
Media	5	19.0	5.0	4.3	68.0	4.4
	11	23.1 ± 1.1	5.3 ± 0.3	5.4 ± 0.2	61.8 ± 1.4	4.3 ± 1.7
	13	19.7 ± 1.4	2.5 ± 0.3	3.1 ± 0.1	70.3 ± 1.4	4.6 ± 0.8
	21	23.0 ± 0.6	3.5 ± 0.2	3.4 ± 0.1	64.7 ± 0.9	5.8 ± 1.1

HIEC were incubated with [^{14}C]oleic acid for 20 h at different periods post-confluence. Lipids of cell homogenates and media were then extracted with chloroform–methanol (2:1), isolated by TLC, and quantitated as described in Methods. Results represent means ± SEM of 3–4 experiments; PL, phospholipids; MG, monoglycerides; DG, diglycerides; TG, triglycerides; CE, cholesteryl esters; FC, free cholesterol.

TABLE 2. Phospholipid synthesis and secretion by HIEC

Samples	Sphingomyelin	Phosphatidylcholine	Phosphatidylserine	Phosphatidylinositol	Phosphatidylethanolamine
Cells	503 ± 31 (1.5 ± 0.1)	28529 ± 444 (79.4 ± 1.2)	1942 ± 67 (5.4 ± 0.2)	2175 ± 156 (6.1 ± 0.4)	2787 ± 220 (7.8 ± 0.6)
Media	247 ± 39[a] (5.7 ± 0.9[a])	2358 ± 242[b] (54.7 ± 5.6[a])	343 ± 19[a] (8.0 ± 0.4[a])	439 ± 24[a] (10.2 ± 0.5[a])	929 ± 204[a] (21.5 ± 4.7[b])

HIEC (21 d) were incubated with [^{14}C]oleic acid and phospholipids were extracted from cells and media. Values are mean ± SE as dpm/mg cell protein for 4 experiments. The distribution in percentage of the total is given in parentheses.
[a] $P < 0.01$, [b] $P < 0.001$ compared with cells.

the predominant fraction of total ^{14}C-labeled PL extracted from cells and media. However, differences were noted in the relative distribution between the two compartments. Taken together, our data demonstrate that despite their immaturity, HIEC are capable of active lipid esterification. However, they display low efficiency of lipid export.

Separation of lipoproteins by agarose column chromatography

The elution profiles of the d < 1.21 g/ml of both [^{14}C]oleate- and [^{3}H]leucine-labeled media of cultured HIEC (13 d) is depicted in **Fig. 2**. Peaks of particles containing labeled proteins and lipids corresponding in size to human plasma CM, VLDL, LDL, and HDL were observed. As illustrated in Fig. 2, the highest proportion of radiolabeled lipids was located in the triglyceride-rich lipoprotein region, whereas the major concentration of radioactive proteins appeared in the HDL site.

Lipoprotein production

Lipoprotein production by HIEC was assessed at three different culture periods: 5, 13, and 21 days. For these experiments, HIEC were incubated with lipid substrate ([^{14}C]oleate) for 20 h and lipoprotein fractions from media were isolated by ultracentrifugation. As illustrated in **Fig. 3**, CM and VLDL were the dominant and minor fractions, respectively, throughout the culture periods studied. Maximal yield of all lipoprotein types including LDL and HDL was observed at 13 d. These data show the potential of proliferative crypt cells to transport lipids in the form of lipoprotein fractions. The composition of the major lipoprotein lipid components at varying periods of culture is shown in **Table 3**. A slight trend toward TG en-

