Cholesterol feeding reduces nuclear forms of sterol regulatory element binding proteins in hamster liver

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ABSTRACT Cholesterol feeding reduces the mRNAs encoding multiple enzymes in the cholesterol biosynthetic pathway and the low density lipoprotein receptor in livers of hamsters. Here we show that cholesterol feeding also reduces the levels of the nuclear NH2-terminal domains of sterol regulatory element binding proteins (SREBPs), which activate transcription of sterol-regulated genes. We show that livers of hamsters, like those of mice and humans, predominantly produce SREBP-2 and the 1c isoform of SREBP-1. Both are produced as membrane-bound precursors that must be proteolyzed to release the transcriptionally active NH2-terminal domains. Diets containing 0.1% to 1.0% cholesterol decreased the amount of nuclear SREBP-1c without affecting the amount of the membrane precursor or its mRNA, suggesting that cholesterol inhibits the proteolytic processing of SREBP-1 in liver as it does in cultured cells. Cholesterol also appeared to reduce the proteolytic processing of SREBP-2. In addition, at high levels of dietary cholesterol the mRNA encoding SREBP-2 declined and the amount of the precursor also fell, suggesting that cholesterol accumulation also may inhibit transcription of the SREBP-2 gene. The high-cholesterol diets reduced the amount of low density lipoprotein receptor mRNA by 30% and produced a more profound 70–90% reduction in mRNAs encoding 3-hydroxy-3-methylglutaryl CoA synthase and reductase. Treatment with lovastatin and Colestipol, which increases hepatic demands for cholesterol, increased the amount of SREBP-2 mRNA as well as the precursor and nuclear forms of the protein. This treatment caused a reciprocal decline in SREBP-1c mRNA and protein. Considered together, these data suggest that SREBPs play important roles in controlling transcription of sterol-regulated genes in liver, as they do in cultured cells.

Since the pioneering work of Gould, carried out more than 40 years ago (1, 2), scientists have known that high-cholesterol diets suppress cholesterol synthesis in the livers of experimental animals. More recently, the converse also was shown to be true, i.e., manipulations that deplete the liver of cholesterol lead to an increase in cholesterol synthesis (see ref. 3 for review). Much of this control is attributable to coordinate changes in the levels of mRNAs encoding multiple enzymes in the cholesterol biosynthetic pathway, including 3-hydroxy-3-methylglutaryl CoA (HMG CoA) synthase, HMG CoA reductase, farnesyl diphosphate synthase, squalene synthase, and others (see ref. 4 for review). The mRNA for the low density lipoprotein (LDL) receptor also is reduced by cholesterol feeding and increased by cholesterol depletion, although the amplitude of these changes is not as profound as that of the cholesterol biosynthetic enzymes, and the changes do not necessarily occur in parallel (4, 5). The changes in hepatic LDL receptors contribute to the elevation in blood cholesterol levels induced by high-cholesterol diets and to the reduction that follows hepatic cholesterol depletion (6).

A potential mechanism for this regulation was disclosed recently through studies of nonhepatic cells in tissue culture. In these cells the transcription of genes encoding cholesterol biosynthetic enzymes and the LDL receptor is controlled by a family of transcription factors designated SREBPs (sterol regulatory element binding proteins) (see ref. 7 for review). The SREBPs are proteins of ∼1,150 amino acids that are bound to membranes of the endoplasmic reticulum. In sterol-depleted cells, proteases release the NH2-terminal domains of the SREBPs, which are transcription factors of the basic-helix-loop-helix-leucine zipper (bHLH-Zip) family. These soluble domains, designated the mature forms of the SREBPs, enter the nucleus where they activate transcription by binding to 10-bp sterol regulatory elements in the enhancer regions of target genes. When cultured cells are overloaded with sterols, the proteolytic process is inhibited, the SREBPs remain bound to endoplasmic reticulum membranes, and transcription of the target genes declines (7).

The three known members of the SREBP family are produced by two genes (7). The SREBP-1 gene gives rise to two transcripts designated SREBP-1a and SREBP-1c, which differ only in the first exon that encodes an acidic transcription activation domain. This domain is much longer in SREBP-1a than in SREBP-1c, and therefore SREBP-1a is a much stronger activator of transcription (8). The third member of the family, designated SREBP-2, has a low activation domain, and its action resembles that of SREBP-1a. Tissue culture cells produce predominantly SREBP-1a and SREBP-2 (9), and the proteolytic processing of the two proteins is regulated in parallel (7).

