Diminished Hepatic Response to Fasting/Refeeding and Liver X Receptor Agonists in Mice with Selective Deficiency of Sterol Regulatory Element-binding Protein-1c*

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Two treatments, fasting/refeeding and administration of liver X receptor (LXR) agonists, elevate the mRNA for sterol regulatory element-binding protein-1c (SREBP-1c) and enhance lipid synthesis in liver. These treatments do not affect the mRNA for SREBP-1a, an alternative transcript from the same gene. Through homologous recombination, we eliminated the exon encoding SREBP-1c from the mouse genome, leaving the SREBP-1a transcript intact. On a normal diet, livers of SREBP-1c−/− mice manifested reductions in multiple mRNAs encoding enzymes of fatty acid and triglyceride synthesis, including acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS). In contrast, SREBP-1c−/− livers showed a compensatory increase in hepatic SREBP-2 mRNA, accompanied by increased mRNA levels for cholesterol biosynthetic enzymes. In fasted/refed animals, ACC and FAS mRNAs rose, but not to the same extent as in wild-type livers. The refeeding-induced increase in SREBP-1c−/− mice was greater than in mice lacking SREBP cleavage-activating protein (SCAP), in which all nuclear SREBPs are absent. Thus, SREBP-2 and/or SREBP-1a can substitute partially for SREBP-1c in permitting an insulin-mediated increase in ACC and FAS mRNAs. In contrast, mRNAs for several other lipogenic enzymes (glucose-6-phosphate dehydrogenase, malic enzyme, glycerol-3-phosphate acyltransferase, and stearoyl-CoA desaturase-1) showed a complete failure of the normal inductive response to refeeding, indicating specific reliance on SREBP-1c. Moreover, these mRNAs, as well as multiple other lipogenic mRNAs, showed a markedly blunted response to the LXR agonist T090137, indicating an essential role of SREBP-1c in the LXR response.

Three sterol regulatory element-binding proteins (SREBPs)† with overlapping functions control lipid synthesis in liver and other tissues of mammals (1). The SREBPs are transcription factors of the basic helix-loop-helix-leucine zipper family. Two of the SREBPs, designated SREBP-1a and SREBP-1c, are derived from a single gene through the use of alternate promoters that give rise to alternate first exons, thereby changing the NH2-terminal sequences of the proteins (1, 2). The NH2 terminus of SREBP-1a contains a long acidic transcription-activating domain, and it is a potent activator of transcription of genes controlling the synthesis of cholesterol, fatty acids, and triglycerides. SREBP-1c contains a shorter transcription-activating domain, and it is a weaker transcription factor with preferential specificity for fatty acid and triglyceride biosynthesis. SREBP-2 is derived from a different gene. With its long transcription-activating sequence, it is a potent activator of cholesterol biosynthesis and a weaker activator of fatty acid and triglyceride synthesis (2–6).

All three SREBPs are synthesized as long inactive precursors that are bound to membranes of the endoplasmic reticulum. There, they form complexes with SREBP cleavage-activating protein (SCAP) (7). SCAP escorts the SREBPs to the Golgi complex, where they are cleaved sequentially by two membrane-bound proteases designated site-1 protease (S1P) and site-2 protease (S2P), thereby liberating the NH2-terminal domain so that it can enter the nucleus to activate transcription (8). The direct targets of SREBPs include multiple enzymes of the cholesterol biosynthetic pathway, such as 3-hydroxy-3-methylglutaryl coenzyme A synthase, 3-hydroxy-3-methylglutaryl coenzyme A reductase, farnesyl diphosphate synthase, and many others. The SREBPs also activate enzymes that play a role in fatty acid biosynthesis, including acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), ATP citrate lyase (which supplies acetyl-CoA), and malic enzyme and glucose-6-phosphate dehydrogenase (both of which produce NADPH). The SREBPs also activate stearoyl-CoA desaturase-1, thereby favoring the synthesis of unsaturated fatty acids, and glycerol-3-phosphate acyltransferase, which converts the excess fatty acids to triglycerides (1, 9–11).

Because of the overlapping functions of the three SREBPs, their individual roles in liver have been difficult to assign precisely. One approach is to produce transgenic mice overex-

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† The abbreviations used are: SREBP, sterol regulatory element-binding protein; ACC, acetyl-CoA carboxylase; ACE, angiotensin-converting enzyme; FAS, fatty acid synthase; IRS, insulin receptor substrate; LXR, liver X receptor; PEPCK, phosphoenolpyruvate carboxykinase; SCAP, SREBP cleavage-activating protein; S1P, site-1 protease; S2P, site-2 protease; plpC, polyinosinic/polycytidylic acid; USF, upstream regulatory factor.
pressing truncated versions of each of the SREBPs that enter the nucleus directly without a requirement for proteolysis. Overexpression of truncated SREBP-1a produced a massive fatty liver due to marked increases in the mRNAs encoding enzymes of fatty acid and triglyceride biosynthesis and to a definite but lesser increase in cholesterologenic enzymes (3). SREBP-1c produced less lipid accumulation, all of which was in the form of triglycerides (12). SREBP-2 also produced a fatty liver, but the enrichment in cholesterol was greater than that of triglycerides (5). These results led to the conclusion that both SREBP-1 isoforms favor triglyceride synthesis, whereas SREBP-2 is more specific for cholesterol (1).

