Regulation of sterol regulatory element-binding proteins by cholesterol flux in CaCo-2 cells

F. Jeffrey Field,[1] Ella Born, Shubha Murthy, and Satya N. Mathur

Department of Internal Medicine, 4-522 JCP, 200 Hawkins Drive, and Veterans Administration, University of Iowa College of Medicine, Iowa City, IA 52242

Abstract **The regulation of sterol regulatory element-binding proteins (SREBP) by cholesterol flux was studied in the intestinal cell line CaCo-2. CaCo-2 cells were incubated for 18 h with micelles containing 5 mM taurocholate and 500 μM oleic acid or micelles containing either 200 μM cholesterol or 150 μM lysophosphatidylcholine. In some incubations, an ACAT inhibitor was added or 25-hydroxycholesterol was substituted for cholesterol. The SREBP-1a transcript was 2-fold more abundant than the SREBP-1c transcript. In cells incubated with micelles containing cholesterol, rates of cholesterol synthesis were decreased and rates of esterification were increased. Cholesterol synthesis was decreased further by ACAT inhibition. Cholesterol influx decreased mRNA levels of SREBP-2, HMG-CoA synthase, HMG-CoA reductase, and fatty acid synthase. ACAT inhibition modestly suppressed gene expression further. Neither SREBP-1a nor SREBP-1c mRNA levels were altered by cholesterol. Despite decreases in gene expression of the sterol-responsive genes by cholesterol, the amounts of precursor and mature forms of SREBP-1 and SREBP-2 were not altered. In contrast, if 25-hydroxycholesterol was substituted for cholesterol, both the precursor and mature forms of SREBP-2 were decreased. The polar sterol decreased the mature form of SREBP-1 but the amount of the precursor form was unchanged. In cells incubated with micelles containing lysophosphatidylcholine, which causes cholesterol to efflux from cells, sterol-responsive gene expression was increased. The amounts of precursor and mature forms of SREBP-1 and SREBP-2, however, were not altered. In contrast, if the cells were depleted of cholesterol by incubating them with lovastatin and cyclodextrin, the mature forms of SREBP-1 and SREBP-2 were increased, as were mRNA levels for the sterol-responsive genes. The data would suggest that cholesterol influx/efflux regulates mRNA levels of sterol-responsive genes independently of changes in the amount of mature SREBP. In contrast, 25-hydroxycholesterol influx or cholesterol depletion alters the amount of mature SREBP, leading to the regulation of sterol-responsive gene expression.**—Field, F. J., E. Born, S. Murthy, and S. N. Mathur. **Regulation of sterol regulatory element-binding proteins by cholesterol flux in CaCo-2 cells.** ***J. Lipid Res.*** **2001.** 42: **1687–1698.**

Supplementary key words 3-hydroxy-3-methylglutaryl coenzyme A reductase • 25-hydroxycholesterol • fatty acid synthase • gene expression • micelles

At the cellular level, the amount of unesterified cholesterol is tightly controlled. Regulating the amount of cholesterol within a cell is dependent on a rather potent end-product feedback regulation of cholesterol synthesis. This is achieved, in part, by inhibiting or enhancing the expression of genes that encode key regulatory proteins responsible for cholesterol synthesis [see review in ref. (1)]. Mechanisms by which a cell recognizes changes in cholesterol flux and how these changes directly influence transcription rates of target genes that control cholesterol synthesis have been elucidated. Three transcription factors, designated sterol regulatory element-binding proteins 1a, 1c, and 2 (SREBP-1a, -1c, and -2) regulate the transcription of genes that encode enzymes in the biosynthetic pathways of both cholesterol and fatty acids [see review in ref. (2)]. SREBP-1a and SREBP-1c are products of a single gene. SREBP-1a is the more potent activator of genes of both synthetic pathways, whereas SREBP-1c is a weaker transcription factor and tends to be more active in enhancing transcription of genes in the fatty acid synthetic pathway (3). SREBP-2 preferentially enhances the expression of genes of the cholesterol synthetic pathway but can also enhance transcription of genes of fatty acid synthesis as well (4–6). Precursor, or a membrane form of SREBP, is bound to membranes of the endoplasmic reticulum and nuclear envelope. When a cell is depleted of cholesterol, a two-step proteolytic process releases a mature, or nuclear, form that then enters the nucleus (7). By binding to a 10-bp sterol regulatory element in the promoter of target genes, transcription is enhanced. In times of cholesterol excess, proteolysis of the precursor form is prevented and transcription of these genes is suppressed (8).

Similar to other cells within the body, cells of the small intestine can procure cholesterol from circulating lipoproteins and de novo synthesis (9). Unlike other cells, however, intestinal cells have a continual source of either dietary or biliary cholesterol that can be absorbed from the lumen. Thus, the regulation of cholesterol synthesis in this organ has another layer of complexity that is not found

Abbreviations: SREBP, sterol regulatory element-binding proteins.

[1] To whom correspondence should be addressed.
e-mail: f-jeffrey-field@uiowa.edu

in other cells (9). It might be assumed that the influx of cholesterol from the lumen into an absorptive cell would contribute to the cholesterol pool and, hence, regulate the synthesis of cholesterol in this organ. Although this assumption seems reasonable, several early studies of intact animals have suggested that this may not be the case (10–12).

In an earlier study performed in the intestinal cell line CaCo-2, changes in apical sterol flux were found to alter the activity of the LDL receptor and HMG-CoA reductase, a key regulatory enzyme in the pathway of cholesterol synthesis (13–15). Loss of cellular cholesterol caused an increase in gene expression of reductase with an increase in enzyme activity (13, 16). In contrast, sterol influx, particularly cholesterol and low concentrations of 25-hydroxycholesterol, suppressed reductase activity without significantly altering gene expression. It was postulated that posttranscriptional events, including decreased translational efficiency and increased degradation of reductase protein, accounted for most of the regulation of reductase activity (13). The present study was performed to investigate whether changes in cholesterol flux in CaCo-2 cells would regulate the SREBP pathway and hence the regulation of expression of sterol-responsive genes. The results suggest that the influx of cholesterol from bile salt micelles does not significantly alter the proteolytic processing of either SREBP-1 or SREBP-2 despite causing an increase in cholesterol esterification, a decrease in cholesterol synthesis, and a decrease in the expression of SREBP-2 mRNA and other sterol-responsive genes, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase. Cholesterol efflux increases mRNA levels of SREBP-2 and sterol-responsive genes but also does not alter SREBP mass. In contrast, if 25-hydroxycholesterol is substituted for cholesterol, sterol-responsive genes and SREBP-2 mRNA levels, both precursor and mature forms of SREBP-2, and the mature form of SREBP-1 are decreased. Cholesterol depletion enhances the amount of the mature forms of SREBP and increases the expression of sterol-responsive genes.

MATERIALS AND METHODS

[9,10-^{3}H(N)]oleic acid (14 Ci/mmol), [^{14}C]acetate (55 mCi/mmol), and [^{32}P]UTP (3,000 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Protease inhibitors, bovine serum albumin, cholesterol, oleic acid, taurocholic acid, methyl-β-cyclodextrin, and TriReagent were purchased from Sigma (St. Louis, MO). 25-Hydroxycholesterol was obtained from Steraloids (Newport, RI). MAXIscript T7 and ribonuclease protection assay (RPA) III kits were supplied by Ambion (Austin, TX). Medium 199 was from GIBCO-BRL Life Technologies (Grand Island, NY). Lovastatin was obtained from Merck Research Laboratories (Rahway, NJ). ACAT inhibitor PD 128,042 was a gift from Parke-Davis Pharmaceutical Research Division (Warner-Lambert, Ann Arbor, MI).

Cell culture

CaCo-2 cells were cultured on plastic six-well trays for 14 days as described previously (17).

Stock solutions of taurocholate, cholesterol, and 25-hydroxycholesterol were prepared in 95% ethanol. Oleic acid and lysophosphatidylcholine were dissolved in chloroform. Appropriate amounts of sodium taurocholate and lipids were mixed and solvents were evaporated under a stream of nitrogen. Medium 199 with 10 mM HEPES was then added to obtain the desired concentrations of the lipids. The solution was warmed to 37°C and stirred vigorously before use.

Cholesterol and fatty acid synthesis

CaCo-2 cells were incubated for 22 h at 37°C in 5% CO_2 with the appropriate treatment. During the last 4 h of the incubation, 2 μCi of [^{14}C]acetate was added in a small volume of medium 199 to each well. The final concentration of acetate was 0.6 mM. At the end of the incubation, cells were rinsed with medium 199, suspended in 1 ml of 90% methanol containing 0.5 M NaOH, and placed in a boiling water bath for 1 h to saponify lipids. After adding an equal volume of acidic water, the lipids were extracted twice at pH 3.0 with 2 ml of hexanes. To remove residual radiolabeled acetate, the combined hexane extract was washed twice with 1 ml of acidic water. Hexanes were then evaporated under a stream of nitrogen and lipids were dissolved in 0.1 ml of chloroform. Fatty acids and cholesterol were separated by thin-layer chromatography on silica gel plates, using hexanes–diethyl ether–acetic acid–methanol 70:30:1:1 (v/v/v/v) as solvent. Lipids were visualized by exposure to iodine and the radioactivity in fatty acids and cholesterol fractions was determined by scraping the bands and counting in a Packard (Downers Grove, IL) Tricarb 2100-TR liquid scintillation counter.