In liver, the pattern of SREBP expression and regulation differs from that observed in cultured cells. In livers of mice and humans, the SREBP-1c mRNA is at least 9-fold more abundant than the SREBP-1a mRNA (9). The abundance of the SREBP-2 transcript appears to be intermediate between these extremes (9). In hamster liver, depletion of cholesterol by treatment with a bile acid binding resin (Colestipol) and an HMG CoA reductase inhibitor (lovastatin) caused a paradoxical decline in the amount of total SREBP-1 protein and in the efficiency of its processing to the mature form (7, 10). At the same time, the total amount of SREBP-2 increased, and its processing to the mature form increased. If hamster liver produces SREBP-1c as its predominant isoform, then the net

Abbreviations: HMG CoA, 3-hydroxy-3-methylglutaryl CoA; LDL, low density lipoprotein; SREBP, sterol regulatory element binding protein.
effect of the lovastatin/Colestipol regimen would be the replacement of a weak activator, SREBP-1c, with a strong activator, SREBP-2.

The current studies were designed to determine whether hamster liver produces SREBP-1c as the predominant isofom and to assess the effects of cholesterol feeding on the pattern of expression and processing of the SREBP1s in livers of these animals. We chose to perform these studies in hamsters because of the extensive previous literature documenting an inhibition of cholesterol synthesis and LDL receptor activity elicited by cholesterol feeding in this species (11, 12). Our results indicate that hamster liver does produce SREBP-1c and that cholesterol feeding reduces the amount of nuclear SREBP-1c and SREBP-2. These changes appear to explain the reduction in mRNAs for the cholesterol biosynthetic enzymes in response to cholesterol feeding.

METHODS

Materials and Procedures. Standard molecular biology techniques were used (13). We obtained all restriction enzymes and modifying enzymes from New England Biolabs, [α-32P]CTP (3,000 Ci/mmol) from Amersham, and other chemicals from Sigma. Plasmid DNA was prepared with Plasmid Maxi kits (Qiagen, Chatsworth, CA). Total RNA was prepared by the guanidinium thiocyanate/phenol/chloroform method (14). The content of cholesterol in plasma and liver was measured as described (15, 16).

Animals. Male and female Golden Syrian hamsters (100–120 g), obtained from Sasco (Omaha, NE), were exposed to a 12-hr light/12-hr dark cycle and fed one of the following diets: standard Teklad 4% Mouse/Rat Diet 7001 (Harlan Teklad, Madison, WI), the same diet supplemented with powdered cholesterol (0.1–1%, wt/wt), or the same diet containing powdered lovastatin (0.05%) (Merck Sharp and Dohme) plus 4% Colestipol (Upjohn). Hamsters had free access to diet and water during the experimental period and were sacrificed without fasting at the midpoint of the dark cycle.

Immunoblot Analysis. Membranes (10 g pellet) and nuclear extracts from hamster livers were prepared immediately after exsanguination as described (10). Aliquots of membranes and nuclear extracts were subjected to 8% SDS/PAGE and transferred to Hybond C extra membranes (Amersham). The following primary antibodies were used: mouse mAb (IgG2A4) against amino acids 301–407 of human SREBP-1a (17) at 5 μg/ml, and a 1:4,000 dilution of a rabbit IgG fraction of antisera against amino acids 32–250 of hamster SREBP-2 (this paper). Immunoblot analysis was carried out with the ECL system. Blots were exposed to film for 60 sec (lanes 1–5) or 120 sec (lanes 6–10). P, precursor form of SREBP; N, nuclear form of cleaved SREBP.

used to generate SREBP-1a and SREBP-1c specific DNA templates for generation of cRNA probes as described below.

RNase Protection Assay. cDNA fragments for Syrian hamster SREBP-1a, SREBP-1c, SREBP-2, LDL receptor, HMG CoA synthase, HMG CoA reductase, and β-actin were amplified by PCR from first-strand cDNA prepared from Syrian hamster liver poly(A)+ RNA by using the following primers: SREBP-1a, 5' primer, 5'-GGGCCATGGGACGTCCCTTGATG-3'; SREBP-1c, 5' primer, 5'-TTGCAGGACAGTCTGGAAC-3'; and SREBP-2, 5' primer, 5'-ACACAGTGCAGAGCATTGGAAGCT-3' (this paper); SREBP-2, 5' primer, 5'-ACACATGCTGGAACACAGTGTGGCACATG-3'. In cases where Syrian hamster cDNA sequences were not available, the primer sequences were derived from known Chinese hamster, mouse, rat, and human cDNA sequences as indicated in the above references. HindIII and EcoRI sites were added to all 5' and 3' primers, respectively. All first-strand cDNAs were prepared with a SuperscriptIII kit (GIBCO/BRL). Amplified cDNAs fragments were subcloned into the pGEM-3ZI(+) vector (Promega). After linearization of plasmid DNA with HindIII, antisense RNA was synthesized with [α-32P]CTP (20 mCi/ml) by using bacteriophage T7 RNA polymerase (Ambion, Austin, TX). Specific activities of the cRNAs were measured in each experiment and were in the range of 1.7–2.6 × 106 cpm/μg except for β-actin, which was 5.3–8.1 × 106 cpm/μg as a result of dilution of the [α-32P]CTP.