A second approach is gene knockout. Germ-line elimination of the SREBP-2 gene was embryonically lethal. Germ line disruption of the SREBP-1 gene, which eliminated both the SREBP-1a and -1c transcripts, was partially lethal (4). About 50–85% of the SREBP-1−/− embryos died prior to birth. Those that survived were phenotypically relatively normal. Their livers contained increased amounts of SREBP-2, which was postulated to compensate for the SREBP-1 deficiency. The livers also contained an increased amount of cholesterol, apparently as a result of the compensatory rise in SREBP-2 (4). Although SREBP-1c has been implicated in differentiation of adipocytes in vitro (hence its alternate name, adipocyte determination and differentiation factor-1, or ADD-1) (13), the surviving SREBP-1−/− mice had normal adipose tissue, possibly due to compensation by SREBP-2 (4).

To circumvent the lethality of germ line knockouts, we recently turned to conditional tissue-specific knockouts using the Cre-loxP system. We initially targeted the SCAP gene by introducing loxP sites flanking crucial exons (14). The animals were bred with mice expressing the Cre recombinase under control of the interferon-inducible MX1 promoter. After the mice had reached maturity, the SCAP gene in liver was inactivated by subcutaneous injection of a double-stranded oligonucleotide (polynosinic/polycytidylic acid (pIpc)), which induces production of interferon in the liver (15). Cre-mediated elimination of SCAP caused a marked reduction in the membrane-bound precursor forms of all three SREBPs, apparently because these proteins are unstable in the absence of SCAP (14). The nuclear forms of all three proteins also were eliminated, due to a failure of the precursors to move to the Golgi apparatus for processing. In the absence of all nuclear SREBPs, the basal levels of mRNAs for all of the known SREBP target genes declined dramatically, and the measured rates of fatty acid and cholesterol synthesis declined by more than 80%. A similar reduction, although less profound, was found in mice with Cre-mediated elimination of S1P in liver (16). These studies showed that nuclear SREBPs are necessary for normal rates of fatty acid and cholesterol synthesis, but they did not distinguish among the SREBPs.

In addition to their roles in permitting basal lipid synthesis, SREBPs have been implicated in lipogenic responses to two agents: insulin and liver X receptor (LXR) agonists. Both of these responses are believed to be specific for SREBP-1c. The involvement of SREBP-1c in insulin response is suggested by the finding that SREBP-1c mRNA and protein levels fall in livers of fasted mice (low insulin; high glucagon) and rise to supranormal levels when the animals are refed a high carbohydrate diet (high insulin; low glucagon) (17). Similar changes occur when rats are treated with streptozotocin (low insulin) followed by insulin injection (18). These changes correlate with changes in mRNAs encoding ACC and FAS, the two predominant enzymes of fatty acid synthesis as well as other lipogenic genes that are SREBP targets. Under these conditions, the mRNA levels for SREBP-1a and SREBP-2 change only slightly, and their levels do not correlate with mRNA levels for enzymes of fatty acid and triglyceride synthesis.

In freshly isolated rat hepatocytes, insulin treatment elevates SREBP-1c mRNA levels (19, 20), and glucagon lowers them (20). Again, the rise in SREBP-1c mRNA is associated with increases in ACC and FAS mRNAs. More importantly, administration of an adenovirus encoding a dominant negative version of SREBP-1c abolished the increase in ACC and FAS mRNAs in response to insulin (21). The latter data indicate that SREBPs are necessary for the insulin response, but they do not distinguish among the three SREBPs, since dominant negative SREBP-1c may block the function of all SREBPs through heterodimerization (22).

Among the insulin-induced genes, FAS is the most intensively studied. In experiments with transgenic mice expressing reporter genes driven by DNA sequences upstream of the FAS mRNA, Sul and colleagues (23, 24) concluded that full insulin-mediated induction of FAS transcription in liver depended on an SREBP binding site at position –150 as well as a more distal element that binds upstream stimulatory factor (USF), another basic helix-loop-helix-leucine zipper protein. When SREBP-1 nuclear levels were maintained by expression of a transgene encoding truncated SREBP-1a, FAS mRNA no longer declined with fasting, suggesting that SREBP-1 expression alone is sufficient to maintain basal levels of FAS expression. The role of USF in the insulin response was suggested by Casado et al. (25), who showed that germ line knockout of either USF-1 or USF-2 largely blocked the induction of FAS transcription in a fasting-refeeding protocol. Considered together with the data in the SCAP-deficient mice, these data indicate that nuclear SREBPs are both necessary and sufficient to maintain basal levels of FAS transcription and that insulin-mediated enhancement of transcription requires both an SREBP and a USF. Whether the function of SREBP is specific to SREBP-1c or whether other SREBPs can substitute is not known.