Lipid synthesis

The sodium salt of oleate was prepared by adding a two times molar excess of NaOH to the oleic acid solution in chloroform. The solvent was evaporated under a stream of nitrogen and the oleate was dissolved in 100 μl of warm water. An appropriate amount of medium 199 containing 10 mM HEPES and bovine serum albumin was added to obtain a fatty acid-to-albumin ratio of 3:1. The final concentration of oleate was 50 μM with 6 μCi of [^{3}H]oleic acid (14 Ci/mmol) per 2 ml per well. During the last 4 h of the 22-h incubation with the respective treatment, the medium was replaced with the labeled oleate-albumin solution. The cells were rinsed with medium 199 and lipids were extracted twice with 1 ml of hexanes–isopropanol–water 3:2:0.1 (v/v/v), pH 3.0. The solvents were evaporated under nitrogen and the lipids were dissolved in 0.1 ml of chloroform. Phospholipids, fatty acids, triacylglycerols, and cholesteryl esters were separated by thin-layer chromatography on silica gel plates, using hexanes–diethyl ether–acetic acid–methanol 70:30:1:1 (v/v/v/v) as solvent. The lipids were visualized by exposure to iodine and the radioactivity in the lipid fractions was determined by scraping the bands and counting.

RT-PCR

DNA-free RNA was extracted from CaCo-2 cells by using a High Pure RNA isolation kit from Boehringer Mannheim (Indianapolis, IN). cDNA were synthesized and amplified with the Thermoscript RT-PCR system from GIBCO-BRL Life Technologies.

RNase protection assays

The plasmid preparations were a gift from M. Brown and J. Goldstein (University of Texas Southwestern Medical School, Dallas, TX). RNase protection assays were performed using antisense RNA probes described in **Table 1**.

A T7 promoter was added to the PCR products, using a Lig'nScribe kit from Ambion. To obtain antisense probes, the products were amplified by PCR, using a 5′ gene-specific primer and PCR adapter primer. The [^{32}P]UTP-labeled RNA probes were synthesized by using a T7 promoter containing the PCR

TABLE 1. Plasmids and primers used in RNase protection assays

Plasmid Used for RNA Probes	Plasmid	Product	Reference
		bp	
Human SREBP-1a	pGEM-3zf(t)	80	(18)
Human SREBP-1c	pGEM-3zf(t)	80	(18)
Primers for PCR Products Used for RNA Probes	**Primer Sequence**		
Human SREBP-2 1297	CTGAAGCTGGCAAATCAAAAG	200	
Human SREBP-2 1496	TCATCCAATAGAGGGCTTCCT		
Human HMG-CoA synthase 1208	TCAGCATTTAGCAGGGAAGAG	303	
Human HMG-CoA synthase 1510	CTGTGCTTTTCATCCACCCTA		
Human HMG-CoA reductase 2280	GGGAGCATAGGAGGCTACAAC	248	
Human HMG-CoA reductase 2528	TGCTCCTTGAACACCTAGCAT		
Human fatty acid synthase 2500	ACAGGGACAACCTGGAGTTCT	159	
Human fatty acid synthase 2658	CTGTGGTCCCACTTGATGAGT		

products or linearized plasmid as template with a MAXIscript in vitro transcription kit from Ambion. The [^{32}P]UTP-labeled RNA probes were purified on denaturing 6% acrylamide-8 M urea gels. Total RNA from CaCo-2 cells was isolated with TriReagent (Sigma). mRNA transcripts were estimated by using an RPA III kit from Ambion. Protected fragments were resolved on denaturing 6% acrylamide-8 M urea gels in Tris-borate-EDTA (TBE) buffer. Eastman Kodak (Rochester, NY) Biomax MS film with TranScreen HE was used to visualize the bands on the gel. In Figs. 2–5, the results are expressed as relative mRNA levels for each gene after estimating the densities of the bands on a given blot, using the NIH Image J program. Except for the probes used to detect SREBP-1a and SREBP-1c transcripts (see below), specific activities of the probes differed. Thus, in Figs. 2–5, comparisons can be made only among or between treatments for a given gene.

The protected fragments for SREBP-1a and SREBP-1c contained 14 and 17 [^{32}P]uridine phosphates, respectively. The values obtained from these transcripts have been corrected to reflect the differences in the number of uridines in the protected fragment.

The validity of the RPA and equal loading were established by using the same amount of total RNA per sample, documenting RNA concentration dependence, running triplicates of a sample on each gel, and estimating 18S rRNA as a control (Ambion Technical Bulletin 151) (19–23). The 18S probe, pTRI RNA 18S, was obtained from Ambion. This probe gives a protected fragment of 80 bp in length. 18S rRNA was estimated in the same tubes as experimental samples, except in the case of SREBP-1a and SREBP-1c, as these probes also give protected fragments of 80 bp.

Immunoblot analysis

Protease inhibitors *N*-acetyl-leucyl-leucyl-norleucinal (50 μg/ml), 0.5 mM phenylmethylsulfonyl fluoride, pepstatin A (5 μg/ml), leupeptin (10 μg/ml), 1 mM Pefabloc, 10 mM DTT, aprotinin (2 μg/ml), 0.1 mM zinc sulfate, and 0.1 mM copper sulfate were added to all buffers used for preparing cell fractions. All procedures were performed at 4°C. To prepare total membrane fractions, the cells were allowed to swell for 30 min on ice in buffer A [10 mM HEPES-NaOH (pH 7.4), 1.5 mM $MgCl_2$, 10 mM KCl, 1 mM EDTA, 1 mM EGTA] followed by homogenization by passage through a 25-gauge needle five times. The whole homogenate was centrifuged at 100,000 *g* for 45 min. The resulting pellet containing all cell membranes was suspended in 150–300 μl of buffer B [125 mM Tris (pH 6.0), 160 mM NaCl, 1% Triton X-100] and sonicated for 10 s. This was subsequently centrifuged for 30 min in a microcentrifuge at 19,000 *g*. The supernatant was designated as the total membrane fraction.

In some experiments nuclear extract and membrane fractions were prepared from CaCo-2 cells by a modification of the procedure described by Wang et al. (8). The cells were allowed to swell for 30 min on ice in buffer A followed by homogenization by passage through a 25-gauge needle 15 times. The homogenate was first centrifuged at 2,000 *g* to obtain a nuclear pellet followed by centrifugation of the supernatant at 100,000 *g* for 45 min to isolate a membrane fraction. The nuclear proteins were extracted with 0.1–0.2 ml of buffer C containing 20 mM HEPES-KOH (pH 7.9), 1.5 mM $MgCl_2$, 25% glycerol, 500 mM NaCl, 1 mM EDTA, and 1 mM EGTA. Membrane proteins were extracted with 0.15–0.2 ml of buffer B. Both fractions were sonicated for 10 s followed by centrifugation in a microcentrifuge for 30 min at 19,000 *g*. Supernatants from these samples were used for analysis of SREBP-1 and SREBP-2 protein mass.

Equal amounts of the protein from each sample were resolved by SDS-PAGE on the same day of preparation and blotted onto a polyvinylidene difluoride membrane. The blot was prepared, dried, stored, and probed by a nonblock technique described by Sadra, Cinek, and Imboden (24). Briefly, the blot was incubated for 30 min with 1× TBS followed by a rinse with water. It was air dried for 15 min followed by three rinses with methanol–water 1:1 (v/v), kept in methanol for 2 min, and air dried. It was stored at room temperature between two sheets of filter paper. All blots were dried for 10 min under vacuum just before probing with antibodies. The blots were incubated for 1 h with primary antibody in TBS containing 1% goat serum, 2% nonfat milk, and 0.1% Triton X-100. They were rinsed with TBS-0.1% Triton X-100 followed by secondary horseradish peroxidase (HRP) antibody for 1 h in TBS containing 1% goat serum, 2% nonfat milk, and 0.1% Triton X-100. Primary polyclonal antibodies raised in rabbits against human SREBP-1 or SREBP-2 were used. Goat anti-rabbit-HRP (A-6154; Sigma) was used as secondary antibody. The HRP signal was detected on blots with SuperSignal West Femto maximum sensitivity substrate (Pierce, Rockford, IL).

Statistical analysis

To determine whether the differences among the three treatments were significant, the data were analyzed at $P < 0.05$ by one-way ANOVA and by the Student-Newman-Keuls method, using SigmaStat software (SPSS, Chicago, IL). A *t*-test was used to compare data from two treatments.

