Gene expression of sterol regulatory element-binding proteins in hamster small intestine

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Abstract **Gene expression of sterol regulatory element-binding proteins 1a, 1c, and 2 (SREBP-1a, -1c, and -2) and of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase, HMG-CoA reductase, and the low density lipoprotein (LDL) receptor was examined in hamster small intestine. SREBP-1c transcript predominated over SREBP-1a. mRNA levels for SREBP-1a, -1c, and -2, LDL receptor, and HMG-CoA synthase were highest in jejunum and ileum. Expression of SREBP-1a and SREBP-1c was highest in cells of the upper villus and decreased in cells of the lower villus. Gene expression of SREBP-2 was lowest in cells of the upper villus and increased in cells of the lower villus. Ileal SREBP-2 gene expression was highest in cells of the midvillus. mRNA levels for HMG-CoA synthase and the LDL receptor followed a pattern similar to that of SREBP-2. A positive correlation existed between SREBP-2 gene expression and rates of cholesterol synthesis. Fatty acid synthesis was highest in jejunum and ileum, correlating positively with the expression of SREBP-1c. Sterol influx into intestinal cells decreased mRNA levels of SREBP-2, HMG-CoA reductase, HMG-CoA synthase, and LDL receptor. In ileum, sterol influx decreased gene expression of SREBP-1a and increased expression of SREBP-1c. The results suggest that SREBP-2 regulates cholesterol synthesis in the small intestine. SREBP-1a is a minor transcript and its expression does not correlate with cholesterol-synthesizing activity. SREBP-1c is a major transcript in small intestine and its expression along the length of the gut correlates with fatty acid synthesis. Sterols regulate gene expression of sterol-responsive genes, including SREBP-2, in small intestine.**—Field, F. J., E. Born, S. Murthy, and S. N. Mathur. **Gene expression of sterol regulatory element-binding proteins in hamster small intestine.** ***J. Lipid Res.*** **2001.** 42: **1–8.**

Supplementary key words cholesterol synthesis • fatty acid synthesis • HMG-CoA reductase • HMG-CoA synthase • LDL receptor

The small intestine is important in maintaining total body cholesterol homeostasis. It is second only to the liver in the rate of de novo cholesterol synthesis, and in some animal models the intestine actually contributes more cholesterol to total body stores than does liver (1, 2). Newly synthesized cholesterol from the small intestine contributes to the plasma pool of cholesterol, suggesting that changes in intestinal cholesterol synthesis will directly alter plasma cholesterol levels (3). Moreover, by absorbing cholesterol and secreting triacylglycerol-rich lipoproteins, the small intestine is responsible for regulating the amount of dietary and biliary cholesterol entering the body. In addition to cholesterol synthesis, cholesterol absorption, and lipoprotein synthesis, the small intestine also takes up and degrades circulating low density lipoprotein (LDL) particles (4).

Because of the complexity of the organ, understanding the regulation of cholesterol metabolism in the small intestine is not a simple matter. For example, cholesterol and fat-soluble vitamins are absorbed in the upper part of the small intestine; whereas structurally related molecules, such as bile acids, are absorbed in the distal ileum (5, 6). Fatty acid influx and lipoprotein assembly and secretion, which likely affect cholesterol metabolism, predominantly occur in the midgut. Functional differences also exist along the villus axis. Most nutrients, including cholesterol, are absorbed in the mid- to upper villus. In the lower regions of the villus and within the crypts, cells are rapidly dividing to replenish cells being lost in the upper regions of the villus. Cells of the crypts are not exposed to lumenal contents and thus, do not actively absorb cholesterol. These cells, however, do require sufficient cholesterol to meet their increased demands for new membrane synthesis. Thus, when investigating the regulation of cholesterol metabolism in the small intestine, the regional and cellular differences that exist within this organ must be considered.

Our understanding of how a cell senses and regulates the amount of cholesterol has been advanced by the description of two transcription factors designated sterol regulatory element-binding proteins 1 and 2 (SREBP-1 and -2) (7). These proteins, derived from separate genes, regulate the transcription of genes that encode enzymes in the biosynthetic pathways of cholesterol and fatty acids (8–12). There are two isoforms of SREBP-1, SREBP-1a and SREBP-

Abbreviations: EDTA, ethylenediaminetetraacetic acid; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low density lipoproteins; SREBP, sterol regulatory element-binding proteins.

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1c (13). SREBP-1a is the more potent activator of genes of both biosynthetic pathways, whereas SREBP-1c is a weaker transcription factor and tends to be more active in enhancing transcription of genes in the fatty acid biosynthetic pathway (14). In contrast, SREBP-2 preferentially enhances several genes of the cholesterol biosynthetic pathway (15–17). SREBPs are bound to membranes of the endoplasmic reticulum and nuclear envelope (precursor or membrane form). The NH_2- and COOH-terminal domains of the proteins project into the cytosol, with a hydrophilic loop residing in the lumen (18, 19). When a cell is depleted of cholesterol, a two-step proteolytic process releases the NH_2-terminal domain (mature or nuclear form), which then enters the nucleus (20). By binding to a 10-base pair sterol regulatory element in the promoter of target genes, transcription is enhanced. With an influx of cholesterol leading to cholesterol excess, the proteolytic processing of SREBPs within cells is prevented and transcription of these genes is decreased (21).