In an attempt to determine the combined role of the two SREBP-1 isoforms, Shimano et al. (26) subjected the above-mentioned SREBP-1 knockout mice to a fasting-refeeding protocol. Surprisingly, the levels of ACC and FAS mRNAs were somewhat elevated in the fasted knockout mice compared with fasted wild-type mice, possibly because of the compensatory increase in SREBP-2. These mRNA levels rose substantially upon refeeding, but the increase was not as great as that in wild-type mice. Other SREBP target genes such as glycerol-3-phosphate acyltransferase, glucose-6-phosphate dehydrogenase, and malic enzyme showed a more severe reduction in insulin responsiveness in the SREBP-1−/− mice. When mice were put on a long-term high carbohydrate diet, the ACC and FAS mRNAs failed to increase in the SREBP-1−/− mice as they did in the wild-type mice. These studies failed to distinguish between the effects of the SREBP-1a and -1c transcripts.

In the current studies, we used the technique of homologous recombination to delete the first exon of the SREBP-1c gene. This strategy abolished the SREBP-1c transcript while leaving the SREBP-1a transcript intact. Heterozygous germ line deletions of SREBP-1c produced no embryonic lethality. Genes involved in fatty acid and triglyceride synthesis showed partially blunted responses to a fasting-refeeding protocol. In contrast to this partial response, the response to an LXR agonist was eliminated nearly completely.

**EXPERIMENTAL PROCEDURES**

**Methods**—Measurements of cholesterol and triglycerides in plasma and liver (14), preparation of nuclear extracts and membrane fractions from mouse liver (27), immunoblot analyses of mouse SREBP-1 and -2 (4), and RNase protection assays of SREBP-1a, -1c, and -2 mRNAs (2) were carried out as described in the indicated references. The LXR...
agonist T0901317 (28) was kindly provided by Dr. Bei Shan of Tularik, Inc. ppC (catalog number P15530) was obtained from Sigma. S1P1(−/−):MX-1-Cre mice (16) and SCAPE(−/−):MX-1-Cre mice (14) were created and treated with ppC as described in the indicated references.

Construction of Targeting Vector for Disruption of SREBP-1c Allele—The SREBP-1c allele of the SREBP-1 gene was disrupted by replacement of exon 1c with a sequence encoding a modified bacterial lacZ enzyme with a nuclear localization signal (nLacZ) (29). Positive selection was achieved with the use of a self-excision cassette, flanked by loxP sites, that contains IACE-Cre and the neo selectable marker gene, referred to as ACN (30) (kindly provided by Dr. Kirk Thomas, Southwestern Medical Center). Both wild-type and disrupted SREBP-1c allele through the germ line. For further breeding, we chose mice that had deleted the neo gene. The targeting vector was constructed in four steps as follows. 1) A 3.5-kb NotI-Xhol fragment (from pBS-nLacZ) containing a modified bacterial lacZ enzyme with a nuclear localization signal was cloned into the NotI site of the gene replacement vector (pGEM3Zf (+) containing the Pol2Sneop expression cassette (31) to generate pLacZ-TK. 2) A 6.5-kb fragment containing the genomic sequence 5’ of exon 1c and the first four amino acids of exon 1c was generated by PCR using SM-1 ES cell genomic DNA (see below) as template and the following primers: 5’-GCCAGCTGCTCAGATGCAAA-3’ and 3’-CCCGTGACATGCGACAAA. The 6.5-kb PCR product was digested with SacI and ligated into the XhoI site of the pLacZ-TK to fuse the first four amino acids of exon 1c in frame with nLacZ. The resulting plasmid was designated pBP1c-nLacZ. 3) A 1.6-kb fragment containing part of the genomic sequence between exon 1c and exon 2 was digested by PCR using SM-1 ES cell genomic DNA as template and the following primers: 5’-ACGGCGCTGAAGCAGTAAGGATGGA-3’ and 3’-CGCTATCCGCGGCAATGG. The 1.6-kb PCR product was digested with EcoRI and ligated into the EcoRI site of ACN (30) to generate plasmid pACN-SA. 4) A 5.3-kb XhoI-Xhol fragment of pACN-SA was ligated into the XhoI site of pbPlc-nLacZ to generate the exon 1c targeting vector. Restriction analysis and DNA sequencing were used to confirm the integrity of all the constructs.

