Regulation of LDL Receptor Expression by Luminal Sterol Flux in CaCo-2 Cells

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The regulation of expression of the intestinal low density lipoprotein (LDL) receptor by luminal (apical) sterol flux was investigated in the human intestinal cell line CaCo-2. Cells were cultured on semipermeable micropore filters, which separated an upper and lower well. To the apical media were added solutions containing either taurocholate micelles alone or micelles containing sterols. Because of an efflux of cholesterol, which occurred from cells incubated with micelles alone, LDL receptor mRNA levels increased threefold. With an influx of micellar sterols, receptor mRNA levels decreased in a dose-dependent manner. Synthesis and degradation of the LDL receptor were addressed by pulse-chase experiments. In cells incubated with micelles containing 25-hydroxycholesterol, the rate of receptor synthesis was significantly decreased, whereas the rate of receptor turnover remained unchanged. As assessed by immunobLOTS and steady-state labeling of proteins followed by immunoprecipitation of the LDL receptor, cells incubated with micellar 25-hydroxycholesterol contained substantially less receptor protein. These cells also bound and degraded less LDL. In contrast, in cells incubated with micelles alone, the rate of receptor synthesis was increased and cells contained more LDL receptor protein, although this was not reflected in an increase in LDL binding. The results suggest that LDL receptor expression in CaCo-2 cells is regulated by luminal sterol flux and that this regulation occurs at the level of transcription. (Arteriosclerosis and Thrombosis 1993;13:729-737)

Key Words • CaCo-2 cells • LDL receptor • sterol flux • intestine • micelles

The small intestine controls several important functions that are involved in the regulation of cholesterol metabolism within the body.1 These functions directly affect plasma cholesterol levels and hence, the risk of atherosclerosis. For example, the intestine is the only organ responsible for the absorption of dietary cholesterol. Loss of cholesterol or its catabolic products, bile acids, from the gastrointestinal tract is the predominant mechanism for eliminating cholesterol synthesis in the gut after lipoprotein lipoproteins. This function of the intestine has not been adequately addressed. The small-intestinal absorptive cell is continuously exposed to exogenous dietary cholesterol and endogenously synthesized biliary cholesterol. This causes a constant influx of sterol from the lumen, which could regulate the expression of the low density lipoprotein (LDL) receptor on the intestinal cell. This is not a minor point. Since humans spend a significant portion of the day in the postabsorptive state, a chronic downregulation of the intestinal LDL receptor by luminal cholesterol influx would significantly decrease the contribution of the intestine to the degradation of circulating LDL.

Because of the difficulties in separating the effects of luminal cholesterol versus lipoprotein cholesterol on the regulation of intestinal LDL receptor expression in vivo, this possibility was explored using the human intestinal cell line CaCo-2. The results suggest that LDL receptor mass and expression are regulated by changes...
in sterol flux occurring at the apical membrane of the cell.

**Methods**

**Materials**

[4-14C]Cholesterol, [5-3H]mevalonic acid, 3-hydroxy-3-methyl-[3-14C]glutaryl coenzyme A, deoxycholate 5'-alpha-[3-3H]triphosphate, and Na[35S] were purchased from New England Nuclear (Boston, Mass.). trans[35S]S-Methionine was from ICN Biomedicals Inc. (Irvine, Calif.). Cholesterol, sodium taurocholate acid, mono-olein, glucose-6-phosphate, glucose-6-phosphate dehydrogenase, nucleotide adenosine diphosphate, dextran sulfate, salmon sperm, protein A-agarose, and sodium dodecyl sulfate (SDS) were purchased from Sigma Chemical Co. (St. Louis, Mo.). HMG-CoA was purchased from P-L Biochemicals Inc. (Milwaukee, Wis.). 25-Hydroxycholesterol was from Steraloids (Wilton, N.H.). The human monoclonal antibody against the LDL receptor (IgG-C7) was prepared from hybridoma cells (CRL 1691, American Type Culture Collection, Rockville, Md.).