There is currently no information on SREBPs in the small intestine. Characterization of these transcription factors and defining their localization within the small intestine will be necessary before studies can be performed that examine their role in regulating cholesterol and fatty acid metabolism in this organ. The results of this study show that gene expression for SREBPs differ along the length of the intestine as well as along the vertical axis of the villus. The SREBP-1c transcript is the dominant form of SREBP-1, with ratios of SREBP-1c to SREBP-1a averaging 3–4. SREBP-2 is likely responsible for regulating cholesterol synthesis in the intestine as there is a correlation between gene expression of SREBP-2 and cholesterol-synthesizing activity. Moreover, changes in sterol flux across the intestinal cell regulate gene expression of sterol-responsive genes, including SREBP-2.

MATERIALS AND METHODS

[^{14}C]acetate and [^{32}P]UTP were purchased from New England Nuclear (Boston, MA). [*Methyl*-^{3}H]thymidine was obtained from Amersham (Arlington Heights, IL). Tri Reagent, cholesterol, methyl-β-cyclodextrin, *p*-nitrophenol phosphate, ATP, phosphocreatine, dithiothreitol, thymidine, and creatine phosphokinase were purchased from Sigma (St. Louis, MO). 25-Hydroxycholesterol was from Steroloids (Wilton, NH). MAXIscript T7 and RPA III kits were supplied by Ambion (Austin, TX). Medium M199 was from GIBCO-BRL Life Technologies (Grand Island, NY). Lovastatin was obtained from Merck Sharp and Dohme Research Laboratories (Rahway, NJ).

Animals

Male golden Syrian hamsters, 90–120 g, were purchased from Harlan Sprague Dawley (Indianapolis, IN) and maintained on NIH-31 modified mouse/rat diet 7013 (Harlan/Teklad, Madison, WI). The animals were fed ad libitum and the light cycle was from 6:00 AM to 6:00 PM.

Isolation of enterocytes

Hamsters were killed between 8:00 and 9:00 AM by inhalation of CO_2. The small intestine was removed in its entirety and placed in a beaker containing cold 0.9% saline. Isolated intestinal cells were prepared by a procedure as described by Cartwright and Higgins (22). The lumen of the intestine was flushed with solution A [117 mM NaCl, 5.4 mM KCl, 0.96 mM NaH_2PO_4, 26 mM $NaHCO_3$, 5 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), 5.5 mM glucose] to remove any fecal material and debris. The intestine was then filled with solution B (67.5 mM NaCl, 1.5 mM KCl, 0.96 mM NaH_2PO_4, 26 mM $NaHCO_3$, 5 mM HEPES, 5.5 mM glucose, 27 mM sodium citrate) and placed in a beaker containing 0.9% saline warmed to 37°C. The beaker was gently agitated for 15 min. Solution B was removed and the intestine was divided into three equal segments. Each segment was filled with solution C [115 mM NaCl, 5.4 mM KCl, 0.96 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1.5 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM dithiothreitol, 5 mM HEPES, 5.5 mM glucose] and placed in a beaker containing 0.9% saline at 37°C. To isolate cells along the villus gradient, the beakers were gently agitated in a shaking water bath. Solution C was emptied into plastic conical centrifuge tubes every 5 min and replaced. The tubes containing the cells were kept on ice until 12 fractions were collected. Two sequential fractions were then pooled to obtain a total of six fractions (23). Cells were collected by centrifugation at 1,000 *g* for 10 min at 4°C.

Assay of thymidine kinase activity

The activity of thymidine kinase was determined as follows (22, 24). Cells were suspended in 10 mM Tris-HCl, 150 mM NaCl, pH 7.4 and homogenized by passage through a 25-gauge needle 20 times. Aliquots of 100–200 μg of protein were incubated for 2 h at 37°C in 0.2 ml of assay buffer containing 90 mM Tris-HCl (pH 7.5), 20 mM ATP, 20 mM phosphocreatine, 10 mM $MgCl_2$, 10 mM NaF, 2 mM dithiothreitol, 1 mM thymidine (containing 4 μCi of [^{3}H]thymidine), and 20 μg of creatine phosphokinase. At the end of the incubation, 1.25 ml of 100 mM lanthanum chloride-5 mM triethanolamine solution was added followed by incubation at 4°C for 30 min. Samples were then centrifuged at 15,000 *g* for 10 min at 4°C. The pellets were washed once with 1.5 ml of 100 mM lanthanum chloride-5 mM triethanolamine and resuspended in 0.3 ml of 0.1 M HCl and mixed with 5 ml of scintillation fluid. The incorporation of [^{3}H]thymidine into thymidine phosphate was determined by liquid scintillation counting.