RESULTS

Effect of micellar cholesterol on cholesterol synthesis and esterification

To address the regulation of cholesterol synthesis by cholesterol influx, CaCo-2 cells were incubated for 22 h

with a micellar solution containing 5 mM taurocholate and 500 μM oleic acid with or without 200 μM cholesterol. Although unsaturated fatty acids alone have been shown to regulate SREBP expression (25–29), oleic acid was included in all micellar solutions because without the fatty acid, the results were inconsistent and changes in cholesterol metabolism were modest. To further expand the intracellular unesterified cholesterol pool, cholesterol esterification was prevented by adding an ACAT inhibitor to some of the incubations. During the last 4 h of the incubation, labeled acetate was added and the incorporation of acetate into cholesterol and fatty acids was estimated (**Fig. 1A** and **B**). Compared with cells incubated with micelles alone, the rate of cholesterol synthesis was significantly decreased in cells incubated with micelles containing cholesterol. This was accentuated in cells incubated with micellar cholesterol together with the ACAT inhibitor. In contrast, there was a modest, but significant, increase in fatty acid synthesis in cells incubated with cholesterol and this was enhanced by the presence of the ACAT inhibitor.

To estimate the rate of cholesterol esterification, labeled oleic acid was added during the last 4 h of the incubation and the incorporation of oleic acid into cholesteryl esters was estimated (Fig. 1C). In cells incubated with micelles containing cholesterol, the rate of cholesterol esterification was increased more than 2-fold compared with cells incubated with micelles alone. As expected, in cells treated with the ACAT inhibitor, the synthesis of cholesteryl esters was markedly decreased. In data not shown, the incorporation of oleic acid into phospholipids or triacylglycerols was not altered by cholesterol or cholesterol plus the ACAT inhibitor.

It is clear that micellar cholesterol expands cellular cholesterol pools in CaCo-2 cells, leading to downregulation of cholesterol synthesis and increased cholesteryl ester synthesis.

Effect of micellar cholesterol or 25-hydroxycholesterol on gene expression of SREBP, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase

To study the regulation of SREBP and sterol-responsive gene expression by cholesterol influx, CaCo-2 cells were incubated for 18 h with micelles alone or micelles containing cholesterol with or without the ACAT inhibitor. After the incubation, mRNA levels were estimated by RNase protection assays for SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase (**Fig. 2**). 18S rRNA was used as a control to ensure that equal amounts of RNA were analyzed. In CaCo-2 cells, there was 2-fold more of the SREBP-1a transcript than the SREBP-1c transcript. Gene expression of SREBP-1a and SREBP-1c was not altered in cells incubated with micelles containing cholesterol with or without the ACAT inhibitor. In contrast, SREBP-2 gene expression was modestly decreased by micellar cholesterol and this was further decreased in the presence of the ACAT inhibitor. In

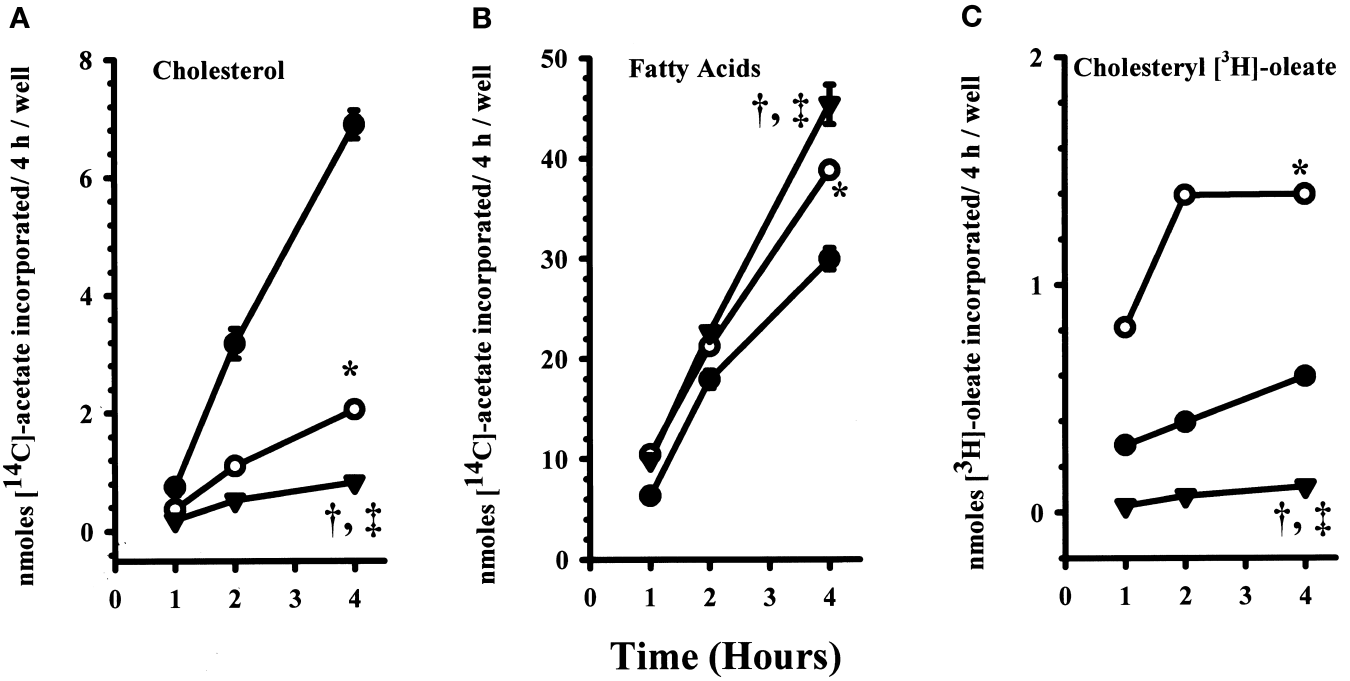

Fig. 1. Effect of micellar cholesterol on cholesterol, fatty acid, and cholesteryl ester synthesis. CaCo-2 cells were incubated for 22 h with 5 mM taurocholate and 500 μM oleic acid (solid circles), 5 mM taurocholate, 500 μM oleic acid, and 200 μM cholesterol (open circles), or 5 mM taurocholate, 500 μM oleic acid, 200 μM cholesterol, and an ACAT inhibitor (PD 128042, 2 μg/ml) (inverted solid triangles). During the last 4 h of incubation, the incorporation of labeled acetate into unesterified cholesterol (A) and fatty acids (B), or of labeled oleate into cholesteryl esters (C), was estimated. Each point represents the mean ± SE of four separate dishes. * Taurocholate + oleate values are significantly different from taurocholate + oleate + cholesterol values at $P < 0.05$. † Taurocholate + oleate values are significantly different from taurocholate + oleate + cholesterol + ACAT inhibitor values at $P < 0.05$. ‡ Taurocholate + oleate + cholesterol values are significantly different from taurocholate + oleate + cholesterol + ACAT inhibitor values at $P < 0.05$.