Selective Knockout of SREBP-1c Gene in Mice

| Category                        | Gene          | Sequence of forward and reverse primers (5’ to 3’) | GenBank accession |
|--------------------------------|---------------|---------------------------------------------------|-------------------|
| Control                        | Cyclophilin   | TGGAGGCACCCAGAAGCAGACA                               | M60456             |
| Fatty acid synthesis           | Glucokinase   | GGAAAACCTGGACAGGATGAG                                 | L38990             |
|                                | ATP citrate lyase | GCCAGCGGAGACATTC                                       | AF332051           |
|                                | Acetyl-CoA synthetase | GCTTGCCAGGGCGACCATG                                     | AF216873           |
|                                | Glucose-6-phosphate dehydrogenase | GACGCAGAATGCGAGAAGGATGAG                                    | Z19111             |
|                                | Malic enzyme  | GCCGGTGCTTCATCTCCCTCCTTTG                               | NM-008615          |
|                                | Glycerol-3-phosphate acyltransferase | TTTTGTAGCTCTTGCACAACTTCTT                                              | NM-008149          |
| Insulin signaling pathway      | Insulin receptor | CGAGTGCCGCTGCTGCAATTTATG                                           | J05149             |
|                                | IRS-1         | GGGCAACCCAGACCTAAGTCT                                 | AF097038           |
|                                | IRS-2         | GATGCCTTTAGGGCCCTTAC                                   | AF09605            |
|                                | PEPCK         | CCACAGCTGTGCGAAGAAC                                     | AF09605            |
|                                | IGFBP-1       | GGAGATCGCCGACCTTCAAG                                    | NM-008341          |
| ABC transporters              | ABCA1         | CTTGCAGTCATCTTCAGCTTATAG                                 | NM-003454          |
|                                | ABCG5         | TGCTAGAGTGAAGAGACGATTG                                  | AF132713           |
|                                | ABCG8         | TGCCCCCTCTCCACATGTC                                    | AF324495           |
| Other                          | CYP7A1        | AGCAGAATCCAGCAGGACTAGA                                  | NM-007824          |
|                                | ApoE          | CGAGGGCAGATCTTCCCA                                    | NM-009696          |

Quantitative Real Time PCR—Total RNA was prepared from mouse tissues using an RNA STAT-60 kit (TEL-TEST B, Friendswood, TX). Equal amounts of RNA from 3–5 mice were pooled and treated with DNase I (DNA-free™, Ambion, Inc.). First strand cDNA was synthesized from 2 μg of DNase I-treated total RNA with random hexamer primer sequences of genes not shown here have been described previously (16).

TABLE I

Quantitative Real Time PCR—Total RNA was prepared from mouse tissues using an RNA STAT-60 kit (TEL-TEST B, Friendswood, TX). Equal amounts of RNA from 3–5 mice were pooled and treated with DNase I (DNA-free™, Ambion, Inc.). First strand cDNA was synthesized from 2 μg of DNase I-treated total RNA with random hexamer primer sequences of genes not shown here have been described previously (16).
Selective Knockout of SREBP-1c Gene in Mice

**Fig. 1. Disruption of SREBP-1c allele.** A, schematic of gene targeting strategy. The map of the wild-type allele spans the region between exons 1a and 2 of the SREBP-1 gene. The sequence replacement vector was constructed as described under “Experimental Procedures.” The targeting vector contains a modified bacterial lacZ gene with a nuclear localization signal (nLacZ) fused in frame with the first four amino acids of exon 1c. This vector also contains two copies of the HSV-TK gene and the self-excision ACN cassette (30). The ACN cassette contains the neo selection marker linked to tACE-Cre (Cre recombinase driven by angiotensin-converting enzyme promoter), flanked by loxP sites (denoted by open triangles). During spermatogenesis, the tACE promoter induces expression of Cre recombinase to excise the ACN cassette, leaving a single loxP site at the targeted chromosomal locus (30). The location of the probe (0.8-kb XhoI-Xhol fragment) used for Southern analysis is denoted by a solid box. B, representative Southern blot analysis ofSacI-digested tail DNA of the offspring from heterozygous matings of SREBP-1c+/− mice. The positions of migration of the fragments derived from wild-type and disrupted alleles are indicated. C, RNase protection assay of SREBP-1a, -1c, and -2 mRNAs. Total RNA from livers of mice (Table II) was pooled, and 15-μg aliquots were subjected to RNase protection assay (2). After RNase digestion, protected fragments were separated by gel electrophoresis and exposed to film for 16 h at −80 °C. The relative intensity of each band is denoted above the band. D, immunoblot analysis of SREBP-1 and -2 from livers of wild-type (+/+), SREBP-1c−/−, and SREBP-1c+/− mice. Livers from four mice in each group (as those described in Table II) were pooled, and aliquots (30 μg of protein) of the membrane and nuclear extract fractions were subjected to SDS-PAGE and immunoblot analysis (4). Filters were exposed to Kodak X-Omat™ Blue film for 5–30 s at room temperature. The precursor and nuclear forms of SREBPs are denoted as P and N, respectively.