Assay of alkaline phosphatase activity

The activity of alkaline phosphatase was determined with 8.44 mM *p*-nitrophenyl phosphate as substrate in 0.2 M glycine buffer, pH 9.8, containing 0.5 mM $MgCl_2$ and 1.6 μM zinc chloride. Aliquots containing 25–50 μg of protein in 50 μl of Tris buffer, pH 7.4, were incubated with 200 μl of the substrate solution at 37°C for 30 min. The incubation mixture was centrifuged at 13,000 *g* for 5 min to remove turbidity. The absorbance of the supernatant was measured at 405 nm to determine the amount of *p*-nitrophenol released by the enzyme.

Cholesterol and fatty acid synthesis

The isolated cells from duodenum, jejunum, or ileum (0.8–4 mg total protein) were incubated for 4 h in 1.5 ml of M199 medium containing 0.6 mM unlabeled acetate and 10 μCi of [^{14}C]acetate at 37°C in 5% CO_2. At the end of the incubation, the cells were isolated by centrifugation at 1,000 *g* for 10 min at 4°C. The cells were suspended in 1 ml of 90% methanol containing 0.5 M NaOH and kept in boiling water for 1 h to saponify the lipids. After adding an equal volume of acidic water, the lipids were extracted twice at pH 3.0 with 2 ml of hexanes. Hexanes were then evaporated in a stream of nitrogen and lipids were dissolved in 0.100 ml of hexanes. The 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. The 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 Packard (Downers Grove, IL) Tricarb 2100-TR liquid scintillation counter.

RNase protection assays

RNA was extracted from isolated cells with Tri Reagent (Sigma). The RNA probes to estimate mRNA for hamster SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA reductase, HMG-CoA synthase, and LDL receptor were prepared as described by Shimomura et al. (16). The RNA probe for SREBP-1c, however, contained 0.207 kb instead of 0.114 kb as described. pTRI RNA 18S probe was obtained from Ambion. The probes were labeled with[^{32}P]UTP, using a MAXIscript T7 kit from Ambion. To calculate the ratio of hamster SREBP-1a to SREBP-1c transcripts, the results were corrected for the difference in number of [^{32}P]UTP incorporated into each protected fragment (42 and 32, respectively). Twenty to 40 μg of total RNA was used per assay. The assay was performed per a protocol for RPA III described by Ambion. The protected fragments were resolved on a 6% polyacrylamide gel. The radioactivity in the bands was measured by autoradiography using a Storm phosphor screen and quantitated with ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

Regulation of gene expression by sterol flux

Hamster intestine was rinsed with solutions A and B as described above for isolation of enterocytes. The duodenal, jejunal, and ileal segments were slit longitudinally to obtain two equal strips from each segment. One of the strips from each segment was incubated in 8 ml of M199 containing 10% delipidated fetal calf serum, 25 μM cholesterol, and 50 μM 25-hydroxycholesterol. The sterols were added in a final concentration of 0.1% ethanol and the same amount of ethanol was added to the strips without sterols.

The other corresponding intestinal strip was incubated with 8 ml of M199 containing 10% delipidated fetal calf serum, 25 μM lovastatin, and methyl-β-cyclodextrin (1 mg/ml). The intestinal strips were then incubated for 8 h at 37°C in 5% CO_2/air mixture. At the end of the incubation, the intestinal strips were transferred to a round-bottom glass tube. The cells from each strip were isolated by adding 2 ml of ice-cold solution C (115 mM NaCl, 5.4 mM KCl, 0.96 mM NaH_2PO_4, 26 mM $NaHCO_3$, 1.5 mM EDTA, 0.5 mM dithiothreitol, 5 mM HEPES, 5.5 mM glucose) to the tube and vortexing vigorously for 15 sec. The medium containing the released cells was transferred to a tube and kept on ice. This procedure was repeated five or six times for each intestinal strip. The cells were collected by centrifugation at 1,000 *g* for 10 min at 4°C. Total RNA was extracted from the cells as described above.

RESULTS

SREBP gene expression

To investigate gene expression of SREBPs down the length of the intestine and along the vertical axis of the villus, the small intestine of hamsters was divided into three equal segments representing duodenum, jejunum, and ileum. From each of the three segments, intestinal cells were sequentially isolated into six fractions (villus tips to crypts). Total RNA was extracted from the six fractions and mRNA levels for SREBP-1a, -1c, -2, HMG-CoA synthase, and LDL receptor were estimated by RNase protection assays. The amount of 18S RNA was also estimated to ensure that similar amounts of RNA were analyzed for each fraction. Alkaline phosphatase and thymidine kinase activities were estimated as marker enzymes to ensure the existence of a gradient from the villus tips to crypts, respectively. The results are shown in **Fig. 1**. In all three segments, alkaline phosphatase activity was highest in fractions 1–3 and progressively declined to its lowest level in fraction 6 (Fig. 1A). Alkaline phosphatase activity was highest in duodenum and lowest in ileum. In contrast, thymidine kinase activity was highest in cells collected in fractions 5 and 6, with lower levels found in fractions 1–4. Thymidine kinase activities were similar in all three segments, duodenum, jejunum, and ileum. Thus, in the six sequential fractions of cells collected, there exists a gradient among cells isolated from the upper villus (fractions 1–3) versus those isolated from the lower villus regions (fractions 5 and 6).