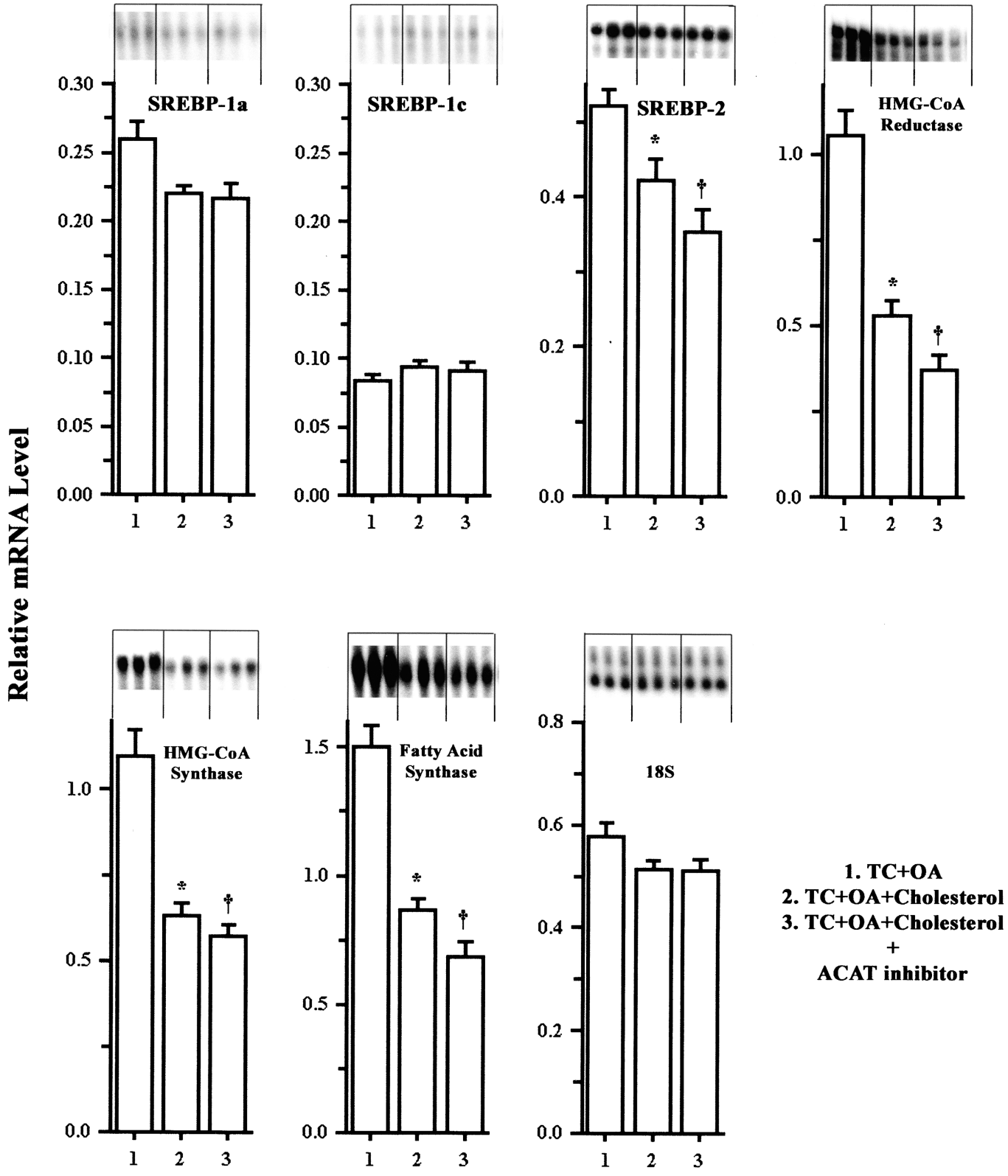

Fig. 2. Effect of micellar cholesterol on mRNA levels of SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase. After an 18-h incubation with the treatments describe in Fig. 1, RNA was isolated from the cells and mRNA levels for the respective genes were estimated by RNase protection assays. Lane 1, taurocholate (TC) and oleic acid (OA); lane 2, TC, OA, and cholesterol; lane 3, TC, OA, cholesterol, and ACAT inhibitor. The data represent means ± SE of nine samples from three individual experiments. A representative autoradiogram of the protected fragments from three separate dishes is shown. * TC + OA values are significantly different from TC + OA + cholesterol values at $P < 0.05$. † TC + OA values are significantly different from TC + OA + cholesterol + ACAT inhibitor values at $P < 0.05$.

cells incubated with micellar cholesterol, mRNA levels of HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase were approximately half those observed in cells incubated with micelles alone. Again, mRNA levels for the three genes were suppressed further in cells incubated with cholesterol and the ACAT inhibitor.

In another set of experiments, cells were incubated with micelles in which cholesterol was replaced with the polar sterol 25-hydroxycholesterol (**Fig. 3**). Compared with cells incubated with micelles alone, mRNA levels of HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase were decreased in cells incubated with micelles containing the hydroxysterol. SREBP-2 gene expression was also decreased by 25-hydroxycholesterol, whereas neither SREBP-1a nor SREBP-1c expression was altered.

Effect of cholesterol deficiency on gene expression of SREBP, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase

The results suggested that influx of both cholesterol and hydroxylated cholesterol suppressed mRNA levels of SREBP-2 and the other sterol-responsive genes. To investigate whether cholesterol loss from CaCo-2 cells would regulate expression of these genes, cells were incubated with taurocholate micelles or micelles containing lysophosphatidylcholine. In previous studies, we have shown that micellar lysophosphatidylcholine causes cholesterol to efflux from cells, leading to upregulation of HMG-CoA reductase activity and mRNA levels (16). The results are shown in **Fig. 4**. In cells incubated with micelles containing lysophosphatidylcholine, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase mRNA levels were significantly increased. The effects of cholesterol efflux on SREBP gene expression, however, were modest. In cells incubated with lysophosphatidylcholine, SREBP-1a and SREBP-2 mRNA levels were increased 40% and 25%, respectively. Gene expression of SREBP-1c was not altered.

To induce cholesterol depletion, CaCo-2 cells were incubated in lipoprotein-deficient serum containing lovastatin and cyclodextrin. After the incubation, mRNA levels of SREBP and the sterol-responsive genes were estimated and compared with levels estimated in cells incubated with

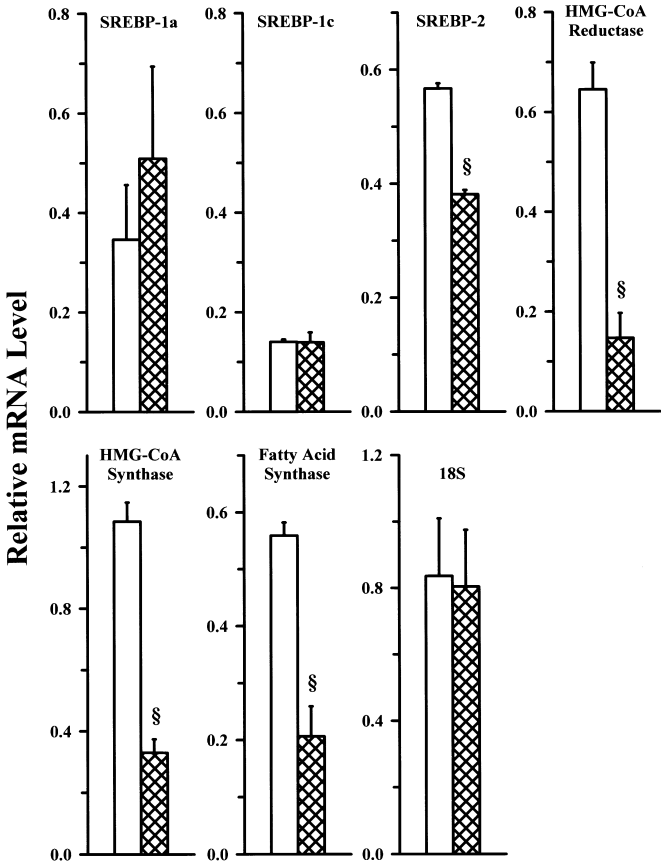

Fig. 3. Effect of micellar 25-hydroxycholesterol on mRNA levels of SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase. CaCo-2 cells were incubated for 18 h with 5 mM taurocholate and 500 μM oleic acid (open columns) or 5 mM taurocholate, 500 μM oleic acid, and 50 μM of 25-hydroxycholesterol (cross-hatched columns). mRNA levels for the respective genes were estimated by RNase protection assays. The data represent means ± SE of six samples from two individual experiments. § Taurocholate + oleate values are significantly different from taurocholate + oleate +25-hydroxycholesterol values at $P < 0.001$.

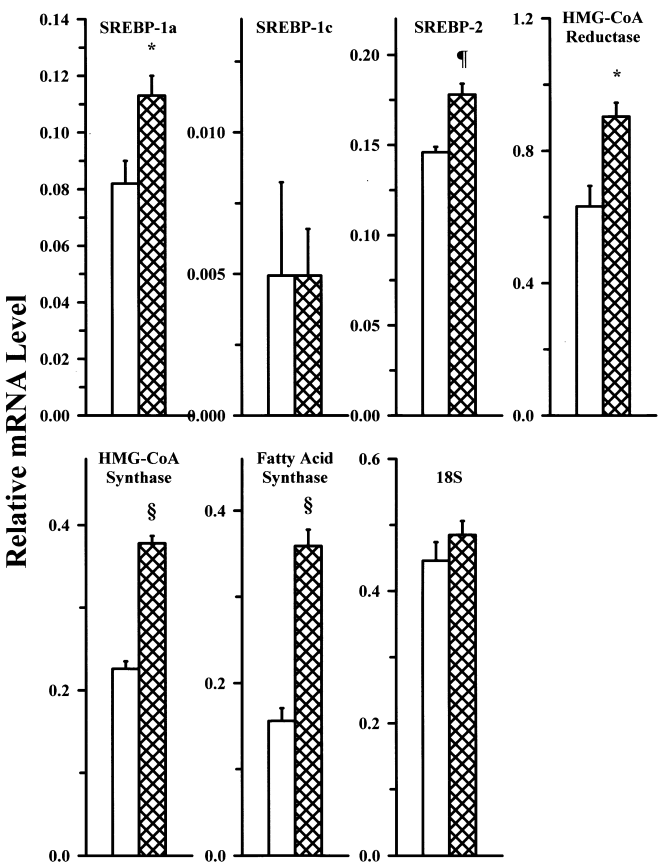

Fig. 4. Effect of micellar lysophosphatidylcholine on mRNA levels of SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA reductase, HMG-Co synthase, and fatty acid synthase. CaCo-2 cells were incubated for 18 h with 1 mM taurocholate (open columns) or 1 mM taurocholate and 200 μM lysophosphatidylcholine (cross-hatched columns). mRNA levels for the respective genes were estimated by RNase protection assays. The data represent means ± SE of six samples from two individual experiments. Taurocholate values are significantly different from taurocholate + lysophosphatidylcholine values at * $P < 0.05$, ¶ $P < 0.01$, and § $P < 0.001$.