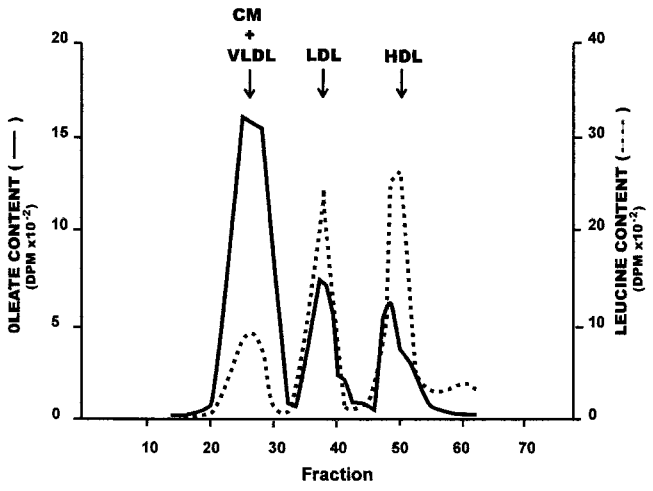

Fig. 2. Agarose column chromatography of newly synthesized lipoproteins. HIEC were radiolabeled for 4 h with [^{14}C]oleic acid (—) and [^{3}H]leucine (---). The media of 6 dishes were pooled and ultracentrifuged (100,000 *g* for 2.26 h with TL 100.3). The top fraction of 1.21 g/ml was concentrated and applied on agarose column chromatography as described in Materials and Methods. Plasma carrier was omitted in this experiment.

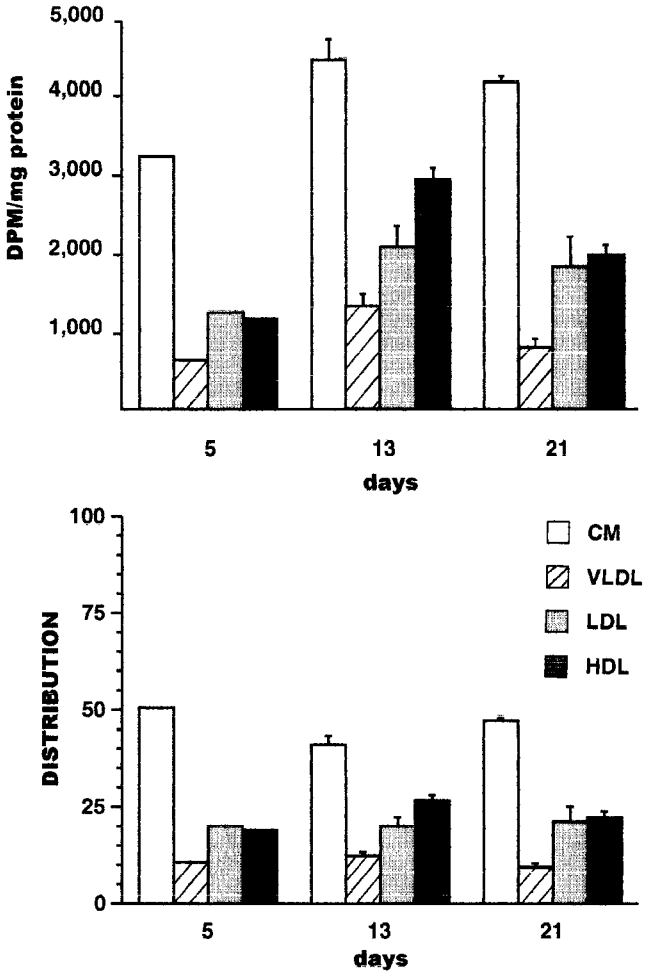

Fig. 3. HIEC lipoprotein secretion (A) and distribution (B). HIEC were cultured in the presence of [^{14}C]oleic acid. After the incubation period, lipoproteins from medium were isolated by ultracentrifugation; CM, chylomicrons; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins. Values are means ± SEM of 4 experiments, expressed as dpm/mg protein (A) and as percentage of total radioactivity recovered in total lipoproteins (B).