Figure 1B shows a representative RNase protection assay from a single animal. Quantitation of the protected bands is shown in the line graph representing an average of three animals. It is clear from these results that in hamster intestine, the SREBP-1c transcript predominates over the SREBP-1a transcript in duodenum, jejunum, and ileum. On average, the ratio of SREBP-1c to SREBP-1a gene expression in duodenum, jejunum, and ileum approximated 3.0, 4.7, and 3.4, respectively (Fig. 1B and C). The results also show that, along the length of the intestine, gene expression for the SREBPs, HMG-CoA synthase, and the LDL receptor differed. Compared with the expression of these genes within the duodenum, mRNA levels for SREBP-1a, -1c, -2, and HMG-CoA synthase were higher in jejunum and ileum. Compared with the duodenum and jejunum, the expression of transcripts for SREBP-2, HMG-CoA synthase, and the LDL receptor was highest in the ileum. In contrast, for the other predominant transcript in intestine, SREBP-1c, gene expression appeared to be somewhat higher overall in the jejunum compared with that observed in either duodenum or ileum. SREBP-1a expression followed a pattern similar to that of SREBP-1c. mRNA levels for HMG-CoA reductase were also highest in ileum, followed by jejunum and duodenum (see Fig. 3, protected bands in intestinal strips incubated in the absence of sterols).

Gene expression for these proteins also differed along the vertical axis of the villus. In the more proximal intestine, duodenum and jejunum, the expression for both SREBP-1a and -1c transcripts was highest in cells of the upper villus (Fig. 1B). Expression then decreased as cells of the crypts were reached. In the ileum, this trend remained but the differences were not as marked as they were in the proximal intestine. Figure 1C also shows the ratio of SREBP-1c to SREBP-1a transcripts along the villus axis. In all three segments, the ratio is highest in cells of the upper villus and decreases as cells of the lower villus are reached. In distinct contrast to SREBP-1 gene expression, the expression for SREBP-2 was lowest in cells of the villus tips and tended to increase as cells of the lower villus were