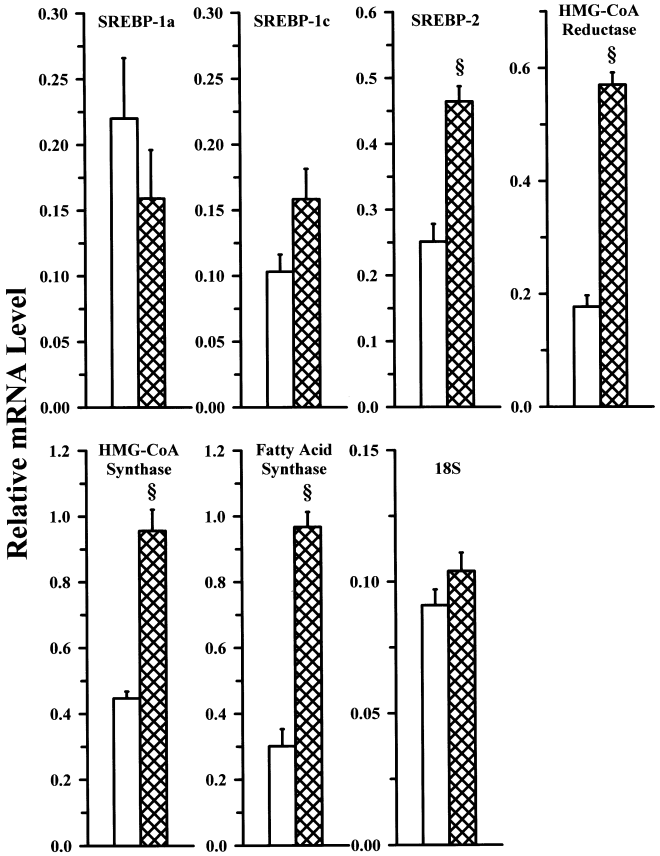

Fig. 5. Effect of cholesterol depletion on mRNA levels of SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase. CaCo-2 cells were incubated for 18 h with 2.5% delipidated fetal calf serum containing 25 μM cholesterol and 50 μM 25-hydroxycholesterol (open columns) or 25 μM lovastatin and cyclodextrin (1 mg/ml) (cross-hatched columns). mRNA levels for the respective genes were estimated by RNase protection assays. The data represent mean ± SE of six samples from two individual experiments. § Cholesterol + 25-hydroxycholesterol values are significantly different from lovastatin + cyclodextrin values at $P < 0.001$.

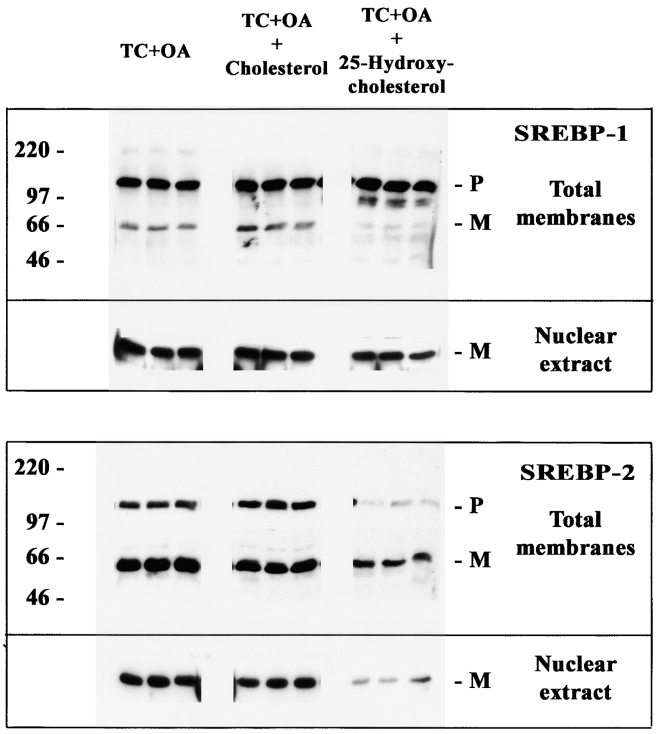

Fig. 6. Effect of micellar cholesterol on (top) SREBP-1 and (bottom) SREBP-2 mass. CaCo-2 cells were incubated for 18 h with 5 mM taurocholate (TC) and 500 μM oleic acid (OA); 5 mM TC, 500 μM OA, and 200 μM cholesterol; or 5 mM TC, 500 μM OA, and 50 μM 25-hydroxycholesterol. After the incubation, the amount of SREBP-1 and SREBP-2 mass was estimated by immunoblotting. An immunoblot of SREBP-1 and SREBP-2 mass estimated in total membranes is shown at the top of each panel. Each blot is representative of three individual experiments showing three separate samples for each treatment. P, Precursor; M, mature. An immunoblot of the mature protein of SREBP-1 and SREBP-2 estimated in nuclear extracts is shown at the bottom of each panel. Each is from a separate experiment showing three separate samples for each treatment. The SREBP bands depicted in each panel are derived from an autoradiogram of a single membrane.

sterols (**Fig. 5**). Compared with cells incubated with sterols, mRNA levels of SREBP-2, HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase were significantly increased in cells depleted of cholesterol. In contrast, both SREBP-1 transcripts were unaltered.

Effect of micellar cholesterol or 25-hydroxycholesterol on SREBP mass

To address the effect of micellar cholesterol on SREBP mass, CaCo-2 cells were incubated for 18 h with micelles alone or with micelles containing either cholesterol or 25-hydroxycholesterol. After the incubation, proteins were extracted from total cellular membranes and the amounts of precursor and mature forms of SREBP-1 and SREBP-2 were estimated by immunoblotting. A representative blot is shown in **Fig. 6**. Compared with cells incubated with micelles alone, neither the precursor nor the mature form of either SREBP-1 or SREBP-2 was altered in cells incubated with micelles containing cholesterol. Similar results were observed in cells incubated with cholesterol and the ACAT inhibitor (data not shown). In contrast, the mature forms of both SREBP-1 and SREBP-2 were decreased in cells incubated with micelles containing 25-hydroxycholesterol. Compared with the precursor form of SREBP-1, however, which was not altered by the hydroxylated sterol, the amount of precursor form of SREBP-2 was decreased by 25-hydroxycholesterol.

It could be argued that estimating the amount of mature SREBP in total membranes may not reflect the amount within the nucleus, its site of action. This was addressed by estimating the mature form of SREBP in nuclear fractions isolated from CaCo-2 cells after the above-described incubations. The results were similar (Fig. 6). The amount of the mature form in the nuclear fraction was unaltered by cholesterol flux but decreased by the influx of the polar sterol.

Effect of cholesterol deficiency on SREBP mass

SREBP mass was estimated in CaCo-2 cells that were incubated with micelles alone or with micelles containing

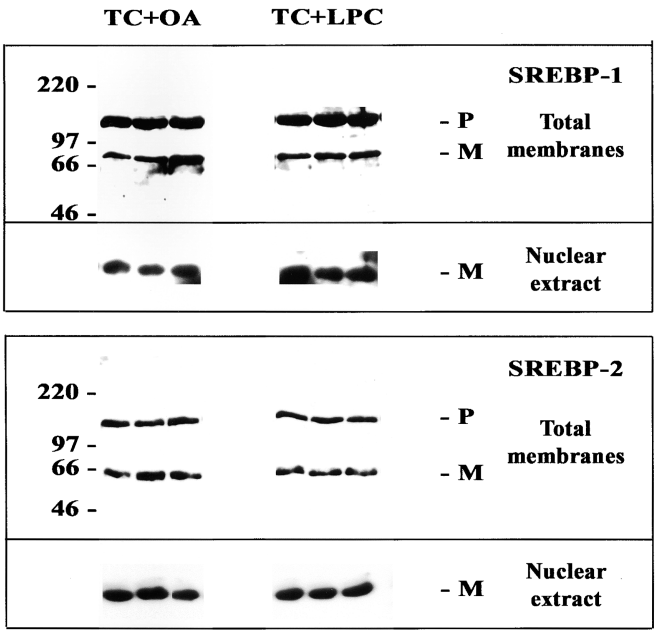

Fig. 7. Effect of micellar lysophosphatidylcholine (LPA) on (top) SREBP-1 and (bottom) SREBP-2 mass. The experimental design is described in Fig. 4. The amount of SREBP-1 and SREBP-2 mass was estimated by immunoblotting. An immunoblot of SREBP-1 and SREBP-2 mass estimated in total membranes is shown at the top of each panel. Each blot is representative of three individual experiments showing three separate samples for each treatment. P, Precursor; M, mature. An immunoblot of the mature protein of SREBP-1 and SREBP-2 estimated in nuclear extracts is shown at the bottom of each panel. Each is from a separate experiment showing three separate samples for each treatment. The SREBP bands depicted in each panel are derived from an autoradiogram of a single membrane.