TABLE 3. Lipid composition in lipoproteins

Lipoproteins	Time	PL + MG	FC	DG	TG	CE
	days			*%*		
CM	5	33.2	13.5	10.7	35.0	7.6
	13	31 ± 1.6	10.5 ± 0.7	8 ± 0.4	43.4 ± 3.1	7.2 ± 0.5
	21	33.4 ± 1.7	9.9 ± 1.1	9.2 ± 0.7	39.1 ± 3.8	8.3 ± 1.1
VLDL	5	15.45	11.2	9.0	51.3	13.0
	13	10.8 ± 0.3	4.9 ± 0.2	6.2 ± 0.3	72.4 ± 1.2	5.7 ± 0.5
	21	14 ± 0.9	6.3 ± 0.4	7.2 ± 0.6	64.9 ± 1.9	7.6 ± 0.3
LDL	5	30.3	12.3	10.6	34.2	12.6
	13	38.6 ± 2.8	7.1 ± 0.6	7.4 ± 0.4	40.3 ± 3.8	6.7 ± 0.2
	21	40.6 ± 4.2	7.8 ± 1	7.5 ± 0.6	37.5 ± 6.7	6.6 ± 1
HDL	5	49.3	12.3	11.5	17.0	9.9
	13	44.9 ± 0.8	4.8 ± 0.1	6.6 ± 1.1	30.1 ± 0.9	5.6 ± 0.3
	21	50.4 ± 2.5	8.9 ± 1.1	8.2 ± 0.5	24.5 ± 1.9	8.0 ± 0.8

HIEC were cultured in the presence of [^{14}C]oleic acid. At the culture periods listed in the table, lipoproteins from medium were isolated by ultracentrifugation; CM, chylomicrons; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoprotein. Values are means ± SEM of 3 experiments. Data are expressed as percentage of total lipids in lipoproteins.

richment as well as DG, FC, and CE depletion was noted in most lipoprotein fractions.

Electron microscopy of HIEC lipoproteins

After determining lipoprotein composition, the form and size of the different lipoprotein classes were assessed by electron microscopy (**Fig. 4**). Several incubation media (13 d) were pooled, concentrated, and adjusted with KBr to d 1.006 g/ml, d 1.063 g/ml, and d 1.21 g/ml for the isolation of lipoproteins by sequential ultracentrifugation. Analyses of these fractions showed that the particles of *1*) d 1.006 g/ml correspond in size (diameter) to CM and VLDL (40–140 nm), *2*) d 1.063 g/ml correspond to LDL (19–26 nm), and *3*) d 1.21 g/ml correspond to HDL (9–11 nm). All particles presented with spherical shape and seemingly normal appearance, except for the HDL fraction. The latter contained two morphologically distinct populations of spherical and discoidal structures.

Detection of apolipoproteins by immunofluorescence

To further establish the expression of the individual apolipoproteins in HIEC, indirect immunofluorescence was carried out in situ using specific monoclonal antibodies. With regard to apoB, the labeling was detected with the 2D8 antibody, which recognizes the NH_2-terminal of the amino acid chain common to both apoB-100 and apoB-48, as well as with 4G3 antibody directed against COOH-terminal of the amino acid chain of apoB-100. **Figure 5** clearly illustrates the immunofluorescence staining related to apoA-I, apoA-IV, and apoB. For each apolipoprotein studied in our experiments, cytoplasmic immunofluorescence staining was noted in all intestinal cells. The staining was detectable with variable intensity. However, taking into account the qualitative nature of this technique, it is not possible to speculate on the quantitative aspects relative to apolipoprotein signals. Intracellular staining with these antibodies was not observed after incubations in which the primary antibodies were omitted or preabsorbed to excess antigen prior to incubations.

De novo synthesis of apolipoproteins

In addition to documenting the presence of apolipoproteins by immunofluorescence, we assessed their biogenesis. For this purpose, HIEC were cultured at different postconfluence periods in the presence of [^{35}S]methionine for 4 h, and newly synthesized apolipoproteins were analyzed by $NaDodSO_4$–PAGE after immunoprecipitation. **Figure 6** reveals the ability of HIEC to produce apolipoproteins A-I, A-IV, B-48, B-100, C, and E. The predominant apolipoproteins in the cells were apoB-100 and apoA-IV, while apo C was the major apolipoprotein in the media at 11- and 21-day postconfluence. A decrease in apoBs was noted in the cells in parallel with an increase of apoC at 11-day postconfluence.