The C7 antibody was coupled to cyanogen bromide-activated Sepharose 4B according to the procedure recommended by Pharmacia (Uppsala, Sweden). A cDNA clone of the human LDL receptor (pLDLR3) was also obtained from American Type Culture Collection. A polyclonal rabbit anti-rat LDL receptor immunglobulin G was a generous gift from Dr. Allen Cooper (Palo Alto Medical Research Foundation, Palo Alto, Calif.).

Human alpha-actin cDNA (PHM alpha-A-1) was graciously supplied by Dr. Peter Rubenstein (Department of Biochemistry, University of Iowa). All other reagents were reagent grade.

**Cell Culture**

CaCo-2 cells were cultured on micropore polycarbonate filters (Transwells, 0.4-μm pore size, inserts of 6.5- or 24.5-mm diameter; Costar, Cambridge, Mass.) as previously described. 10 25-Hydroxycholesterol micelles with and without the sterols were prepared as described. 11 All treatments were added to the apical medium. The lower wells contained control medium alone (medium 199/Earle’s [M199], Gibco, Grand Island, N.Y.).

**LDL Receptor mRNA Quantification**

Total cellular RNA was extracted from CaCo-2 cells by the method described by Chomczynski and Sacchi 12 using guanidium thiocyanate. Northern blot hybridizations were performed exactly as described. 11

**Pulse-Chase Experiments**

For these experiments, CaCo-2 cells were grown on the smaller (6.5 mm) inserts. Because of toxicity effects observed with 5 mM taurocholate, sterols were solubilized in a 1 mM solution of the bile salt. No toxicity was observed with this concentration, as assessed by lactate dehydrogenase release and trypsin blue exclusion, and the amount of cellular protein did not vary from filter to filter. To estimate the rate of synthesis of the LDL receptor, cells were incubated for 18 hours in control medium or medium containing micelles with or without 150 μM cholesterol or 25 μM 25-hydroxycholesterol. The cells were then depleted of methionine by incubating them for 1 hour in methionine-free medium. After this preincubation, 100 μCi[35S]methionine was added to the media, together with the appropriate treatments added back. After 0.5-2 hours of incubation, the cells were washed twice with phosphate-buffered saline and harvested immediately for immunoprecipitation.

To estimate the rate of turnover of the LDL receptor, the cells were preincubated with methionine-free medium for 1 hour, followed by a 3-hour pulse with 200 μCi[35S]methionine. The inserts containing[35S]methionine-labeled cells were transferred to new wells and incubated for up to 20 hours in M199 supplemented with 2% delipidated bovine serum, 1 mM unlabeled methionine, 0.1 mM cysteine, and the treatments described in the figure legends. 35S-Labeled LDL receptor was then immunoprecipitated from the cells.

**Immunoprecipitation of [35S]Methionine-Labeled LDL Receptor**

Cells were solubilized in 1 mL of the following cell lysis buffer: 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), pH 7.4, 200 mM NaCl, 2.5 mM MgCl2, 2.0 mM CaCl2, 1% Triton X-100, 1 mM methionine, 0.1 mM cysteine, 1.0 mM phenylmethylsulfonyl fluoride, 10 μg soybean trypsin inhibitor, and 20 μM leupeptin. The cells were vigorously vortexed and centrifuged for 30 minutes at 15,000g. An aliquot of the supernatant was used to determine the incorporation of [35S]methionine into total cellular protein after trichloroacetic acid precipitation. The supernatant was incubated on a rocker for 16 hours at 4°C with 10 μg C7 antibody coupled to Sepharose. The amount of antibody required to immunoprecipitate 95-98% of the labeled receptor was determined by titration. The antigen-antibody-Sepharose complex was sedimented by centrifugation for 30 seconds at 15,000g. The pellet was washed six to eight times with 1 mL cell lysis buffer. The receptor was dissociated from the antibody by adding 5 μL of 0.1 M glycine, pH 2.5, 5 μL of 5x Laemmli buffer, and 30 μL of 1x Laemmli buffer. SDS-polyacrylamide gel electrophoresis (PAGE) was performed without heating and in the absence of reducing agents. The proteins were separated on a 5% stacking and 8% separating porous gel as described by Doucet et al. After electrophoresis, the gels were treated for 5 minutes with fixative solution (40% methanol, 7% acetic acid) followed by three washings with water and a 30-minute incubation with 1 M sodium salicylate solution. The gels were dried and placed on Kodak X-Omat AR film for 6-24 hours at -70°C. The bands corresponding to the LDL receptor were then cut from the gel and the radioactivity determined by liquid scintillation spectrometry.