**Table II**

Comparison of wild-type and SREBP-1c−/− mice

| Parameter                     | Wild type | SREBP-1c−/− |
|-------------------------------|-----------|-------------|
| Number of mice                | 4         | 4           |
| Body weight (g)               | 29.7 ± 0.6| 26.4 ± 0.5  |
| Liver weight (g)              | 1.6 ± 0.1 | 1.5 ± 0.1   |
| Liver weight/body weight (%)  | 5.5 ± 0.3 | 5.7 ± 0.1   |
| Epididymal fat weight (g)     | 0.37 ± 0.06| 0.27 ± 0.05 |
| Epididymal fat weight/body weight (%) | 1.3 ± 0.2 | 1.0 ± 0.2  |
| Liver cholesterol content (mg/g) | 1.9 ± 0.1 | 2.0 ± 0.1** |
| Liver triglyceride content (mg/g) | 7.8 ± 1.0 | 5.9 ± 0.9   |
| Total plasma cholesterol (mg/dl) | 86.6 ± 5.0| 73.0 ± 1.7* |
| Total plasma triglyceride (mg/dl) | 130 ± 19  | 57 ± 5**    |
| Plasma free fatty acids (mM)  | 0.4 ± 0.04| 0.4 ± 0.02  |
| Plasma insulin (ng/ml)        | 1.4 ± 0.2 | 0.8 ± 0.04  |
| Plasma glucose (mg/dl)        | 210 ± 8   | 240 ± 9     |

**RESULTS**

Fig. 1A summarizes the strategy that we used to eliminate the SREBP-1c transcript in mice. The SREBP-1a transcript begins with exon 1a and splices into exon 2, removing exon 1c as part of a 13.2-kb intron. The SREBP-1c transcript initiates from its own promoter at exon 1c and also splices into exon 2 (1, 2). As an aid in eliminating exon 1c, we used the removable neo gene cassette developed by Bunting et al. (30). This cassette, designated ACN, is flanked by loxP sites, and it encodes the neo gene and the Cre recombinase, the latter under control of the ACE promoter. This cassette is eliminated when it passes through the mouse germ line, due to Cre-mediated recombinase in the testis. The strategy results in a selective replacement of the first exon of the SREBP-1c transcript with a sequence encoding bacterial β-galactosidase with a nuclear localization signal (nLacZ). The nLacZ should be expressed from the SREBP-1c promoter. The SREBP-1a transcript should continue to be expressed normally, since it splices around the nLacZ exon. Representative Southern blots of genomic DNA from several SREBP-1c−/− mice are shown in Fig. 1B. Using an RNase protection assay, we confirmed that the SREBP-1c transcript was absent from livers of the SREBP-1c−/− mice (Fig. 1C). The SREBP-1a transcript, which is normally present at much lower levels than the SREBP-1c transcript, was reduced slightly in the SREBP-1c−/− mice (70% of wild-type). In contrast, the SREBP-2 transcript was increased by 60%. Immunoblot analysis was performed with an antibody that detects both SREBP-1a and -1c (Fig. 1D). The results demonstrated that the total amount of SREBP-1 protein was reduced markedly in the SREBP-1c−/− mice. The amounts of the membrane-bound precursor and nuclear forms of SREBP-2 were both increased.

primers using the ABI cDNA synthesis kit (catalog no. N808-0234; Applied Biosystems). Specific primers for each gene (Table I and Ref. 16) were designed using Primer Express software (Applied Biosystems). The real time PCR contained, in a final volume of 20 μl, 20 ng of reverse transcribed total RNA, 167 nM forward and reverse primers, and 10 μl of 2× SYBR Green PCR Master Mix (catalog no. 4312704; PerkinElmer Life Sciences). PCR was carried out in 384-well plates using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). All reactions were done in triplicate. The relative amount of all mRNAs was calculated using the comparative C_T method (41). Cyclophilin mRNA was used as the invariant control for all studies except for the fasting/refeeding study (see Fig. 3), in which apoB mRNA was used as the invariant control.
slightly (Fig. 1D). β-Galactosidase staining of frozen sections of liver revealed that the enzyme was present in nuclei of many, but not all, hepatocytes of the SREBP-1c−/− mice, indicating that the SREBP-1c promoter remained active (data not shown).

The SREBP-1c−/− mice appeared normal at birth and throughout their life spans. Matings between wild-type mice, the liver cholesterol content in pads, but the histology of the tissue was normal. As compared insignificantly reduced in the weights of the epididymal fat pads, but the histology of the tissue was normal. As compared with wild-type mice, the liver cholesterol content in SREBP-1c−/− mice was significantly increased (p < 0.01), and the levels of plasma cholesterol (p < 0.05) and of plasma triglycerides (p < 0.01) were significantly reduced. Plasma insulin concentration was slightly reduced, and plasma glucose was elevated, but these differences were not statistically significant. Table II lists several relevant attributes of the SREBP-1c−/− mice. Of note, body weights and liver weights were normal. There was a slight, statistically insignificant reduction in the weights of the epididymal fat pads, but the histology of the tissue was normal. As compared with wild-type mice, the liver cholesterol content in SREBP-1c−/− mice was significantly increased (p < 0.01), and the levels of plasma cholesterol (p < 0.05) and of plasma triglycerides (p < 0.01) were significantly reduced. Plasma insulin concentration was slightly reduced, and plasma glucose was elevated, but these differences were not statistically significant.