Regulation of cholesterol synthesis

HIEC were evaluated for their ability to synthesize cholesterol at subconfluent to confluent growth. Cells were deprived of exogenous sterols for 24 h in LPDS-based DMEM and then subjected to subsequent treatments (**Fig. 7**). With FCS that provides cells with a sterol-rich medium, HIEC exhibited an endogenous cholesterol synthesis of 79 ± 13 nmol of [^{14}C]acetate incorporated into cholesterol. The replacement of FCS by LPDS did not significantly change the incorporation of [^{14}C]acetate into cholesterol. Similarly, when HIEC grown in LPDS were treated with LDL, their cholesterol synthesis was not suppressed, indicating the inability of LDL to regulate endogenous cholesterol synthesis. However, HIEC showed a decrease in cholesterol synthesis in the presence of 25-hydroxycholesterol, suggesting that oxysterol regulatory mechanisms remained functional. The impact of an HMG-CoA reductase inhibitor upon cholesterol synthesis was also assessed. HIEC responded by suppressing cholesterol production with mevinolin administration, and concurrent addition of LDL remained without effect.

Regulation of LDL receptor activity

LDL receptor activity was analyzed in HIEC (**Fig. 8**). Cells treated with increasing concentrations of LDL (5,

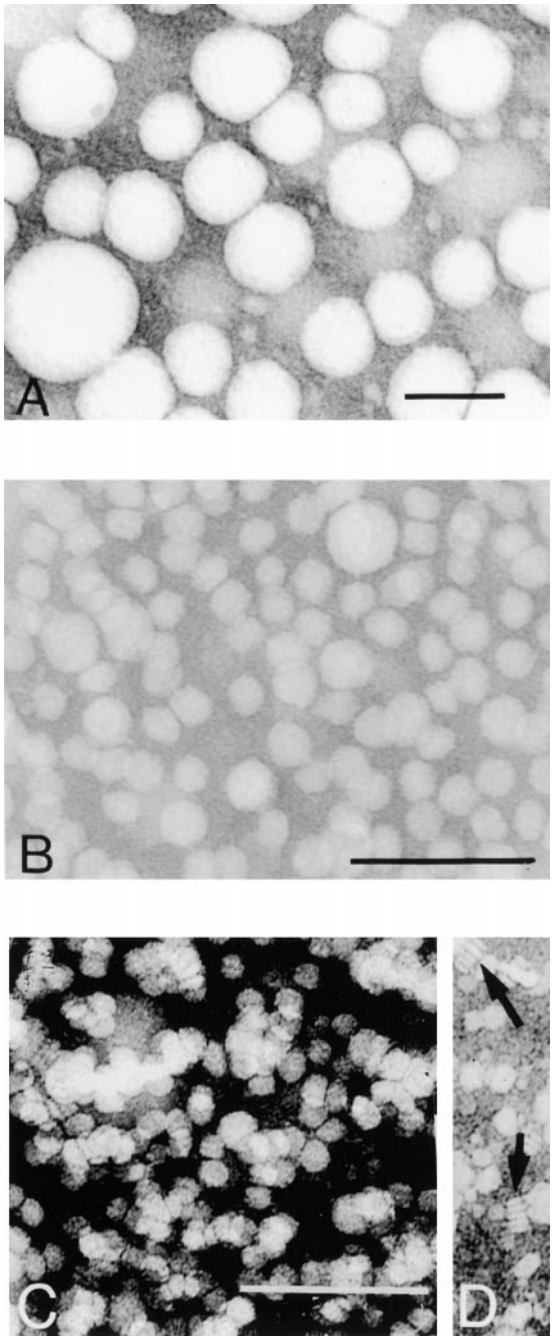

Fig. 4. Electron micrographs of HIEC lipoproteins. Cells were cultured for 20 h after 8 days of confluence. Lipoproteins were isolated and examined by electron microscopy; (A) d 1.006 g/ml (CM + VLDL); (B) d 1.063 g/ml (LDL). Two representative electron microscopic images of HDL (d 1.21 g/ml) are illustrated: (C) spherical and flattened HDL (D) discoidal structures. These abnormal structures are shown by arrowheads. Bar markers represent 100 nm.