**LDL Binding Studies**

CaCo-2 cells grown on 24.5-mm-diameter filters were incubated for 18 hours with the respective treatments added to the apical media. The cells were then washed twice with M199, and 1.5 mL Dulbecco’s modified Eagle’s essential medium (DMEM) was added to the apical side. To the lower well was added 2.6 mL DMEM containing 4 mg/mL bovine serum albumin, 2.5 mM HEPES, pH 7.4, 137 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl2, 0.8 mM MgSO4, and increasing concentrations of [125I]-LDL. Binding assays were done in triplicate. To estimate the amount of nonspecific binding, a 30-fold
excess of unlabeled LDL was added to some wells. After a 5-hour incubation at 37°C, the basolateral media were precipitated with trichloroacetic acid and the trichloroacetic acid–soluble counts quantified to estimate the amount of LDL degraded. The cells were washed three times with a HEPES–saline solution containing 136 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl₂, 0.8 mM MgSO₄, 3.6 mM HEPES, pH 7.4, and 2 mg/mL bovine serum albumin. The cells were then rinsed twice with phosphate-buffered saline containing 0.9 mM CaCl₂ and 0.08 mM MgSO₄. After the five washes, the cells were incubated for 1 hour in 10 mM HEPES, pH 7.4, containing 50 mM NaCl and 4 mg/mL sodium heparin. The amount of heparin-releasable radioactivity was quantified to estimate surface-bound LDL. The cells were then lysed in 2 mM EDTA, and the amount of heparin-resistant radioactivity was quantified as an estimate of internalized LDL.

**Immunoblot of the LDL Receptor**

LDL receptor protein mass was determined by Western blot hybridization. CaCo-2 cells were scraped into the cell lysis buffer. The cell lysate was vortexed vigorously and centrifuged for 30 minutes at 13,000g. To the supernatant containing 200 or 600 μg protein was added 10 μg C7 antibody coupled to Sepharose. After an 18-hour incubation on a rocker at 4°C, the antigen–antibody–Sepharose complex was sedimented by centrifugation at 13,000g for 30 seconds. The sediment was washed once with the cell lysis buffer, and the receptor was eluted and analyzed by SDS-PAGE as described for the immunoblot of the receptor. The PVDF membranes were blocked for 30 minutes at room temperature with 5 µL rabbit immunoglobulin G-peroxidase, affinity purified from goat serum (dilution, 1:2,000; Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The filters were again washed six times with the blocking buffer. After three to four washings with the blocking buffer, the membranes were incubated for 30 minutes with anti-rabbit immunoglobulin G–peroxidase, affinity purified from goat serum (dilution, 1:2,000; Boehringer Mannheim Biochemicals, Indianapolis, Ind.). The filters were again washed six times with the blocking buffer supplemented with 0.05% Tween 20. Phosphate-buffered saline was used for the final two washings to remove the Tween 20. The peroxidase activity was detected by exposing the filters for 1–2 minutes to a high-sensitivity enzygraphic web (Kodak, International Biotechnologies, Inc., New Haven, Conn.). Because of the low abundance of the receptor protein in CaCo-2 cells, use of this film was necessary to detect the 10–100 fg peroxidase on the filters.