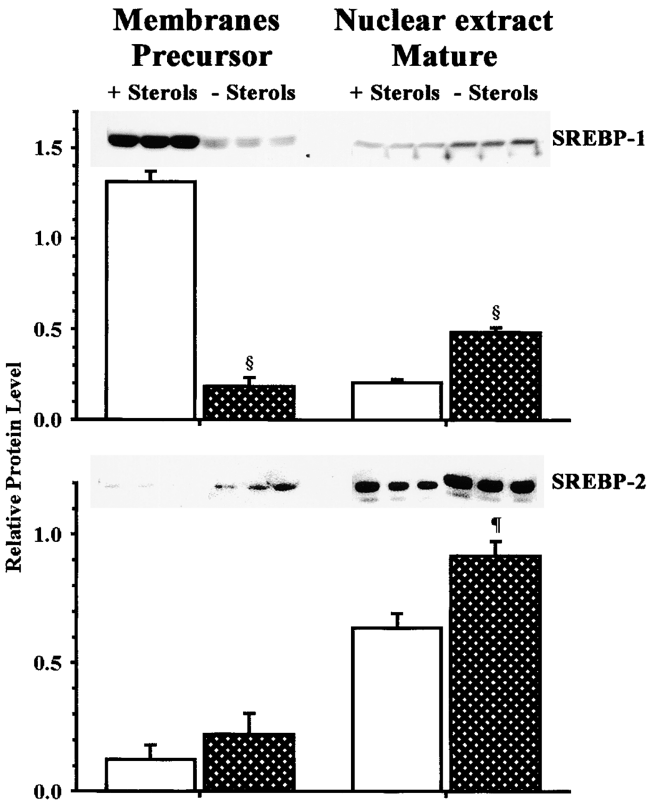

Fig. 8. Effect of cholesterol depletion on SREBP-1 and SREBP-2 mass. The experimental design is described in Fig. 5. After the incubation, nuclear and membrane fractions were prepared as described in Materials and Methods and the amount of SREBP-1 and SREBP-2 mass was estimated by immunoblotting. The data represent means ± SE of six samples from two individual experiments. A representative immunoblot for three separate samples from each group is shown. Cholesterol + 25-hydroxycholesterol values are significantly different from lovastatin + cyclodextrin values at ¶ $P < 0.01$ and § $P < 0.001$.

lysophosphatidylcholine (**Fig. 7**). Compared with CaCo-2 cells incubated with micelles alone, the amounts of the precursor and mature (from total and nuclear membranes) forms of SREBP-1 or SREBP-2 were unaltered by lysophosphatidylcholine.

Cells were then depleted of cholesterol by incubating them in delipidated fetal calf serum containing lovastatin and cyclodextrin (**Fig. 8**). After the incubation, nuclear and membrane fractions were isolated. Compared with cells incubated with sterols, there was more mature SREBP-1 and SREBP-2 and less precursor SREBP-1 in cells depleted of cholesterol.

DISCUSSION

Similar to other cell culture models, the SREBP-1c transcript in CaCo-2 cells was a minor transcript compared with SREBP-1a (18). The ratio of SREBP-1c to SREBP-1a in CaCo-2 cells remained constant whether the cells were confluent or subconfluent (3, 5, 7, 10, and 14 days in culture) or whether they were grown on plastic or semipermeable filters (data not shown). In contrast, it has been observed that in organs of intact animals, the SREBP-1c transcript predominates over the SREBP-1a transcript (18). This was also true in cells prepared from hamster intestine although the ratio of SREBP-1c to SREBP-1a transcripts differed in cell populations recovered along the villus axis (30). A reason for the observed difference in the ratio of SREBP-1c to SREBP-1a transcripts between cell culture models and cells of intact organs remains unexplained.

Cholesterol, absorbed by CaCo-2 cells from bile salt micelles, suppressed cholesterol synthesis and enhanced cholesterol esterification. Preventing cholesterol esterification further suppressed the rate of cholesterol synthesis. These results clearly support the notion that cholesterol expanded regulatory pools of intracellular cholesterol, causing the expected changes in cholesterol metabolism. Moreover, the absorbed cholesterol caused a decrease in the expression of all the sterol-responsive genes: HMG-CoA reductase, HMG-CoA synthase, and fatty acid synthase. Neither of these observations was totally unexpected, as in other cell culture models the addition of sterols clearly downregulates cholesterol synthesis and sterol-responsive gene expression [see reviews in refs. (31) and (32)]. What was unexpected, however, was the observation that these changes in gene expression were not associated with changes

in the proteolytic processing of the precursor form of SREBP. In cell culture, it has been clearly established that changes in cholesterol flux coordinately regulate the proteolytic processing of both SREBP-1 and SREBP-2 (18, 33, 34). In CaCo-2 cells, this did not occur. Our experimental conditions, however, differed from those of previous reports. To investigate the regulation of SREBP expression by changes in cholesterol flux, our intention was to simulate in cell culture what an intestinal absorptive cell might normally encounter in vivo. In fact, in past studies, we have used a similar experimental design to document the regulation of expression of HMG-CoA reductase by cholesterol flux (13–16). One difference from other studies is the use of taurocholate micelles to solubilize cholesterol. This, as opposed to cholesterol added in ethanol, will likely increase the amount of cholesterol that crosses the apical membrane. In cell culture, however, bile salts have been shown to alter numerous other cellular metabolic pathways (35–41). In CaCo-2 cells, for example, taurocholate micelles cause cholesterol to efflux from cells, resulting in increased HMG-CoA reductase activity and gene expression (13). Amounts of the mature forms of SREBP-1 and SREBP-2, however, were not altered in cells incubated with taurocholate micelles alone (F. J. Field, E. Born, S. Murthy, and S. N. Mathur, personal observations). Moreover, even if bile acids prevented in some way the proteolytic processing of SREBP or altered the rate of degradation of the mature protein, it still does not explain the dichotomy observed between the changes in sterol-responsive gene expression and no change in the amount of mature SREBP mass. Another difference in our study was that the effect of unmodified cholesterol was investigated. Cholesterol is rarely used alone to demonstrate regulation of SREBP and/or sterol-responsive gene expression. It is felt that cholesterol is so poorly soluble that it will not adequately cross cell membranes to cause regulation. Others have suggested that unmodified cholesterol is not the regulatory sterol at all, but that oxygenated cholesterol accounts for the observed changes in cholesterol metabolism (42–48). Thus, in most studies to date, 25-hydroxycholesterol is added together with cholesterol (usually in ethanol) to potentiate the desired regulation (7, 8, 49–51). This assumes that cholesterol and hydroxylated cholesterol regulate cholesterol metabolism by similar mechanisms. Although difficult to test experimentally, this assumption has not been systematically challenged. The present results would suggest, however, that cholesterol absorbed at the apical membrane of CaCo-2 cells does regulate the rate of cholesterol synthesis and sterol-responsive gene expression. This regulation by cholesterol appears to be independent of a measurable change in the SREBP pathway. Furthermore, the enhancement of expression of sterol-responsive genes by cholesterol efflux in CaCo-2 cells also occurs independently of measurable changes in the amount of mature SREBP.

Do these results with unmodified cholesterol suggest that the SREBP pathway is not functional or is not regulated in CaCo-2 cells? Results from experiments in which micellar cholesterol was replaced with 25-hydroxycholesterol, and from experiments in which cells were depleted of cholesterol by lovastatin/cyclodextrin, strongly argue that the SREBP pathway is operative in CaCo-2 cells. Similar to results in other cell models, the polar sterol coordinately decreased the amount of mature protein of both SREBP, whereas cholesterol depletion increased the amount of the mature form (18, 33, 34). The effect of the hydroxylated sterol on SREBP-1 and SREBP-2 expression, however, differed. The addition of the polar sterol caused a marked decrease in mRNA abundance of SREBP-2 without altering gene expression of either SREBP-1a or SREBP-1c. This has been observed in other cell culture models and makes good sense because the SREBP-2 gene contains a sterol-response element in its promoter, whereas SREBP-1 does not (52, 53). In contrast to SREBP-2, in which both the precursor and mature forms were decreased by 25-hydroxycholesterol, only the mature form of SREBP-1 was decreased by the polar sterol. The data would suggest that in CaCo-2 cells, the amount of the mature form of SREBP-1 is regulated predominantly by proteolytic processing of the precursor form. The amount of the mature form of SREBP-2 is regulated, at least partially if not entirely, at the transcriptional level.

There are other possible explanations for the dichotomy observed between changes in sterol-responsive gene expression and lack of changes in mature SREBP mass by cholesterol flux. It has been suggested that SREBP alone are weak transcription factors and that coregulatory transcriptional factors, which bind to nearby DNA sequences, are required to potentiate the effects of SREBP. Proven coregulators include Sp1 for the LDL receptor gene and CBF/NF-Y for the fatty acid synthase, HMG-CoA synthase, farnesyl-diphosphate synthase, and squalene synthase genes (54–58). The yin yang 1 protein, in contrast, is a coregulator that acts to repress the transcription of HMG-CoA synthase, LDL receptor, and farnesyl-diphosphate synthase genes (59). Perhaps changes in cholesterol flux alter the amounts or DNA binding of these coregulators. This would, in turn, lead to the suppression or enhancement of sterol-responsive gene expression independently of changes in proteolysis of the precursor SREBP. There is also evidence that SREBP can be modified by phosphorylation that may alter the efficiency of SREBP to bind DNA (60, 61). Thus, in response to changes in cholesterol flux in CaCo-2 cells, either coregulator activation/deactivation or phosphorylation modification of SREBP may play a more important role in regulating the activity of SREBP rather than by proteolysis of the membrane-bound protein. Studies are currently being performed to address both of these possibilities.