25, 50, 500 μg/ml) displayed a progressive decrease of LDL binding and internalization compared with HIEC supplemented with LPDS alone. Similarly, the addition of 25-hydroxysterol caused a significant inhibition of LDL receptor activity compared with the LPDS control group. These data therefore show the ability of LDL to down-regulate its own receptor activity.

DISCUSSION

The intestinal epithelium is continually renewed from stem cells located in intestinal crypts, giving rise to enterocytes. Although many intestinal functions are in theory restricted to mature villus enterocytes, little attention has been paid to crypt cell functions because of the lack of an appropriate human model. Our observations have shown that immature HIEC were capable of synthesizing all lipid classes and apolipoproteins. They also exhibited the capacity of assembling lipid and apolipoprotein moieties into the four major lipoprotein types. Nevertheless, the efficiency of their lipid export remained limited. In addition, HIEC were shown to have an altered regulation of cholesterol metabolism.

A considerable body of evidence supports the concept that intestinal cell differentiation is an obligatory prerequisite for apolipoprotein biogenesis. Studies using pulse-labeling experiments in isolated rat enterocytes (32) and dot-blot hybridization analysis of rat mucosal scrapings (33) showed that apoB synthesis was primarily localized in villus-associated enterocytes. Furthermore, rat intestinal cell line IEC-6, which retains the characteristics of normal rat crypt jejunal cells (34), was unable to synthesize apolipoproteins and lipoproteins (35). However, the current results add another dimension to the understanding of the regional identity of apoprotein expression along the crypt-to-villus axis in human. Indeed, we recently reported an increased gradient of apolipoprotein expression from the crypt to the tip of the villus in the human fetal small intestine and colon (24), indicating that undifferentiated human crypt cells would be able to synthesize various apolipoprotein species. Not only do these cells keep growing in culture and retain the ability to express specific intestinal crypt cell markers, but they are also devoid of villus differentiation features, such as brush border microvilli, tight junctions, dome formation, and other maturational characteristics. The use of HIEC reveals that normal human crypt cells are able to express apolipoproteins distinctly, therefore, suggesting that the synthesis of these important proteins is not restricted to villus mature enterocytes.

Both apoB-100 and apoB-48 are elaborated by HIEC. However, HIEC predominantly produced apoB-100. These results validate the concept that the editing mechanisms, occurring in the apoB mRNA of the intestine, are modulated by differentiation (36). As suggested by the studies in Caco-2 cells, the degree of maturation seems to promote the editing of apoB mRNA, leading to translation of apoB-48. This switch in dominance from apoB-100 to apoB-48 does not occur in proliferating, non-differentiated HIEC. Whether the apolipoprotein and lipoprotein synthesis, together with the apoB mRNA editing, are also regulated in HIEC by growth factors and hormones (37–40) remains to be investigated.

The analysis of labeled fatty acid incorporation into cellular lipids established the ability of HIEC to synthesize the major products, i.e., TG, PL, and CE. However, the proportion of lipoproteins, as vehicles of these lipid classes, exported into the medium, was very low. This is