The amount of the receptor protein in the bands was quantified by densitometry using the Ambis Image Acquisition and Analysis System (Ambis Inc., San Diego, Calif.).

**Ligand Blot of the LDL Receptor**

To obtain sufficient mass of the receptor for ligand blotting, cells were pooled from 12 wells of a 24-well plate. The receptor was immunoprecipitated from 12 mg cell protein with 250 μg C7 antibody coupled to Sepharose. The immunoprecipitate was separated by SDS-PAGE and transferred to a PVDF membrane as described above for the immunoblot of the receptor. The PVDF membranes were blocked for 30 minutes at 37°C in blocking buffer containing 50 mM Tris base, pH 8.0, 80 mM NaCl, 2 mM CaCl₂, 2 mL Polydet P-40/1, and 3% bovine serum albumin. This buffer was also used during the incubation with radiolabeled LDL and for the washings to follow. The filters were incubated for 1 hour at room temperature with 14 μg 125I-LDL (441 cpm per nanogram) per 10 mL of buffer. The filters were washed eight to 10 times and exposed to Kodak X-Omat AR film for 20 hours at ~70°C.

**Enzyme Assay, LDL Preparation, and Protein Measurement**

HMG-CoA reductase activity was measured as previously described. Human LDL was isolated and iodinated as described. Protein was determined according to the method of Lowry et al.

**Statistical Analysis**

Student’s unpaired t test was used to determine significance.

**Results**

**Effect of Sterol Flux on HMG-CoA Reductase Activity**

To investigate the regulation of LDL receptor expression in CaCo-2 cells, it was first necessary to determine whether changes in apical sterol flux altered the rates of cholesterol synthesis. Previous data that were generated in CaCo-2 cells grown on plastic clearly showed that HMG-CoA reductase activity decreased with sterol influx and increased with sterol efflux. Sterol efflux occurred under conditions in which cells were incubated with taurocholate micelles alone that were devoid of sterol. Figure 1 shows data on the effect of apical sterol flux on HMG-CoA reductase activity in cells grown on semipermeable micropore filters. Under conditions of cholesterol efflux (micelles alone), reductase activity increased from 1.64 to 2.93 nanomoles of mevalonate formed per milligram per 30 minutes. In contrast, the highest concentration of cholesterol that could be solubilized within the micelle (150 μM), HMG-CoA reductase activity decreased to 1.70. Moreover, 25-hydroxycholesterol solubilized within the micelle caused a more profound inhibition of reductase activity at concentrations well below that of cholesterol.

**Effect of Sterol Flux on LDL Receptor mRNA Levels**

CaCo-2 cells cultured on filters for 14 days were incubated for 18 hours in control medium, medium containing micelles, or medium containing micelles and increasing concentrations of cholesterol. After the incu-
FIGURE 1. Bar graph showing regulation of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity by micelles ± sterols. Solutions containing medium 199/Earle’s (control), 5 mM taurocholate and 30 μM mono-olein (micelles), or micelles and sterols were added to the apical media of CaCo-2 cells grown on semipermeable filters. After 18 hours, HMG-CoA reductase activities were determined in total membranes prepared from the cells. n=3, with assays performed in triplicate.

bation, total RNA was extracted from the cells and steady-state levels of mRNA for the LDL receptor were estimated by Northern hybridization. α-Actin mRNA abundance for each sample was used to correct for inequalities in transfer or loading of total RNA per sample (Figure 2 and Table 1). The levels of mRNA for the LDL receptor increased threefold in cells that were losing cholesterol into the medium (micelles alone, lanes 3 and 4). In contrast, LDL receptor mRNA levels decreased in CaCo-2 cells incubated with micelles containing 75 and 150 μM native cholesterol (lanes 7-10).