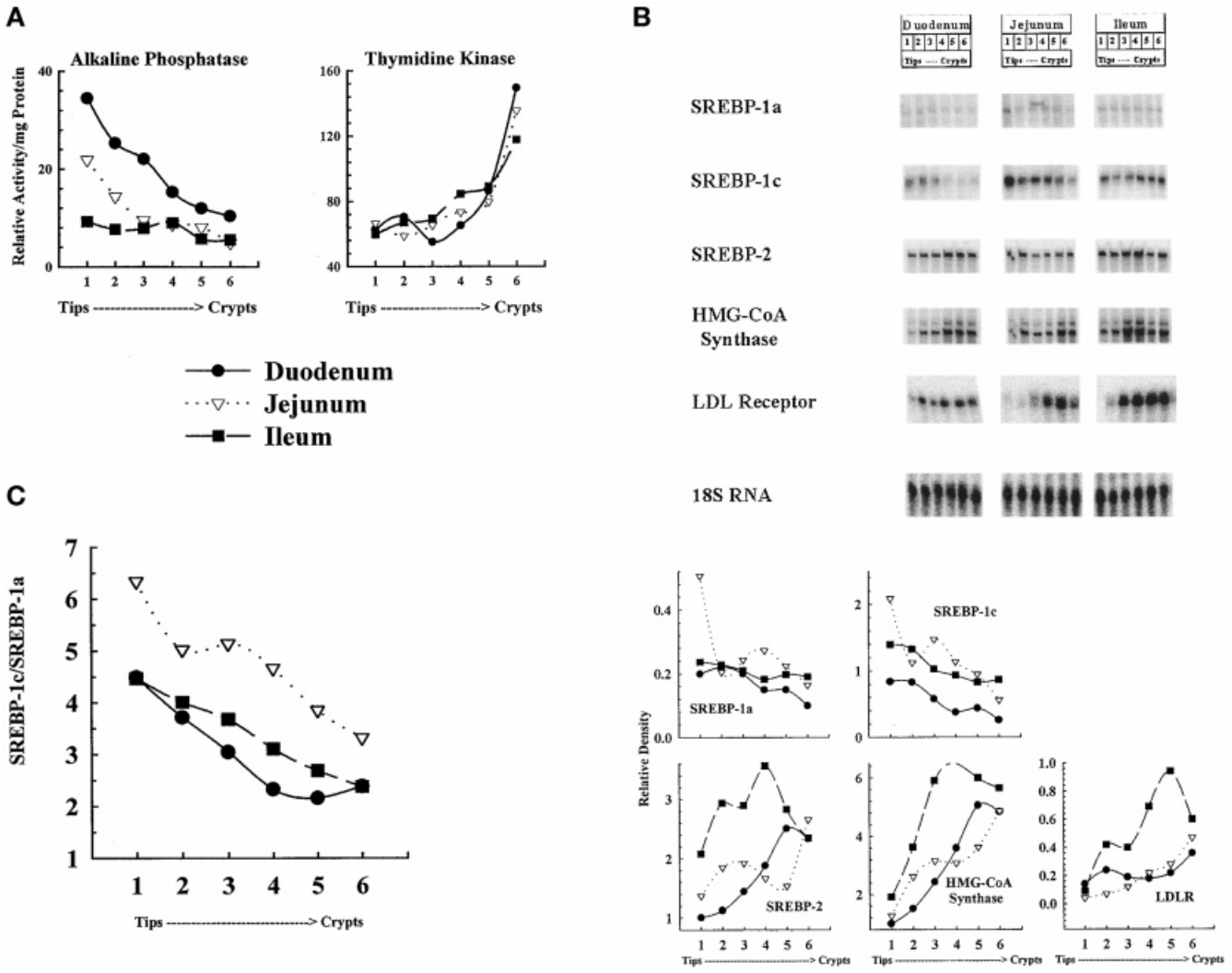

Fig. 1. Gene expression for SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA synthase, and LDL receptor (LDLR) in hamster intestine. Isolated intestinal cells were prepared from duodenum (solid circles), jejunum (open inverted triangles), and ileum (solid squares) as described in Materials and Methods. (A) Alkaline phosphatase and thymidine kinase activities were estimated in cells prepared from villus tips to crypts. (B) RNase protection assays were performed to estimate mRNA levels for SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA synthase, and LDL receptor. Each autoradiogram shows a representative RNase protection assay from a single animal. The line graphs show the average of three individual animals. (C) The ratio of SREBP-1c to SREBP-1a is calculated from the results presented in (B).

reached (Fig. 1B). This trend was clearest in the duodenum. In the ileum, the segment with the greatest amount of SREBP-2 expression, cells of the midvillus, appeared to have more of the transcript than cells of either the tips or the crypts. mRNA levels for HMG-CoA synthase and the LDL receptor followed a pattern similar to that of SREBP-2. HMG-CoA synthase and LDL receptor expression were lowest in cells of the villus tips and progressively increased as cells of the crypts were reached (Fig. 1B).

Cholesterol and fatty acid synthesis

Because SREBPs regulate the transcription of genes that encode enzymes in both the cholesterol and fatty acid synthetic pathways, it was next examined whether the differences observed in the expression of SREBPs in the intestine correlated with the total rate of synthesis of these two lipids. Again, hamster intestine was divided into three equal segments and cells were isolated into six sequential fractions representing cells of the villus tip to crypt. The incorporation of acetate into total fatty acids and cholesterol was then estimated in each of the cell fractions from duodenum, jejunum, and ileum. **Figure 2** shows the results of this experiment. Values depicted in parentheses represent total synthesizing activity for that segment. Compared with cholesterol-synthesizing activity in the duodenum and jejunum (which were similar in cholesterol-synthesizing activity), significantly more cholesterol was synthesized in the ileum. Moreover, along the length of the villus, cholesterol-synthesizing activity was lowest in villus tip cells and increased as cells of the midvillus were reached. Synthesis then modestly decreased in cells isolated from the lower villus. This pattern of cholesterol synthesis along the villus axis was similar in all three segments.