Table III shows the relative amounts of various mRNAs in pooled samples of liver from ad libitum fed wild-type and SREBP-1c−/− mice as determined by quantitative real time PCR. Notable changes were a slight reduction in SREBP-1a mRNA, a 44% increase in SREBP-2 mRNA, and slight reductions in the mRNAs encoding SCAP and S1P. The mRNAs encoding enzymes of fatty acid and triglyceride synthesis were significantly reduced, generally to values that were 25–40% of normal. All of these genes are known to be controlled by SREBPs (1, 9). In sharp contrast, there was a definite increase in mRNAs encoding enzymes of cholesterol synthesis. There was no change in the mRNA for the low density lipoprotein receptor or in the mRNAs for apoB and apoE. We found increases of ~2-fold in mRNAs encoding three proteins that are normally down-regulated by insulin (IRS-2, PEPCK, and insulin-like growth factor-binding protein-1) (20, 32). The significance of this latter observation is not clear inasmuch as the steady-state expression of these insulin-regulated genes was variable from experiment to experiment in wild-type as well as SREBP-1c−/− mice on an ad libitum diet.

To determine whether SREBP-1c deficiency interferes with the lipogenic response to carbohydrate feeding, we measured various mRNAs in ad libitum fed mice, fasted mice, and fasted mice that were refed a high carbohydrate diet. Table IV shows the physiologic parameters of the mice in this experiment. We again observed an increase in hepatic cholesterol content and decreases in plasma levels of cholesterol and triglyceride in the ad libitum fed SREBP-1c−/− mice. During fasting, plasma triglycerides rose in the SREBP-1c−/− mice, and they fell dramatically upon refeeding. In wild-type mice, there was little change in plasma triglycerides on fasting and an increase upon refeeding, which was opposite to the change in the SREBP-1c−/− mice.

As expected (17), in wild-type mice the precursor and nuclear forms of hepatic SREBP-1 were reduced upon fasting and restored to supernormal levels upon refeeding, as determined by immunoblotting (Fig. 2A). We visualized no SREBP-1 in the SREBP-1c−/− mice. Nuclear SREBP-2 was slightly increased in the ad libitum fed SREBP-1c−/− mice. The protein fell somewhat upon fasting and was restored partially by refeeding. For comparative purposes, we also measured SREBP levels in new studies of livers from two previously described lines of mice that have conditional disruption of the genes encoding SCAP or S1P. These floxed mice carry recombinogenic loxP sites in each of these genes, and they undergo deletions when the expression of Cre recombinase is induced in hepatocytes by treatment with poly(I)-poly(C) (14, 16). As previously reported (14), SCAP−/− mice manifested a major reduction in the precursor and nuclear forms of SREBP-1 and -2, and there was no effect of fasting or refeeding (Fig. 2B). The S1P−/− mice had a partial defect, with moderate reductions in nuclear SREBP-1 and -2. Both proteins declined with fasting and were restored only partially with refeeding (Fig. 2C). The partial effect of S1P deficiency was noted previously (16).

Fig. 3 shows the relative levels of various mRNAs in the livers from each of the three lines of knockout mice upon fasting and refeeding. The mRNAs are divided into four pathways: the SREBP pathway, the pathway of fatty acid and triglyceride synthesis, the pathway for cholesterol synthesis and uptake, and the insulin signaling pathway. The level of mRNA in nonfasted wild-type mice studied in the same experiment is designated as 1. In the SREBP-1c−/− mice, the SREBP-1c mRNA was undetectable, and it did not rise upon refeeding as it did in normal mice (Fig. 3A). In wild-type mice, fasting lowered all of the mRNAs for the fatty acid and triglyceride-synthesizing enzymes, and refeeding raised the levels to supernormal values. Surprisingly, some of these mRNAs appeared to rise to nearly normal values in the refed SREBP-1c−/− mice. This was particularly true of the mRNAs for glucokinase, ACC, and FAS. Other mRNAs failed to rise with refeeding in the SREBP-1c−/− mice. These included glucose-6-phosphate dehydrogenase, malic enzyme, stearoyl-CoA desaturase-1, and glycerol-3-phosphate acyltransferase. The mRNAs for the cholesterol biosynthetic enzymes tended to rise to supernormal levels upon refeeding in the SREBP-1c−/− mice (Fig. 3A).

The results in the SCAP−/− mice (Fig. 3B) were markedly
Male mice (8–10 weeks of age) were subjected to fasting and refeeding as described under "Experimental Procedures." Each value represents the mean ± S.E. Asterisks denote the level of statistical significance between the wild-type and SREBP-1c" mice (Student’s t test); *, p < 0.05; **, p < 0.01; ***, p < 0.001.