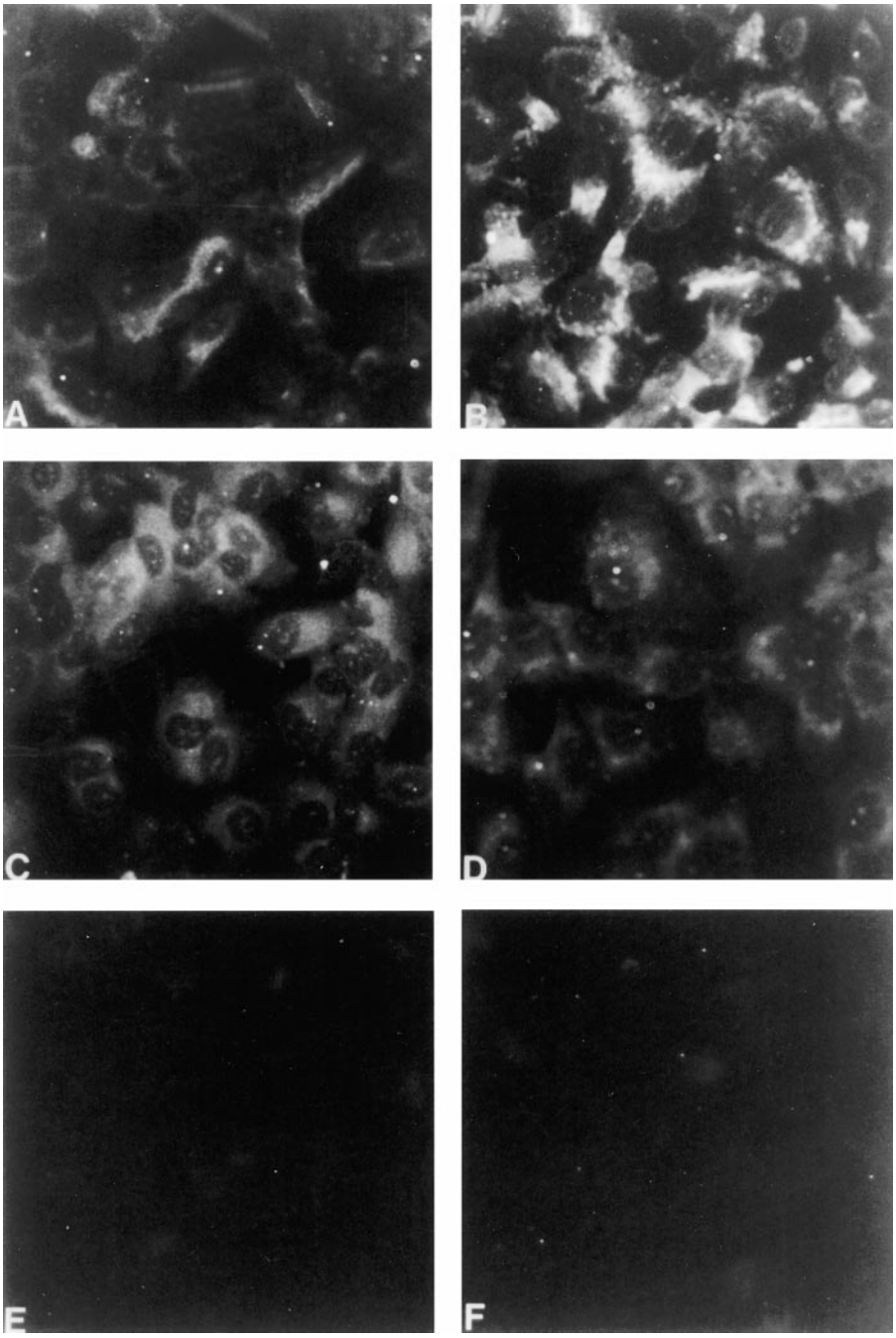

Fig. 5. Immunodetection of apolipoproteins in human intestinal epithelial cells (HIEC). Cells were grown on 8-well Lab-Tek slides using DMEM supplemented with 5% FBS until confluence. Cells were washed twice with incomplete DMEM before use. Cells were then fixed in ethanol before immunostaining. Cryosections were stained with anti-apoA-IV (A), and anti-apoA-I (B), anti-apoB-48 and B-100 (2D8) (C), anti-apoB-100 (4G3). In all cases, no fluorescent staining was observed when the primary antibodies were replaced with the appropriate nonimmune sheep (E) or mouse sera (F).

reminescent of what has been reported in differentiated Caco-2 cells (3). One has to keep in mind that the well accepted in vivo counterpart of Caco-2 cells, human fetal colonocytes (24, 41), also exhibits a reduced ability to secrete lipoproteins (41). In contrast, mature human enterocytes are able to both synthesize and efficiently secrete all lipoprotein species (26, 29, 42). Much investigation is needed to establish whether the lack of an efficient secretory process in HIEC is due to their undifferentiated nature, as other factors involved in cell preparation and culture may influence exocytosis.