Fatty acid-synthesizing activity was significantly higher

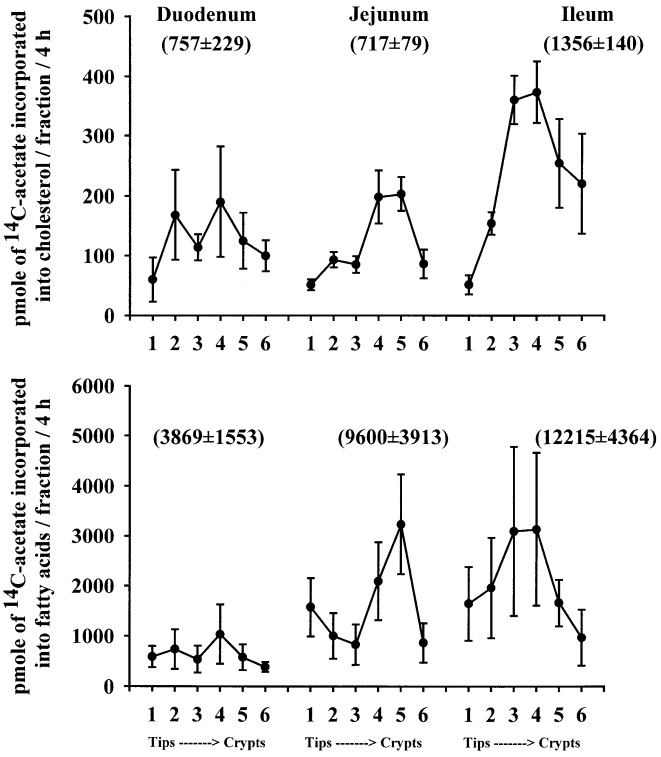

Fig. 2. Cholesterol and fatty acid synthetic activity in hamster intestine. Isolated intestinal cells were prepared from duodenum, jejunum, and ileum as described in Materials and Methods. Isolated cells were incubated with [^{14}C]acetate for 4 h. Lipids were extracted and the incorporation of [^{14}C]acetate into total fatty acids and cholesterol was estimated as described in Materials and Methods. The data represent means ± SE of four animals. The data were analyzed by one-way analysis of variance with the Student-Newman-Keuls test. Rates of total cholesterol synthesis in ileum were significantly different from rates of total cholesterol synthesis in duodenum and jejunum at $P < 0.05$. Rates of cholesterol synthesis in jejunal fraction 1 versus 4 and 5 and ileal fraction 1 versus 3 and 4 were significantly different at $P < 0.05$. Rates of total fatty acid synthesis in jejunum and ileum were significantly different from rates of fatty acid synthesis in duodenum at $P < 0.05$.

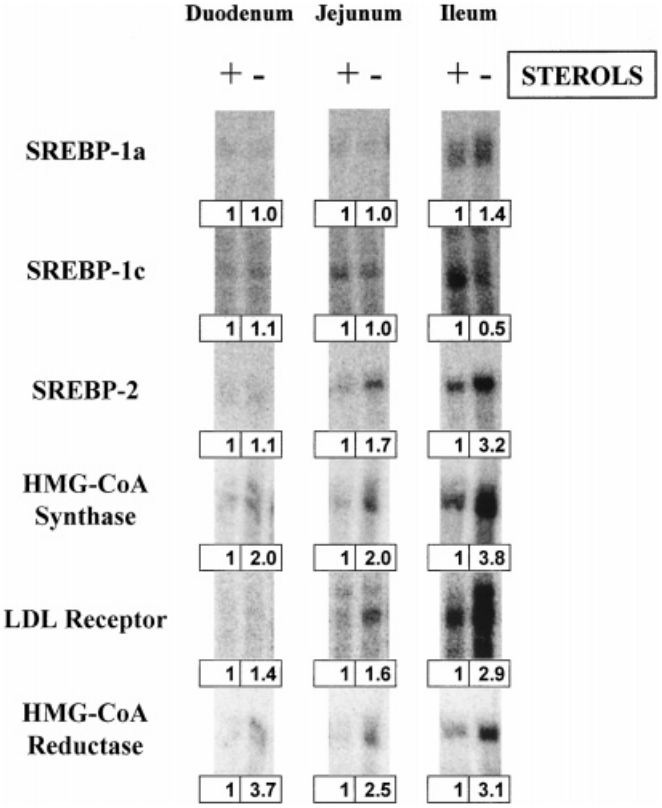

Fig. 3. Regulation of gene expression of SREBP-1a, SREBP-1c, SREBP-2, HMG-CoA synthase, LDL receptor, and HMG-CoA reductase by sterol flux. Equal segments from duodenum, jejunum, and ileum were incubated in medium containing 10% delipidated fetal calf serum containing 25 μM cholesterol and 50 μM 25-hydroxycholesterol or 10% delipidated fetal calf serum containing 25 μM lovastatin and methyl-β-cyclodextrin (1 mg/ml). After 8 h of incubation, isolated cells were prepared from the segments and RNA was extracted. RNase protection assays were performed to estimate mRNA levels. The data are from one experiment representative of two, both showing similar results.

in jejunum and ileum compared with that observed in duodenum. In contrast to cholesterol-synthesizing activity, however, there was no clear discernible gradient in fatty acid-synthesizing activity along the villus axis. In the jejunum and ileum, there was a tendency for the rates of fatty acid synthesis to be higher in cells of the midvillus region compared with tips.

Regulation of SREBP gene expression by sterol flux

To address the regulation of intestinal SREBP gene expression by changes in cholesterol flux, hamster intestine was divided into three equal segments. The segments were opened and cut longitudinally into equal strips. One strip from each segment was incubated in medium containing 10% delipidated fetal calf serum and sterols, whereas the other half was incubated in medium containing delipidated fetal calf serum and lovastatin and cyclodextrin. After an 8-h incubation, isolated intestinal cells were prepared from each strip and total RNA was extracted. mRNA levels for SREBP-1a, -1c, -2, HMG-CoA synthase, the LDL receptor, and HMG-CoA reductase were estimated by RNase protection assays. The results are shown in **Fig. 3**. In cells isolated from duodenum and jejunum, changes in sterol flux did not alter gene expression of SREBP-1a or SREBP-1c. In the ileum, however, sterol influx decreased expression of SREBP-1a and increased expression of SREBP-1c. In contrast, sterol influx decreased gene expression of HMG-CoA synthase, the LDL receptor, and HMG-CoA reductase in cells isolated from all three segments and expression of SREBP-2 in jejunum and ileum.