Aliquots of total RNA (10–15 μg) were assayed by RNase protection by using a HybSpeed RPA kit (Ambion) as de-
RESULTS

Fig. 1 shows immunoblots of the precursor and mature forms of SREBP-1 and SREBP-2 in livers of male hamsters fed varying amounts of cholesterol for 10 days. The amounts of the mature forms of SREBP-1 and SREBP-2 in nuclear extracts declined markedly at the lowest cholesterol concentration (0.1%), and there was no further change at cholesterol concentrations as high as 5%. The amounts of the full-length precursor forms of SREBP-1 in cell membranes were unchanged at 0.1% cholesterol and declined by about 50% at 1% cholesterol. The SREBP-2 precursor was visualized as the lower band of a doublet. This declined somewhat at 0.1% cholesterol and declined by about 50% at 1% cholesterol. The SREBP-2 precursor formed the SREBP-2 precursor forms of SREBP-1 and SREBP-2 in nuclear extracts from livers of female hamsters fed the indicated diet for 12 days (same animals as in Table 1 and Figs. 3 and 6). Each lane contained pooled samples from livers of five hamsters that were fed normal chow (lanes 1 and 5), 0.25% cholesterol (lanes 2 and 6), 0.5% cholesterol (lanes 3 and 7), and lovastatin/Colestipol (lanes 4 and 8). Aliquots (30 μg protein) of membranes (Upper) and nuclear extracts (Lower) were subjected to SDS/PAGE. Immunoblot analysis was performed with either 5 μg/ml of mouse mAb (IgG-2A4) against amino acids 301–407 of human SREBP-1a (lanes 1–4) or 1:4,000 dilution of rabbit IgG against amino acids 32–250 of hamster SREBP-2 (lanes 5–8). Bound antibodies were visualized with the ECL system. Blots were exposed to film for 60 sec (lanes 1–4) or 120 sec (lanes 5–8). P, precursor form of SREBP-2. Nuclear extracts were prepared from livers of five female hamsters that were fed the indicated diet for 12 days (same animals as in Figs. 2, 3, and 6). Each value represents the mean ± SEM of five animals.

Cholesterol content of plasma and liver in hamsters on different diets

<table>
<thead>
<tr>
<th></th>
<th>Control diet</th>
<th>0.25% Cholesterol</th>
<th>0.5% Cholesterol</th>
<th>Lovastatin/Colestipol</th>
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<tbody>
<tr>
<td>Plasma (mg/dl)</td>
<td>162 ± 12</td>
<td>220 ± 12*</td>
<td>306 ± 30*</td>
<td>129 ± 7.8*</td>
</tr>
<tr>
<td>Liver (mg/g)</td>
<td>2.0 ± 0.03</td>
<td>13.9 ± 0.80*</td>
<td>13.4 ± 0.24*</td>
<td>1.9 ± 0.05</td>
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Five female hamsters were fed the indicated diet for 12 days (same animals as in Figs. 2, 3, and 6). Each value represents the mean ± SEM of five animals.*P < 0.01 (Student’s t test); compared with control diet.
The major conclusion of the current studies is that high-cholesterol diets reduce the amounts of SREBP-1c and SREBP-2 in hamster liver nuclei. This reduction likely explains the concomitant reduction in the mRNAs encoding enzymes of cholesterol biosynthesis and the LDL receptor. The reduction in mRNAs, together with posttranscriptional events that reduce HMG CoA reductase activity (4, 30), lead to a profound fall in cholesterol synthesis when hamsters consume cholesterol (31).

The high-cholesterol diets reduced the nuclear forms of the two SREBPs by somewhat different mechanisms. The mechanism for down-regulation of SREBP-1c appeared to be relatively simple. Dietary cholesterol produced a clear reduction in the mature nuclear form of SREBP-1c without a significant change in the amount of precursor (Figs. 1 and 2) or the amount of SREBP-1c mRNA (Fig. 5). The most likely mechanism for this change is a reduction in the rate of proteolytic processing of the precursor to the mature form, as occurs in cultured cells that are treated with sterols (7).