Figure 3 shows data from a similar experiment, except that 25-hydroxycholesterol at concentrations of 2.5 and 25 μM was substituted for the native cholesterol in the micelle. Again, steady-state levels of LDL receptor mRNA increased threefold in cells incubated with micelles alone (lanes 3 and 4). At concentrations substantially below those of cholesterol, 25-hydroxycholesterol caused significant reductions in LDL receptor mRNA levels (lanes 5-8). The first column of Table 1 shows the relative densities for the mRNA levels of the LDL receptor from these experiments. Densities for mRNA abundance in control cells incubated in medium alone are expressed as 1.00. All densities are relative to the densities of the α-actin mRNA for that sample. In data not shown, results obtained from dot blots for LDL receptor protein were consistent with the Northern blot data for mRNA. Again, steady-state levels of LDL receptor mRNA in cells grown in micelles alone (lanes 3 and 4) increased threefold compared to control incubations (lanes 1 and 2). In contrast, LDL receptor mRNA levels decreased in cells grown in micelles containing 75 and 150 μM native cholesterol (lanes 7-10).

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### Table 1. Effect of Micelles With or Without Sterols on LDL Receptor mRNA and Protein Levels

<table>
<thead>
<tr>
<th>Condition</th>
<th>LDL receptor mRNA (relative densities)</th>
<th>LDL receptor Protein (relative densities)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Micelles</td>
<td>3.00</td>
<td>1.75</td>
</tr>
<tr>
<td>Micelles + Cholesterol (25 μM)</td>
<td>3.57</td>
<td>ND</td>
</tr>
<tr>
<td>Cholesterol (75 μM)</td>
<td>2.37</td>
<td>ND</td>
</tr>
<tr>
<td>Cholesterol (150 μM)</td>
<td>1.66</td>
<td>1.25</td>
</tr>
<tr>
<td>25-OH cholesterol (2.5 μM)</td>
<td>2.18</td>
<td>ND</td>
</tr>
<tr>
<td>25-OH cholesterol (25 μM)</td>
<td>0.64</td>
<td>0.40</td>
</tr>
</tbody>
</table>

LDL, low density lipoprotein. CaCo-2 cells were incubated for 18 hours in control medium or medium containing micelles ± sterols. After the incubation, total RNA was extracted from the cells and the steady-state levels of mRNA for the LDL receptor were estimated by Northern hybridization. The densities are relative to densities of α-actin mRNA for the same sample. LDL receptor protein was estimated by immunoblot as described in "Methods." ND, not determined.
receptor mRNA (1, 2, 4, and 8 μg total RNA) were confirmatory.

Effect of Sterol Flux on LDL Receptor Synthesis and Degradation

The effect of sterol flux on the rate of synthesis of the LDL receptor was estimated by incubating CaCo-2 cells for 18 hours with micelles alone or micelles containing 150 μM cholesterol or 25 μM 25-hydroxycholesterol. After the incubation, the incorporation of [35S]methionine into immunoprecipitable LDL receptor protein was measured during a 2-hour pulse. Figure 4 shows the resulting autoradiogram after PAGE of the immunoprecipitate. There were two distinct bands at apparent molecular weights of 160 and 120 kD. In cells incubated with micelles containing 25-hydroxycholesterol, the incorporation of radioactivity into the LDL receptor was significantly diminished compared with that observed in control cells or cells incubated with micelles alone. There was a 50% increase in the incorporation of label into the receptor in cells incubated with taurocholate micelles. The rate of incorporation of methionine was not altered by 150 μM cholesterol solubilized within the micelle. These treatments did not affect the incorporation of [35S]methionine into total trichloroacetic acid-precipitable proteins.

To estimate turnover rates of the LDL receptor, CaCo-2 cells were pulsed for 3 hours with [35S]methionine. After the pulse period, the cells were chased for up to 20 hours with medium containing the various treatments and excess unlabeled methionine. Figure 5 shows the mean loss of label within the LDL receptor after immunoprecipitation and PAGE from three separate experiments. The rate of turnover of the labeled LDL receptor was similar in all four treatment groups.
The half-life of the receptor in CaCo-2 cells approximated 19 hours.