The present results in CaCo-2 cells also differed somewhat from results obtained in isolated cells of hamster intestine. Compared with cells prepared from intestinal strips that were depleted of cholesterol by incubation with lovastatin and cyclodextrin, gene expression of SREBP-1c was increased and SREBP-2 gene expression was decreased in cells isolated from strips incubated with sterols (62). Moreover, in a dietary study, compared with SREBP-1c and SREBP-2 gene expression in intestines of hamsters

fed a cholesterol-depletion diet, SREBP-1c gene expression was increased and SREBP-2 expression was decreased in intestines of animals fed a cholesterol-enriched diet (30). Thus, in two separate studies performed in hamster intestine, reciprocal regulation of SREBP-1c and SREBP-2 gene expression by cholesterol flux has been demonstrated. A study demonstrating that the SREBP-1c gene is a target of the sterol-binding nuclear hormone receptor, RXR/LXR transcription factor, explains the enhancement of SREBP-1c gene expression by sterols in intestine in vivo (63). It does not, however, explain the failure of sterols to regulate SREBP-1c expression in cell culture. In addition, in the previous dietary study, compared with hamsters fed a normal chow diet, cholesterol feeding did not significantly alter expression of sterol-responsive genes in intestine (30). Obviously, this is in contrast to what was observed in the present study of CaCo-2 cells.

In hamster intestinal cells, the amount of the mature form of both SREBP was in low abundance and difficult to detect. The amount of precursor mass, in contrast, was in abundance (30). This suggests that in intestinal cells of intact animals, cholesterol is in excess and the proteolytic processing of the precursor form is chronically suppressed. In CaCo-2 cells, however, this was not the case. The mature form of both SREBP was in abundance and easy to detect, suggesting either avid proteolysis of the precursor form or suppressed degradation of the mature form. In either case, ample amounts of the mature form would suggest chronic enhancement of transcription of sterol-responsive genes; just the opposite of what would be expected in intestines of intact animals. These differences observed between results obtained in cell culture versus results obtained in intact animals suggest that caution should be used when findings obtained in cell culture are applied to the in vivo situation.

Finally, we cannot completely exclude the possibility that after unmodified cholesterol flux, modest changes in the proteolytic processing of SREBP occurred, leading to small, undetectable changes in the amount of SREBP mature protein and, thus, regulation of gene expression. We find this difficult to believe, however. The absorption of cholesterol caused a 2-fold decrease in sterol-responsive gene expression, not too unlike the extent of suppression of these genes by 25-hydroxycholesterol. If the SREBP pathway were playing a role in regulating gene expression after cholesterol flux, changes of this degree should have been reflected in the amount of mature SREBP protein, similar to that seen with the oxygenated sterol. Perhaps unmodified and hydroxylated cholesterols regulate gene expression and cholesterol biosynthesis by different mechanisms. This would challenge the assumption that hydroxylated sterols act through the same metabolic pathways as cholesterol. Another possibility is that cholesterol is being hydroxylated either during the incubation period or within the cell, which then leads to suppression of sterol-responsive genes. Even if this were true, it still does not explain the dichotomy observed between changes in gene expression without changes in mature SREBP mass after cholesterol flux.

The authors thank Dr. Jay Horton for helpful discussions throughout this work and Drs. Brown and Goldstein for the SREBP-1a and SREBP-1c plasmids and polyclonal antibodies against SREBP-1 and SREBP-2. This work was supported by the Veterans Administration and by National Institutes of Health grants HL 49264 and 56032.

Manuscript received 26 March 2001, in revised form 31 May 2001, and in re-revised form 29 June 2001.

REFERENCES

1. Goldstein, J. L., and M. S. Brown. 1990. Regulation of the mevalonate pathway. *Nature.* **343:** 425–430.
2. Brown, M. S., and J. L. Goldstein. 1997. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell.* **89:** 331–340.
3. Shimano, H., J. D. Horton, I. Shimomura, R. E. Hammer, M. S. Brown, and J. L. Goldstein. 1997. Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J. Clin. Invest.* **99:** 846–854.
4. Sheng, Z., H. Otani, M. S. Brown, and J. L. Goldstein. 1995. Independent regulation of sterol regulatory element-binding proteins 1 and 2 in hamster liver. *Proc. Natl. Acad. Sci. USA.* **92:** 935–938.
5. Shimomura, I., Y. Bashmakov, H. Shimano, J. D. Horton, J. L. Goldstein, and M. S. Brown. 1997. Cholesterol feeding reduces nuclear forms of sterol regulatory element binding proteins in hamster liver. *Proc. Natl. Acad. Sci. USA.* **94:** 12354–12359.
6. Horton, J. D., I. Shimomura, M. S. Brown, R. E. Hammer, J. L. Goldstein, and H. Shimano. 1998. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. *J. Clin. Invest.* **101:** 2331–2339.
7. Sakai, J., E. A. Duncan, R. B. Rawson, X. Hua, M. S. Brown, and J. L. Goldstein. 1996. Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavages, one within a transmembrane segment. *Cell.* **85:** 1037–1046.
8. Wang, X., R. Sato, M. S. Brown, X. Hua, and J. L. Goldstein. 1994. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell.* **77:** 53–62.
9. Field, F. J., N. T. Kam, and S. N. Mathur. 1990. Regulation of cholesterol metabolism in the intestine. *Gastroenterology.* **99:** 539–551.
10. Dietschy, J. M. 1968. The role of bile salts in controlling the rate of intestinal cholesterogenesis. *J. Clin. Invest.* **47:** 286–300.
11. Dietschy, J. M., and M. D. Siperstein. 1967. Effect of cholesterol feeding and fasting on sterol synthesis in seventeen tissues of the rat. *J. Lipid Res.* **8:** 97–104.
12. Stange, E. F., K. E. Suckling, and J. M. Dietschy. 1983. Synthesis and coenzyme A-dependent esterification of cholesterol in rat intestinal epithelium. Differences in cellular localization and mechanisms of regulation. *J. Biol. Chem.* **258:** 12868–12875.
13. Field, F. J., T. Shreves, D. Fujiwara, S. Murthy, E. Albright, and S. N. Mathur. 1991. Regulation of gene expression and synthesis and degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by micellar cholesterol in CaCo-2 cells. *J. Lipid Res.* **32:** 1811–1821.
14. Field, F. J., D. Fujiwara, E. Born, D. A. Chappell, and S. N. Mathur. 1993. Regulation of LDL receptor expression by luminal sterol flux in CaCo-2 cells. *Arterioscler. Thromb.* **13:** 729–737.
15. Field, F. J., E. Born, and S. N. Mathur. 1997. Effect of micellar beta-sitosterol on cholesterol metabolism in CaCo-2 cells. *J. Lipid Res.* **38:** 348–360.
16. Muir, L. V., E. Born, S. N. Mathur, and F. J. Field. 1996. Lysophosphatidylcholine increases 3-hydroxy-3-methylglutaryl-coenzyme A reductase gene expression in CaCo-2 cells. *Gastroenterology.* **110:** 1068–1076.
17. 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.
18. Shimomura, I., H. Shimano, J. D. Horton, J. L. Goldstein, and M. S. Brown. 1997. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J. Clin. Invest.* **99:** 838–845.
19. Battelle, B. A., C. D. Williams, J. L. Schremser-Berlin, and C. Cacciatore. 2000. Regulation of arrestin mRNA levels in *Limulus* lateral