The regulation of SREBP-2 appeared to be more complex. Low levels of dietary cholesterol elicited a profound reduction in the amount of nuclear SREBP-2, but there was also a drop in the amount of precursor and a slight fall in the amount of SREBP-2 mRNA. At high levels of cholesterol the amount of SREBP-2 mRNA declined by about 50% (Fig. 5). These findings suggest that cholesterol may control the amount of nuclear SREBP-2 by two mechanisms: (i) regulation of proteolytic processing; and (ii) regulation of the level of SREBP-2 mRNA and hence the rate of precursor production. Evidence for this dual control also is supplied by the cholesterol depletion experiments. Lovastatin/Colestipol increased the mRNA for SREBP-2 (Fig. 5), and this was associated with an increase in both the precursor form and the mature nuclear form of the protein (Figs. 1 and 2).

The current studies also establish that hamster liver, like previously studied livers of mice and humans (9), produces SREBP-1c as the predominant form of SREBP-1. The conservation of the SREBP-1a/1c dichotomy in three species attests to the physiologic importance of the two forms of the proteins. The sequence comparisons show that the 1c-specific amino acids are completely conserved in the three species, and the overall acidic nature of the 1a region also is preserved (Fig. 4). One can infer, therefore, that in hamsters, as in humans and mice (8), the SREBP-1c transcript is a weaker transcriptional activator than is the SREBP-1a transcript. Previous studies have shown that SREBP-1a is produced predominantly in cultured cells and in testis, spleen, jejunum, and ileum, whereas SREBP-1c predominates in most organs, including the liver, adrenal, and both white and brown adipose tissue (9).
suppresses cholesterol synthesis markedly. This finding is in contrast to the finding in cultured cells, where LDL receptors and cholesterol biosynthetic enzymes decline in parallel (4, 35). It is possible that transcription of the LDL receptor gene in liver is maintained by the low levels of nuclear SREBPs, especially SREBP-2, that persist after cholesterol feeding. Alternatively, the transcription of the LDL receptor gene in liver may be driven by another factor, in addition to SREBPs, whose activity persists after cholesterol feeding. We note that the LDL receptor mRNA increased by 2.8-fold in response to the lovastatin/Colestipol treatment (Fig. 6), a finding that is consistent with the sensitivity of this gene to increased levels of nuclear SREBP-2.

Although the current paper reports only two independent studies, in one males and one in females, we have repeated the essential features of these experiments on six other occasions, and the major conclusions were similar to those shown here. Considered together with previous data, the experiments support the notion that SREBPs are important control elements for the enzymes of cholesterol biosynthesis and LDL receptors in livers of experimental animals.

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Studies with transgenic mice have shown that overexpression of nuclear SREBP-1a stimulates fatty acid synthesis as well as cholesterol synthesis in liver (19). This is likely attributable to the ability of SREBP-1a to bind to elements in the promoters of the fatty acid synthase and acetyl CoA carboxylase genes (7, 32–34). Animals overexpressing the nuclear form of SREBP-1c had a moderate increase in fatty acid synthesis and no demonstrable increase in cholesterol synthesis, suggesting that SREBP-1c is a relatively specific activator of the fatty acid biosynthetic pathway (8).

In the experiment with male hamsters, treatment with lovastatin/Colestipol led to a selective down-regulation of the amount of SREBP-1c mRNA without affecting the mRNA for SREBP-1a (Fig. 5). This was associated with a profound fall in the amount of both the precursor and mature nuclear forms of SREBP-1 (Fig. 1). In the experiment with females the changes in SREBP-1 protein were in a similar direction, but not as profound as those in males (Fig. 2). These data indicate that the promoter that gives rise to the SREBP-1c transcript responds to a regulatory signal that is not detected by the promoter that gives rise to the 1a transcript. The nature of this signal and the mechanism of its suppression by lovastatin and Colestipol are presently unknown.

An important aspect of these studies was the finding that cholesterol feeding suppressed the LDL receptor mRNA by only 30% at a time when the HMG CoA synthase and reductase mRNAs were suppressed by 90% and 70%, respectively (Fig. 6). The incomplete suppression of LDL receptor mRNA presumably explains the failure of the plasma cholesterol to rise much above 300 mg/dl in the cholesterol-fed hamsters (Table 1). When the LDL receptor is reduced profoundly, as in mice homozygous for an LDL receptor gene knockout, high-cholesterol diets raise the plasma cholesterol level to over 2,000 mg/dl (16).

The current findings on LDL receptor mRNA are consistent with those of Spady et al. (11), who showed that cholesterol suppresses LDL receptor activity in animal livers only after it