**Effect of Sterol Flux on LDL Receptor Mass**

CaCo-2 cells were incubated for 18 hours in control medium, in medium containing micelles alone, or in medium with micelles containing sterols. After the incubation, the cells were solubilized and the amount of LDL receptor protein was estimated by immunoblotting (right panel, Figure 6). The amount of the receptor mass on the blots was analyzed in three separate experiments by densitometry. LDL receptor mass in cells incubated with micelles alone was increased 75% compared with the amount found in control cells. The influx of 150 μM micellar cholesterol decreased LDL receptor protein by approximately 30% compared with the levels observed in cells incubated with micelles alone. Moreover, the influx of the hydroxylated cholesterol decreased receptor protein more than fourfold (last column, Table 1).

The left panel of Figure 6 shows a ligand blot. In this experiment, the LDL receptor was first immunoprecipitated from 12 mg cell protein. After PAGE and transfer to a membrane, the membrane was probed with 125I-LDL in the presence or absence of 10 mM EDTA. One band of 160 kD was observed. No band was visualized from the incubation containing 125I-LDL and EDTA. Thus, the 160-kD band observed by the immunoblot binds 125I-LDL and represents the mature LDL receptor.

LDL receptor protein was estimated by another technique. CaCo-2 cell proteins were labeled to steady state with [35S]methionine. The LDL receptor was then immunoprecipitated after the various treatments (Figure 7). In control cells, most of the label was incorporated

### Table 2. Effect of Sterol Flux on HMG-CoA Reductase and the LDL Receptor in CaCo-2 Cells

<table>
<thead>
<tr>
<th></th>
<th>Activity</th>
<th>mRNA</th>
<th>Protein</th>
<th>Synthesis</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HMG-CoA reductase</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Micelles</td>
<td>2.0</td>
<td>2.5</td>
<td>3.0</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Micelles+25-hydroxycholesterol</td>
<td>0.12</td>
<td>1.3</td>
<td>ND (0.5)</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td><strong>LDL receptor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Micelles</td>
<td>1.0</td>
<td>3.0</td>
<td>2.0</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Micelles+25-hydroxycholesterol</td>
<td>0.5</td>
<td>0.6</td>
<td>0.40</td>
<td>↓</td>
<td>↔</td>
</tr>
</tbody>
</table>

HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LDL, low density lipoprotein.
*From published data (Reference 11).
†Not determined (at lower concentration of 25-hydroxycholesterol, protein mass approximated 0.5).
FIGURE 7. Analysis of low density lipoprotein (LDL) receptor mass by steady-state labeling. CaCo-2 cells were cultured for 1 hour in 0.25 mL methionine-free medium containing 1% delipidated fetal calf serum, 5% Dulbecco's modified Eagle's essential medium, and the following: lane 1, control; lane 2, taurocholate micelles; lane 3, micelles and 150 μM cholesterol; lane 4, micelles and 25 μM 25-hydroxycholesterol. After 1 hour, 150 μCi [35S]methionine was added to the treatments and the incubations continued for 24 hours. The amount of radioiodinated receptor was estimated by immunoprecipitation using the polyclonal antibody as described in the legend for Figure 5. The immunoprecipitated receptor was analyzed on 8% porous gels as described in "Methods."

into a band corresponding to a molecular weight of 160 kD. A faint band was observed at 120 kD. In cells incubated with micelles, irrespective of the presence of sterols, significantly more of the label was incorporated into the lower-molecular-weight band compared with controls. Compared with controls, cells incubated with micelles alone contained significantly more label in both the 160-kD and 120-kD bands. In contrast, in cells incubated with micelles containing 25-hydroxycholesterol, there was a marked decrease in the amount of label found in both bands compared with cells incubated with micelles alone. The addition of 150 μM cholesterol did not significantly alter the amount of label in the receptor.