In mammalian cells, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme for the synthesis of cholesterol. This endogenous synthetic pathway along with the exogenous LDL-receptor-mediated process, which delivers cholesterol to most cells,

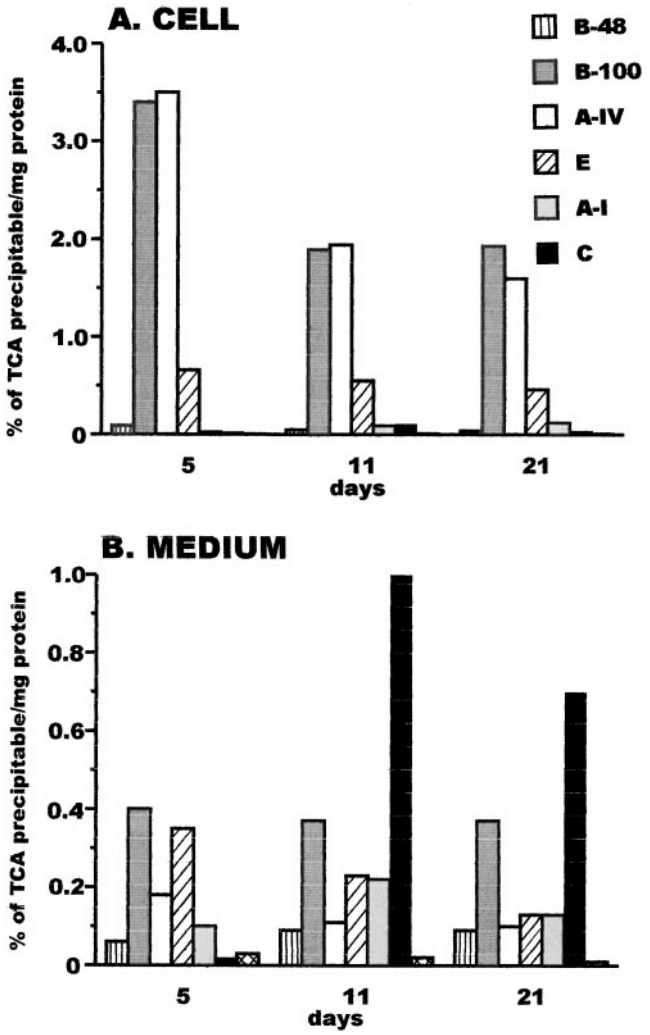

Fig. 6. [^{35}S]methionine incorporation by HIEC into immunoprecipitable apolipoproteins. Cells were incubated with methionine-free medium containing unlabeled oleic acid and [^{35}S]methionine for 4 h. Apolipoproteins were immunoprecipitated and analyzed by SDS-PAGE. Data from a representative experiment are illustrated; means ± SEM of 4 experiments for cells and media (expressed as a percent of total [^{35}S]methionine-labeled protein (TCA)/mg tissue protein) are presented in A and B, respectively.