**TABLE IV**
Effects of fasting and refeeding in wild-type and SREBP-1c" mice

| Parameter            | Wild type | SREBP-1c" |
|----------------------|-----------|-----------|
|                      | Nonfasted | Fasted    | Refed    | Nonfasted | Fasted | Refed    |
| Number of mice       | 4         | 4         | 4        | 4         | 4      | 4        |
| Body weight (g)      | 26.7 ± 0.3| 20.5 ± 0.6| 27.9 ± 0.7| 30.3 ± 1.2| 22.2 ± 1.1| 24.1 ± 0.7|
| Liver weight (g)     | 1.44 ± 0.03| 1.03 ± 0.02| 1.88 ± 0.10| 1.73 ± 0.10 | 1.08 ± 0.08 | 1.68 ± 0.07 |
| Liver weight/body weight (%) | 5.39 ± 0.15 | 5.02 ± 0.09 | 6.72 ± 0.28 | 5.68 ± 0.18 | 4.85 ± 0.16 | 6.98 ± 0.08 |
| Liver cholesterol content (mg/g) | 2.00 ± 0.03 | 2.61 ± 0.02 | 1.71 ± 0.08 | 2.63 ± 0.08**** | 3.56 ± 0.22*** | 2.42 ± 0.08**** |
| Liver triglyceride content (mg/g) | 8.4 ± 1.1 | 66 ± 2.5 | 8.7 ± 0.9 | 6.6 ± 0.1 | 74 ± 2.8 | 17.2 ± 0.5** |
| Total plasma cholesterol (mg/dl) | 105 ± 8 | 89 ± 5 | 87 ± 9 | 71 ± 2** | 80 ± 5 | 53 ± 2** |
| Total plasma triglycerides (mg/dl) | 110 ± 10 | 111 ± 3 | 170 ± 28 | 58 ± 1** | 128 ± 12 | 74 ± 7** |
| Plasma free fatty acids (mm) | 0.5 ± 0.02 | 1.0 ± 0.05 | 0.2 ± 0.02 | 0.5 ± 0.04 | 1.3 ± 0.12** | 0.3 ± 0.02 |
| Plasma insulin (ng/ml) | 1.05 ± 0.08 | 35 ± 0.01 | 11.2 ± 4.7 | 1.00 ± 0.09 | 0.42 ± 0.05 | 3.96 ± 0.75 |
| Plasma glucose (mg/dl) | 212 ± 18 | 126 ± 4 | 202 ± 7 | 237 ± 12 | 130 ± 11 | 243 ± 6** |

FIG. 2. Effect of fasting and refeeding on SREBP proteins in livers of wild-type mice and different knockout mice. A, wild-type (WT) versus SREBP-1c" mice. B, wild-type versus SCAP"/MX1-Cre (SCAP"/C) mice. C, wild-type versus S1P"/MX1-Cre (S1P"/C) mice. The mice used in A are described in Table IV. Livers from each group (four mice per group) were separately pooled, and 30-μg aliquots of the membrane and nuclear extracts fractions were subjected to SDS-PAGE and immunoblot analysis. Filters were exposed to film for 5–30 s at room temperature. The precursor and nuclear forms of SREBPs are denoted as P and N, respectively. For studies in B and C, 8–10-week-old male mice were injected intraperitoneally four (B) or five (C) times with plpC (300 μg/injection). On day 14 after the last injection, the mice were subjected to fasting and refeeding protocol. The nonfasted group (N) was maintained ad libitum, the fasted group (P) was fasted 12 h, and the refeed group (R) was fasted for 12 h and then refed a high carbohydrate/low fat diet for 12 h prior to study. Immunoblot analysis was done as described above.

**DISCUSSION**

The current data demonstrate that basal levels of mRNAs encoding enzymes of fatty acid and triglyceride synthesis are reduced in livers of mice lacking SREBP-1c, but some of these mRNAs rise to nearly normal levels when fasted. SREBP-1c" mice are refed a high carbohydrate diet that elevates plasma insulin and suppresses plasma glucagon. When mouse livers lack all forms of nuclear SREBPs as a result of SCAP deficiency, the response to refeeding is essentially abolished (14). Considered together, these data indicate that one of the three SREBP enzymes is essential for an increase in ACC and FAS mRNAs in response to bolus feeding, but that this is not necessarily SREBP-1c. In the absence of SREBP-1c, this function can be supplied by SREBP-1a or SREBP-2. On the other hand, SREBP-1c is uniquely required for a lipogenic response to the LXR agonist T090137.
actually rise in order to have this effect (active role) or whether they may remain constant, their presence being necessary to allow some other insulin-induced factor to act (permissive role). In the refed SREBP-1c/KO mice, there was a consistent increase in the mRNAs encoding SREBP-1a and SREBP-2 (Fig. 3A), but we were unable to detect a consistent increase in the nuclear forms of these proteins by immunoblotting (Fig. 2). Correlative evidence suggests that the nuclear content of SREBP-2 did increase in response to refeeding but that the immunoblotting technique was not sensitive enough to detect it. This conclusion is supported by the observation that the mRNAs encoding enzymes of cholesterol biosynthesis rose to a greater extent after refeeding in the SREBP-1c/KO mice than they did in the wild-type mice (Fig. 3A). These mRNAs are thought to be particularly responsive to SREBP-2 (5, 6). If SREBP-2 did rise after refeeding, then this might explain the increase in ACC and FAS mRNAs. Alternatively, if nuclear SREBP-1a and SREBP-2 remained constant after refeeding, the data would suggest that these proteins play a permissive role in the insulin response. In this case, insulin may have enhanced transcription by activating other transcription factors, such as USF (38). These other factors cannot act without SREBPs, as indicated by the failure of the ACC and FAS mRNAs to rise upon refeeding in the SCAP-deficient mice (Fig. 3B) (14). Either the active role or the permissive role of SREBPs is consistent with data in rat hepatocytes, which show that a dominant-negative form of SREBP-1c that blocks all SREBP action can abolish the insulin-induced increase in FAS mRNA (21).