DISCUSSION

This is the first study that characterizes gene expression of SREBPs in small intestine. The results clearly demonstrate that SREBP mRNA levels differ along the length of the gut as well as along the vertical axis of the villus. In previous studies performed in rat, the ileum was found to

have the highest rates of cholesterol synthesis compared with either the duodenum or jejunum (25, 26). In other studies, using immunohistochemical techniques, staining of HMG-CoA reductase protein was maximal in ileum, followed by jejunum and duodenum (27). Thus, there was earlier evidence to suggest that in rat, at least, the ileum contributes more to the synthesis of cholesterol than either duodenum or jejunum. In the present study, we also observed that hamster ileum contained the highest cholesterol-synthesizing activity. This corresponded to higher expression in the ileum of transcripts for HMG-CoA synthase, HMG-CoA reductase, LDL receptor, and SREBP-2 compared with expressions found in duodenum or jejunum. From earlier results, it is believed that SREBP-2 is more potent than SREBP-1 in regulating genes that encode enzymes of the cholesterol biosynthetic pathway (17). It is not surprising, therefore, that mRNA levels for SREBP-2 were highest in the ileum. That LDL receptor mRNA abundance was also highest in hamster ileum agrees with previous results obtained in rats (28). An increase in ileal gene expression of proteins that regulate cholesterol synthesis and lipoprotein cholesterol uptake suggests that compared with proximal intestine, cholesterol flux in the ileum is less. This makes good sense as proximal gut is responsible for cholesterol absorption and little lumenal cholesterol reaches the distal ileum (5).

SREBP-1a is also believed to regulate the transcription of genes in the cholesterol biosynthetic pathway (29). In studies performed in cultured cells, the SREBP-1a transcript predominates over the SREBP-1c transcript (30). In human and mouse organs, however, the reverse is true. In most of the organs where this has been analyzed, the SREBP-1c transcript predominates over the SREBP-1a transcript. One of the exceptions, however, was observed in mouse intestine. In both jejunum and ileum, the ratio of SREBP-1c to SREBP-1a transcripts was 0.5 (30). Our results do not agree with the data generated in mice. In hamster intestine, the SREBP-1a transcript is a minor transcript compared with SREBP-1c, with an approximate ratio of SREBP-1c to SREBP-1a of 3–4. The reasons for the observed discrepancy between the two studies are not certain. One obvious explanation is the difference in animal models. When studying cholesterol metabolism in the intestine, differences among species are the norm rather than the exception (31). Another possible explanation may be technical. In the previous study performed in mouse, RNA was extracted from the entire segment of intestine and not from isolated intestinal cells. Thus, RNA from nonintestinal cells was used for analysis. Because SREBP-1a is such a minor transcript in hamster intestine, our results strongly suggest that compared with SREBP-1a, SREBP-2 is playing a more important role in the regulation of cholesterol synthesis in intestine.

It has been postulated that SREBP-1c regulates genes encoding enzymes in the fatty acid biosynthetic pathway more so than enzymes in the cholesterol synthetic pathway (14). In hamster intestine, most of the fatty acid synthetic activity resided in the jejunum and ileum; an observation that agrees with an early study performed in rats (32). These two regions of the gut are also the areas in which SREBP-1c gene expression was the highest. Thus, along the length of the small intestine, gene expression of SREBP-1c correlates positively with fatty acid-synthesizing activity. Because most lipoprotein assembly and secretion occurs within the midgut, jejunum, and proximal ileum, it could be argued that SREBP-1c is playing a role in regulating fatty acid synthesis in those segments of the small intestine responsible for the transport of lipids.

There remains controversy regarding cholesterol-synthesizing activity in intestinal cells that line the villus. In studies performed in rats, whereby cells were isolated from the villus using physical techniques (similar to what was done in the present study), most cholesterol-synthesizing activity was found in cells of the lower villus or crypts (25, 26, 33). Other studies have refuted these findings, however, and have demonstrated more cholesterol synthesis in cells of the upper or apical portions of the villus (34, 35). In studies of intact intestine using antibodies against HMG-CoA reductase, it was observed that most of the reductase protein existed in cells of the middle and upper regions of the villus (27, 36). In the present study, the highest amount of cholesterol-synthesizing activity was found in cells of the midvillus. Compared with cholesterol-synthesizing activity in villus tip cells, cells of the crypts synthesized more cholesterol in all three segments of the small intestine. We also observed a correlation between cholesterol-synthesizing activity along the villus axis and gene expression for HMG-CoA synthase and SREBP-2. In contrast, no correlation existed between cholesterol-synthesizing activity and the expression of transcripts for either SREBP-1a or -1c along the villus axis. In fact, SREBP-1 expression was highest in cells of the upper villus and least in cells of the crypts. The results would again support the notion that SREBP-2, and not SREBP-1, is the transcription factor responsible for regulating cholesterol synthesis in the intestine.