Effect of Sterol Flux on LDL Binding to CaCo-2 Cells

To estimate the functional expression of the LDL receptor on CaCo-2 cells, cells cultured on filters were incubated for 18 hours in control medium, medium containing micelles alone, or medium containing micelles and 25-hydroxycholesterol. As in the previous experiments, the treatments were added only to the apical side. 125I-labeled LDL was then added to the lower well, and the amount of total bound LDL, heparin-releasable LDL, and degraded LDL was determined after a 5-hour incubation at 37°C (Figure 8). Non-specific binding accounted for only 10% of the total. In cells incubated with micelles containing 25-hydroxycholesterol, there was a 40–50% decrease in the amount of total bound and degraded LDL. In contrast, the amounts of LDL bound and degraded by control cells and cells incubated with micelles alone were similar.

In data not shown, binding experiments were performed in which the 125I-labeled LDL was added to the apical surface. Under these experimental conditions, non-specific binding was 25% of the total and LDL degradation was 1/10th of that shown in Figure 8, suggesting that the functional LDL receptor is localized...
predominantly to the basolateral membrane of CaCo-2 cells.

**Discussion**

The results of the present study demonstrate that the expression of the LDL receptor in the human intestinal cell line CaCo-2 is regulated by changes in luminal (or apical) sterol flux. Although this might seem like a rather simple and obvious conclusion, it is not without controversy. Stange and Dietschy, using [14C]sucrose-LDL to estimate cellular LDL uptake, found that LDL uptake by the rat small intestine was independent of changes in mucosal cholesterol flux. In animals fed cholesterol, LDL uptake by the intestine was similar to that of animals fed a control diet. It was their conclusion that the intestine met its cholesterol requirements by altering rates of cholesterol synthesis rather than by altering LDL uptake. In contrast, Fong et al recently demonstrated a decrease in LDL receptor mRNA levels in enterocytes prepared from rats fed cholesterol, suggesting that the intestinal LDL receptor was sensitive to cholesterol influx. The difficulty arises, however, in attempting to differentiate the contribution of dietary (luminal) cholesterol from that of lipoprotein cholesterol to the regulation of LDL receptor expression in the intestine. Recent documentation of the presence of LDL receptors on the basolateral surface of intestinal cells, in addition to data demonstrating the regulation of intestinal cholesterol metabolism by plasma lipoproteins, makes this a real issue. CaCo-2 cells were used in the present study to address this question because these cells maintain their polarity in culture. By growing cells on semipermeable membranes, apical versus basolateral regulation of the LDL receptor can be readily examined. Extrapolating results from CaCo-2 cells and applying them directly to the human enterocyte, however, should be done with caution, because differences in LDL receptor expression may exist. To this point, Fabricant and Broitman studied changes in the expression of the LDL receptor protein and its function in a variety of human colonic carcinoma cell lines. Only CaCo-2 cells were shown to use LDL for growth and to have demonstrable LDL receptors that responded appropriately to lipoprotein cholesterol deprivation.

In CaCo-2 cells, the luminal uptake of micellar 25-hydroxycholesterol resulted in a marked decrease in LDL receptor protein. This was reflected functionally, as cells incubated with the sterol bound and degraded significantly less LDL compared with their controls. The sterol-mediated decrease in receptor protein can be primarily attributed to a decrease in LDL receptor mRNA abundance and a decrease in the rate of receptor synthesis. Although there is some evidence for posttranscriptional regulation of the LDL receptor, most studies have shown that sterols regulate LDL receptor expression primarily at the transcriptional level by interacting with the LDL receptor 5' upstream promoter. There is no evidence for posttranslational control of the LDL receptor. Our results in intestinal cells would agree with these conclusions.