eye: separate and combined influences of circadian efferent input and light. *Vis. Neurosci.* **17:** 217–227.
20. Blondin, P., P. W. Farin, A. E. Crosier, J. E. Alexander, and C. E. Farin. 2000. In vitro production of embryos alters levels of insulin-like growth factor-II messenger ribonucleic acid in bovine fetuses 63 days after transfer. *Biol. Reprod.* **62:** 384–389.
21. Dattilo, J. B., M. P. Dattilo, D. R. Yager, and R. G. Makhoul. 1998. Hypercholesterolemia alters the gene expression of novel components of the extracellular matrix in experimental vein grafts. *Ann. Vasc. Surg.* **12:** 168–173.
22. Davis, M. J., C. S. Bailey, and C. K. Smith. 1997. Use of internal controls to increase quantitative capabilities of the ribonuclease protection assay. *BioTechniques.* **23:** 280–285.
23. Maitra, A., H. A. LaVoie, R. N. Day, J. C. Garmey, and J. D. Veldhuis. 1995. Regulation of porcine granulosa cell 3-hydroxy-3-methylglutaryl coenzyme A reductase by insulin and insulin-like growth factor I: synergism with follicle-stimulating hormone or protein kinase A agonist. *Endocrinology.* **136:** 5111–5117.
24. Sadra, A., T. Cinek, and J. B. Imboden. 2000. Multiple probing of an immunoblot membrane using a non-block technique: advantages in speed and sensitivity. *Anal. Biochem.* **278:** 235–237.
25. Hannah, V. C., J. Ou, A. Luong, J. L. Goldstein, and M. S. Brown. 2001. Unsaturated fatty acids down-regulate SREBP isoforms 1a and 1c by two mechanisms in HEK-293 cells. *J. Biol. Chem.* **276:** 4365–4372.
26. Thewke, D. P., S. R. Panini, and M. Sinensky. 1998. Oleate potentiates oxysterol inhibition of transcription from sterol regulatory element-1-regulated promoters and maturation of sterol regulatory element-binding proteins. *J. Biol. Chem.* **273:** 21402–21407.
27. Worgall, T. S., S. L. Sturley, T. Seo, T. F. Osborne, and R. J. Deckelbaum. 1998. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. *J. Biol. Chem.* **273:** 25537–25540.
28. Xu, J., M. T. Nakamura, H. P. Cho, and S. D. Clarke. 1999. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids. A mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J. Biol. Chem.* **274:** 23577–23583.
29. Xu, J., M. Teran-Garcia, J. H. Park, M. T. Nakamura, and S. D. Clarke. 2001. Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating transcript decay. *J. Biol. Chem.* **276:** 9800–9807.
30. Field, F. J., E. Born, S. Murthy, and S. N. Mathur. 2001. Regulation of sterol regulatory element binding proteins in hamster intestine by cholesterol flux. *J. Biol. Chem.* **276:** 17576–17583.
31. Brown, M. S., and J. L. Goldstein. 1980. Multivalent feedback regulation of HMG CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. *J. Lipid Res.* **21:** 505–517.
32. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
33. Kawabe, Y., M. Honda, Y. Wada, Y. Yazaki, T. Suzuki, Y. Ohba, H. Nabata, A. Endo, A. Matsumoto, H. Itakura, and T. Kodama. 1994. Sterol mediated regulation of SREBP-1a,1b,1c and SREBP-2 in cultured human cells. *Biochem. Biophys. Res. Commun.* **202:** 1460–1467.
34. Hua, X., C. Yokoyama, J. Wu, M. R. Briggs, M. S. Brown, J. L. Goldstein, and X. Wang. 1993. SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. *Proc. Natl. Acad. Sci. USA.* **90:** 11603–11607.
35. Coleman, R. 1987. Bile salts and biliary lipids. *Biochem. Soc. Trans.* **15:** 68S–80S.
36. Hofmann, A. F., and D. M. Small. 1967. Detergent properties of bile salts: correlation with physiological function. *Annu. Rev. Med.* **18:** 333–376.
37. Abramson, J. J., and A. E. Shamoo. 1979. Anionic detergents as divalent cation ionophores across black lipid membranes. *J. Membr. Biol.* **50:** 241–255.
38. Saitoh, O., K. Nakagawa, K. Sugi, R. Matsuse, K. Uchida, K. Kojima, S. Tanaka, T. Teranishi, I. Hirata, and K. Katsu. 1998. Bile acids inhibit tumour necrosis factor alpha-induced interleukin-8 production in human colon epithelial cells. *J. Gastroenterol. Hepatol.* **13:** 1212–1217.
39. Anderberg, E. K., C. Nystrom, and P. Artursson. 1992. Epithelial transport of drugs in cell culture. VII. Effects of pharmaceutical surfactant excipients and bile acids on transepithelial permeability in monolayers of human intestinal epithelial (Caco-2) cells. *J. Pharm. Sci.* **81:** 879–887.
40. Levin, M. S., V. D. Talkad, J. I. Gordon, and W. F. Stenson. 1992. Trafficking of exogenous fatty acids within Caco2 cells. *J. Lipid Res.* **33:** 9–19.
41. Field, F. J., E. Born, H. Chen, S. Murthy, and S. N. Mathur. 1994. Regulation of apolipoprotein B secretion by biliary lipids in CaCo-2 cells. *J. Lipid Res.* **35:** 749–762.
42. Saucier, S. E., A. A. Kandutsch, A. K. Gayen, D. K. Swahn, and T. A. Spencer. 1989. Oxysterol regulators of 3-hydroxy-3-methylglutaryl-CoA reductase in liver. Effect of dietary cholesterol. *J. Biol. Chem.* **264:** 6863–6869.
43. Rennert, H., R. T. Fischer, J. G. Alvarez, J. M. Trzaskos, and J. F. Strauss. 1990. Generation of regulatory oxysterols: 26-hydroxylation of cholesterol by ovarian mitochondria. *Endocrinology.* **127:** 738–746.
44. Johnson, K. A., C. J. Morrow, G. D. Knight, and T. J. Scallen. 1994. In vivo formation of 25-hydroxycholesterol from endogenous cholesterol after a single meal, dietary cholesterol challenge. *J. Lipid Res.* **35:** 2241–2253.
45. Kandutsch, A. A., and H. W. Chen. 1973. Inhibition of sterol synthesis in cultured mouse cells by 7alpha-hydroxycholesterol, 7beta-hydroxycholesterol, and 7-ketocholesterol. *J. Biol. Chem.* **248:** 8408–8417.
46. Schroepfer, G. J. 2000. Oxysterols: modulators of cholesterol metabolism and other processes. *Physiol. Rev.* **80:** 361–554.
47. Breslow, J. L., D. A. Lothrop, D. R. Spaulding, and A. A. Kandutsch. 1975. Cholesterol, 7-ketocholesterol and 25-hydroxycholesterol uptake studies and effect on 3-hydroxy-3-methylglutaryl-coenzyme A reductase activity in human fibroblasts. *Biochim. Biophys. Acta.* **398:** 10–17.
48. Cavenee, W. K., G. F. Gibbons, H. W. Chen, and A. A. Kandutsch. 1979. Effects of various oxygenated sterols on cellular sterol biosynthesis in Chinese hamster lung cells resistant to 25-hydroxycholesterol. *Biochim. Biophys. Acta.* **575:** 255–265.
49. Yang, J., R. Sato, J. L. Goldstein, and M. S. Brown. 1994. Sterol-resistant transcription in CHO cells caused by gene rearrangement that truncates SREBP-2. *Genes Dev.* **8:** 1910–1919.
50. Luskey, K. L., J. R. Faust, D. J. Chin, M. S. Brown, and J. L. Goldstein. 1983. Amplification of the gene for 3-hydroxy-3-methylglutaryl coenzyme A reductase, but not for the 53-kDa protein, in UT-1 cells. *J. Biol. Chem.* **258:** 8462–8469.
51. Osborne, T. F., G. Gil, M. S. Brown, R. C. Kowal, and J. L. Goldstein. 1987. Identification of promoter elements required for in vitro transcription of hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase gene. *Proc. Natl. Acad. Sci. USA.* **84:** 3614–3618.
52. Sato, R., J. Inoue, Y. Kawabe, T. Kodama, T. Takano, and M. Maeda. 1996. Sterol-dependent transcriptional regulation of sterol regulatory element-binding protein-2. *Cell.* **87:** 377–389.
53. Miserez, A. R., G. Cao, L. C. Probst, and H. H. Hobbs. 1997. Structure of the human gene encoding sterol regulatory element binding protein 2 (SREBF2). *Genomics.* **40:** 31–40.
54. Sanchez, H. B., L. Yieh, and T. F. Osborne. 1995. Cooperation by sterol regulatory element-binding protein and Sp1 in sterol regulation of low density lipoprotein receptor gene. *J. Biol. Chem.* **270:** 1161–1169.
55. Dooley, K. A., S. Millinder, and T. F. Osborne. 1998. Sterol regulation of 3-hydroxy-3-methylglutaryl-coenzyme A synthase gene through a direct interaction between sterol regulatory element binding protein and the trimeric CCAAT-binding factor/nuclear factor Y. *Nature.* **391:** 339–340.
56. Ericsson, J., S. M. Jackson, B. C. Lee, and P. A. Edwards. 1996. Sterol regulatory element binding protein binds to a cis element in the promoter of the farnesyl diphosphate synthase gene. *Biochem. Mol. Biol. Int.* **38:** 31–36.
57. Ericsson, J., S. M. Jackson, and P. A. Edwards. 1996. Synergistic binding of sterol regulatory element-binding protein and NF-Y to the farnesyl diphosphate synthase promoter is critical for sterol-regulated expression of the gene. *J. Biol. Chem.* **271:** 24359–24364.
58. Guan, G., G. Jiang, R. L. Koch, and I. Shechter. 1995. Molecular cloning and functional analysis of the promoter of the human squalene synthase gene. *J. Biol. Chem.* **270:** 21958–21965.
59. Ericsson, J., A. Usheva, and P. A. Edwards. 1999. YY1 is a negative regulator of transcription of three sterol regulatory element-binding protein-responsive genes. *J. Biol. Chem.* **274:** 14508–14513.

60. Roth, G., J. Kotzka, L. Kremer, S. Lehr, C. Lohaus, H. E. Meyer, W. Krone, and D. Muller-Wieland. 2000. MAP kinases Erk1/2 phosphorylate sterol regulatory element-binding protein (SREBP)-1a at serine 117 in vitro. *J. Biol. Chem.* **275:** 33302–33307.
61. Kotzka, J., D. Muller-Wieland, G. Roth, L. Kremer, M. Munck, S. Schurmann, B. Knebel, and W. Krone. 2000. Sterol regulatory element binding proteins (SREBP)-1a and SREBP-2 are linked to the MAP-kinase cascade. *J. Lipid Res.* **41:** 99–108.
62. Field, F. J., E. Born, S. Murthy, and S. N. Mathur. 2001. Gene expression of sterol regulatory element-binding proteins in hamster small intestine. *J. Lipid Res.* **42:** 1–8.
63. Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J. M. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein, and D. J. Mangelsdorf. 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev.* **14:** 2819–2830.