In cells along the villus axis, gene expression for SREBP-1c was highest in cells of the upper villus. Expression then decreased sequentially in cells of the lower villus and crypts. This makes good sense as cells in the upper villus primarily function to transport nutrients and fatty acid synthesis is important for the normal assembly and secretion of lipoprotein particles. Because there was a positive correlation between SREBP-1c gene expression and fatty acid synthesis in jejunum and ileum, we expected a correlation between gene expression for SREBP-1c and fatty acid synthesis along the villus axis. This turned out not to be the case, however. When fatty acid-synthesizing activity was estimated in cells along the villus axis, the highest activity was not in cells of the upper villus, as would have been predicted from expression of SREBP-1c. Instead, fatty acid synthesis was highest in cells of the midvillus region. Thus, there was not a correlation between SREBP-1c gene expression and fatty acid-synthesizing activity in cells along the villus axis. This would suggest that additional factors other than SREBP-1c gene expression are playing a role in regulating fatty acid synthesis in the different cell populations that line the villus.

During times of increased sterol flux, cells "sense" sterol

excess and decrease sterol synthesis by inhibiting the activities of several key enzymes in the cholesterol synthetic pathway (37). There has been controversy concerning whether this feedback regulation of cholesterol synthesis by cholesterol exists in intestine. In rats, for example, dietary cholesterol does not significantly alter rates of intestinal cholesterol synthesis (1, 25). Only when rats are fed cholesterol together with a bile acid (conditions that increase cholesterol mass within intestinal cells) does intestinal cholesterol synthesis decrease (38). In hamsters, however, there is evidence to suggest that the ingestion of cholesterol alone does cause a decrease in intestinal cholesterol synthesis (39, 40). The present results clearly show that the influx of sterols into intestinal cells causes a decrease in gene expression of HMG-CoA synthase, HMG-CoA reductase, and the LDL receptor, genes that contain sterol response elements in their promoters. Moreover, mRNA levels of SREBP-2 are also decreased by sterol influx. Like the other sterol responsive genes, the promoter of the SREBP-2 gene contains a sterol response element (41, 42). Thus, sterol excess within an intestinal cell will decrease the transcription of SREBP-2, the predominant transcription factor that regulates intestinal cholesterol synthesis. In the ileum, sterol influx decreases gene expression of SREBP-1a while increasing the expression of SREBP-1c. In previous studies performed in cell culture, in which the predominant SREBP-1 transcript is SREBP-1a, changes in sterol flux alter the amount of the SREBP-1a transcript without affecting SREBP-1c (30). In contrast, in livers of mice that are fed cholesterol-depletion diets, SREBP-2 gene expression increases whereas SREBP-1c expression modestly decreases (16). The reverse occurs when cholesterol is fed, SREBP-2 gene expression decreases and SREBP-1c expression modestly increases (16). Thus, despite the lack of a sterol response element in the SREBP-1 gene (42), there are previous data to suggest that changes in sterol flux regulate SREBP-1 gene expression. For reasons that are not clear, in animal models, it appears that changes in sterol flux cause a reciprocal change in gene expressions of SREBP-2 and SREBP-1c.

The distribution in intestine of mRNA for SREBPs and genes that encode enzymes that regulate cholesterol synthesis makes sense if one considers where in the intestine cholesterol is absorbed. In animals fed a low cholesterol chow diet, essentially all lumenal cholesterol is derived from that contained in bile. Thus, enterocytes of the upper villus in duodenum (distal to the ampulla) and proximal jejunum are exposed continuously to lumenal cholesterol from bile. In contrast, undifferentiated cells of the crypts and enterocytes of the distal jejunum and ileum are not exposed to lumenal cholesterol. Thus, cholesterol flux through these cells will be minimal. Indeed, this has been demonstrated experimentally in hamsters, using a fluorescent cholesterol analog to trace cholesterol absorption (43). It would be expected, therefore, that in enterocytes continuously absorbing cholesterol, gene expression of SREBP-2 and other sterol-responsive genes would be chronically depressed. In turn, cholesterol-synthesizing activity would be less in these regions of the small intestine. Our data suggest that this is, indeed, the case. It is also unlikely that increasing cholesterol flux would cause a further decrease in gene expression and cholesterol synthesis in cells in which gene expression and synthesis are already at a nadir. This likely explains why it is difficult to demonstrate the regulation of cholesterol synthesis in the intestine by feeding cholesterol (1, 25, 38–40). In contrast, in enterocytes of the ileum and cells of the crypts, lack of lumenal cholesterol flux results in enhanced expression of SREBP-2 and sterol response genes, causing an increase in cholesterol synthesis in these cells. Moreover, in the ileum, our data would indicate (Fig. 3) that once expression of sterol-responsive genes is enhanced by lack of cholesterol flux, it is not difficult to demonstrate regulation of gene expression by sterol influx.

The regulation of cholesterol and fatty acid synthesis in cells by SREBPs is dependent on the proteolytic cleavage of the membrane-bound protein (precursor) to its nuclear form (mature). It is the nuclear form that enters the nucleus to bind to the sterol response element of the target gene to enhance transcription (7). We are currently investigating this proteolytic process in intestinal cells of hamsters ingesting diets that will alter cholesterol flux across the intestine.

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