Cholesterol efflux occurring in cells incubated with micelles alone caused a threefold increase in LDL receptor mRNA abundance and modestly increased the rate of receptor synthesis. LDL receptor protein also increased. LDL binding and degradation, however, were similar to controls. Two possible explanations could account for the discrepancy observed between receptor protein and functional expression. As demonstrated by the immunoblot (Figure 6) and perhaps more convincingly shown in the gel of the LDL receptor after steady-state labeling and immunoprecipitation (Figure 7), substantially more of the lower-molecular-weight protein band (120 kD) was present in cells incubated with micelles compared with that observed in controls. This 120-kD protein was previously shown to represent a precursor to the mature form of the receptor (160 kD). The precise mechanism that underlies the maturation of the receptor and the addition of 40 kD to the immature form is not clear. What is understood, however, is that the 120-kD receptor protein is not functional and does not bind LDL (Reference 28 and ligand blot in Figure 6). In CaCo-2 cells incubated with micelles alone, the “machinery” to turn on LDL receptor synthesis is appropriately activated by cholesterol efflux; however, the normal posttranscriptional processing of the receptor appears to be disrupted by the presence of bile salts. Thus, although more receptor protein is detected in these cells, the newly synthesized receptor is not functional.

An alternative explanation is that the amount of cellular LDL receptor protein estimated by the immunoblot may not reflect what exists on the cell surface and therefore, what is available to bind LDL. In fibroblasts, for example, Hare has estimated that between 39% and 49% of the LDL receptor exists on the cell surface. The remainder resides within the cell. It is possible that bile salts interfere with the normal transport of the newly synthesized receptor to the cell surface. The possibility was excluded that under conditions of cholesterol efflux, more receptors were expressed on the apical surface. The binding and degradation of apical LDL were 1/10th of that observed when LDL was added to the basolateral medium, and no differences were found between control cells and cells incubated with micelles.

HMG-CoA reductase, the product of another sterol-responsive gene, is also regulated by sterol flux in CaCo-2 cells. In these cells, mechanisms for the regulation of LDL receptor expression and the expression of HMG-CoA reductase differ. These differences might provide some insight as to how the enterocyte responds to acute changes in its requirement for cholesterol. Table 2 summarizes these data. Cholesterol efflux (micelles alone) caused a similar increase in mRNA levels for both proteins. Changes in mRNA abundance in response to apical sterol influx, however, differed markedly. Compared with their controls, LDL receptor mRNA levels decreased fivefold compared with the modest twofold reduction observed in mRNA levels for HMG-CoA reductase. However, the effect of sterol influx on the functional expression of the two proteins was much greater for HMG-CoA reductase. Thus, compared with the predominantly transcriptional regulation of LDL receptor expression in CaCo-2 cells, posttranscriptional control of reductase plays a more dominant role in its regulation. Indeed, with sterol influx, reductase protein was less stable than receptor protein, and translational efficiency of reductase mRNA was markedly decreased compared with that observed.
for receptor mRNA. Similar observations were made in Chinese hamster ovary cells.20

The estimated half-life of the LDL receptor in CaCo-2 cells was 19 hours. This is similar to the 21-25-hour half-life observed by Hare29 in fibroblasts but somewhat longer than the 10-13-hour half-life found by Tam et al.31 in fibroblasts and hepatoma cells. In contrast, in CaCo-2 cells the half-life of HMG-CoA reductase was 2.1 hours.11 Thus, in light of the data presented here, it would seem reasonable to assume (in agreement with the general conclusions of Stange and Dietschy19) that after the ingestion of a meal containing cholesterol, the enterocyte could more rapidly turn off cholesterol synthesis than regulate the amount of LDL taken up. However, intestinal cells are continually exposed to biliary cholesterol and throughout the day are frequently exposed to dietary cholesterol as well. This would result in the chronic suppression of intestinal LDL receptor expression and cause a decrease in the contribution of the intestine to the clearance of plasma lipoproteins.

Acknowledgments

We wish to thank Glenna Fry for technical assistance and Mrs. Joan Ockenfels for assistance in the preparation of this manuscript.

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doi: 10.1161/01.ATV.13.5.729
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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