Another variation of the permissive role of SREBP in the refeeding response is the possibility that refeeding induces or activates a co-activator that enhances the transcription-activating capacity of SREBP-1c and/or USF, both of which are basic helix-loop-helix-leucine zipper proteins. This role would be analogous to the recently described role of PGC-1, a coactivator that works together with nuclear receptors in the liver to enhance the transcription of genes encoding gluconeogenic enzymes (39). PGC-1 is down-regulated in refed mice, and it is suppressed by insulin in HepG2 cells (40), whereas the postulated SREBP/USF coactivator should be up-regulated by refeeding and by insulin. A search for such a factor is clearly warranted by the available data.

For several SREBP-responsive genes, the deficiency in the refeeding response in the SREBP-1c/KO mice was much more severe than observed for ACC and FAS in mRNAs. The severely affected mRNAs included glucose-6-phosphate dehydrogenase, malic enzyme, stearoyl-CoA desaturase-1, and glycerol-3-phosphate acyltransferase. Shimano et al. (26) also noted a severe defect in the regulation of these genes in SREBP-1c−/− mice. Thus, expression of these genes appears to be specifically...
Selective Knockout of SREBP-1c Gene in Mice

In experiments not shown, we found that SREBP-1c/−/− mice fed T0901317 (~15 mg/kg per day) for 7 days had a 3-fold lower content of triglycerides in the liver than wild-type controls (10.5 ± 0.9 versus 29.8 ± 3.2 mg/kg, p < 0.001; 11 mice in each group). In contrast to these lipogenic actions, SREBP-1c was not essential for the induction of mRNAs in nonlipogenic pathways, all of which appear to be direct targets of LXR (Fig. 4).

The absence of embryonic lethality in the SREBP-1c/−/− mice stands in sharp contrast to the 50–85% embryonic lethality observed when the entire SREBP-1 gene is abolished (4). These data indicate that SREBP-1a plays a more essential role than SREBP-1c during embryonic development. This is consistent with the observation that SREBP-1a is the predominant transcript in rapidly growing cells in tissue culture, and SREBP-1c becomes predominant only in adult organs like liver and adipose tissue (2). Neither SREBP-1a nor SREBP-1c is essential for the normal development of either of these organs, as illustrated by the findings in this paper and by previous observations in the few surviving mice with complete SREBP-1 deficiency (4). Whether the postulated differentiation function of SREBP-1c in adipose tissue (13) is compensated by expression of SREBP-2 or whether SREBPs are not required for adipocyte differentiation in vivo remains to be determined.

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REFERENCES

1. Brown, M. S., and Goldstein, J. L. (1997) Cell 89, 331–340
2. Shimomura, I., Shimano, H., Horton, J. D., Goldstein, J. L., and Brown, M. S. (1997) J. Clin. Invest. 99, 838–845
3. Shimano, H., Horton, J. D., Hammer, R. E., Herz, J., Goldstein, J. L., Brown, M. S., and Horton, J. D. (1997) J. Clin. Invest. 98, 1575–1584
4. Shimano, H., Shimomura, I., Hammer, R. E., Herz, J., Goldstein, J. L., Brown, M. S., and Horton, J. D. (1997) J. Clin. Invest. 98, 1575–1584
5. Horton, J. D., Shimomura, I., Brown, M. S., Hammer, R. E., Goldstein, J. L., and Shimano, H. (1998) J. Clin. Invest. 101, 2331–2339
6. Pai, J., Guruv, O., Brown, M. S., and Goldstein, J. L. (1998) J. Biol. Chem. 273, 26138–26148
7. Sakai, J., Nohturfft, A., Goldstein, J. L., and Brown, M. S. (1998) J. Biol. Chem. 273, 5785–5793
Diminished Hepatic Response to Fasting/Refeeding and Liver X Receptor Agonists in Mice with Selective Deficiency of Sterol Regulatory Element-binding Protein-1c
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