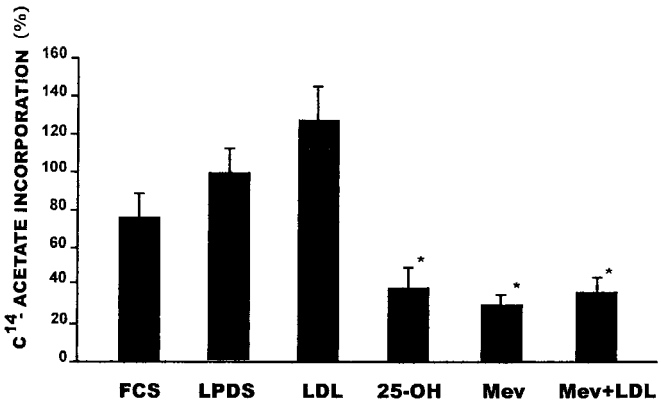

Fig. 7. Cholesterol synthesis by HIEC. Cells were grown to subconfluency (80%) and cultured with 10% LPDS. They were then incubated with 5 μCi/ml [^{14}C]acetate (sp. act. 50–62 mCi/mmol) and subjected to the following treatments: LDL (50 μg protein/ml), 25-hydroxysterol (25-OH, 5 μg/ml), mevinolin (1 μm). All data were normalized as a percentage of the LPDS control group. In the presence of fetal bovine serum (which provided HIEC with a sterol-rich medium), reference values for endogenous cholesterol synthesis were 61 ± 17 nmoles of [^{14}C]acetate incorporated into cholesterol. The data presented are the mean values of three separate experiments ± SEM.

can fulfill cellular cholesterol requirements. In addition, intestinal absorptive cells receive a high influx of cholesterol from dietary intake. In general, the synthesis of cholesterol is regulated by LDL cholesterol after the internalization of this lipoprotein particle bound to its surface receptor. However, it seems that cholesterol synthesis is modulated differently in cells derived from crypts and villi (43, 44). Our study shows the ability of crypt intestinal cells to synthesize cholesterol and to express the classic LDL receptor. These cells bind and internalize exogenous LDL via specific high-affinity LDL receptors, but marked differences could be observed. First, HIEC exhibited no down-regulation of cholesterol synthesis by LDL in contrast to the effect of 25-hydroxycholesterol sterol or mevinolin. Also, no significant differences in cholesterol synthesis by HIEC were noted with FCS or LPDS. On the other hand, LDL pretreatment caused a reduction in LDL receptor activity. These findings confirm and extend the observations suggesting that HMG-CoA reductase is controlled by sterols (45) and that LDL uptake and cholesterol synthesis are independently regulated (46).

In conclusion, this investigation reveals that human intestinal crypt cells exhibit the ability to synthesize the major lipid classes and various types of apolipoproteins and display the intracellular mechanisms required for lipoprotein assembly. Furthermore, the lack of apoB editing, the

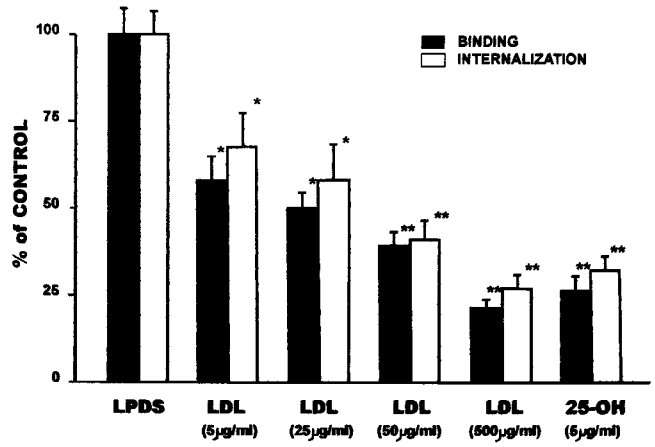

Fig. 8. HIEC LDL receptor studies. HIEC were cultured with LPDS-supplemented media for 24 h, then supplemented with increasing concentrations of LDL or with 25-hydroxycholesterol (1 h). The characteristics of the LDL receptor, either binding or internalization, were determined as described in Methods. Activity is reported as % of LPDS control for each experiment. Values are means (±SEM) of three assays; * $P < 0.05$; ** $P < 0.01$.

low level of lipoprotein secretion, and the unresponsiveness of cholesterol synthesis to LDL underline the undifferentiated states of human intestinal crypt cells.

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