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Sterol Regulatory Element-binding Protein-1 as a Key Transcription Factor for Nutritional Induction of Lipogenic Enzyme Genes*

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To elucidate the physiological role of sterol regulatory element-binding protein-1 (SREBP-1), the hepatic mRNA levels of genes encoding various lipogenic enzymes were estimated in SREBP-1 gene knockout mice after a fasting-refeeding treatment, which is an established dietary manipulation for the induction of lipogenic enzymes. In the fasted state, the mRNA levels of all lipogenic enzymes were consistently low in both wild-type and *SREBP-1*$^{-/-}$ mice. However, the absence of SREBP-1 severely impaired the marked induction of hepatic mRNAs of fatty acid synthetic genes, such as acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase, that was observed upon refeeding in the wild-type mice. Furthermore, the refeeding responses of other lipogenic enzymes, glycerol-3-phosphate acyltransferase, ATP citrate lyase, malic enzyme, glucose-6-phosphate dehydrogenase, and S14 mRNAs, were completely abolished in *SREBP-1*$^{-/-}$ mice. In contrast, mRNA levels for cholesterol biosynthetic genes were elevated in the refed *SREBP-1*$^{-/-}$ livers accompanied by an increase in nuclear SREBP-2 protein. When fed a high carbohydrate diet for 14 days, the mRNA levels for these lipogenic enzymes were also strikingly lower in *SREBP-1*$^{-/-}$ mice than those in wild-type mice. These data demonstrate that SREBP-1 plays a crucial role in the induction of lipogenesis but not cholesterol biosynthesis in liver when excess energy by carbohydrates is consumed.

Cholesterol and fatty acids are the primary lipids synthesized in liver. However, biosynthetic pathways for cholesterol and fatty acids are under distinct and separate regulation (for a review, see Ref. 1). In contrast to cholesterol synthesis, which is tightly regulated by a feedback system to maintain cellular cholesterol levels, fatty acid synthesis is driven primarily by the availability of carbohydrates and the actions of hormones such as insulin. Despite these different patterns of regulation, recent evidence suggests that both biosynthetic pathways can be controlled by a common family of transcription factors designated sterol regulatory element binding proteins (SREBPs)[1] (reviewed in Ref. 2). SREBPs belong to a large class of transcription factors containing basic helix-loop-helix-leucine zipper. Unlike other members of this class, SREBPs are synthesized as membrane-bound precursors that require cleavage by a two-step proteolytic process in order to release their amino-terminal basic helix-loop-helix-leucine zipper-containing domain into the nucleus to activate their target genes in a sterol-regulated manner (2). Another unique feature of SREBPs is that they have a dual binding specificity to both classic palindromic E-boxes and nonpalindromic sterol regulatory elements (SREs) (2–4).

The SREBP target genes include enzymes of cholesterol biosynthesis: HMG-CoA reductase, HMG-CoA synthase, farnesyl diphosphate synthase, squalene synthase, and SREBP-2 itself, each of which contains a SRE or SRE-like sequence in its promoter (5–9). The SREBPs also bind to regulatory sequences in the promoters of the genes involved in the biosynthesis of fatty acids: acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase (3, 10–12) as well as glycerol-3-phosphate acyltransferase, a gene involved in the production of triglycerides (13).

To date, three SREBPs have been identified: SREBP-1a and SREBP-1c, produced from a single gene through the use of alternate promoters, and SREBP-2 from a separate gene (2). The rat homologue of SREBP-1c, named ADD1, was cloned independently as a protein that binds to E-boxes and presumably promotes adipocyte differentiation (14). All actively growing cultured cells so far studied produce predominantly SREBP-1a and SREBP-2, whereas most organs including liver from adult animals predominantly synthesize SREBP-1c and SREBP-2 (15). All three SREBPs are capable of activating each of the known target genes, although with differing efficiencies. SRBP-1c is weaker than SREBP-1a and SREBP-2 due to its shorter transactivation domain (16, 17).

To gain insight into the distinct roles of each SREBP isoform *in vivo*, transgenic mice that overexpress truncated, active nuclear forms of human SREBP-1a, -1c, or -2 in liver were produced and characterized (16, 18, 19). The different SREBP-overexpressing transgenic animals showed a different pattern of increase in hepatic synthesis and accumulation of cholesterol and/or fatty acids. These data suggest that the SREBP-1 isoforms are more selective in activating fatty acid biosynthetic genes, while SREBP-2 is more specific for controlling cholesterol biosynthesis. The mechanism for the relative specificity of

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[1] The abbreviations used are: SREBP, sterol regulatory element-binding protein; SRE, sterol regulatory element; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD, stearoyl-CoA desaturase; CYP4A2, cytochrome P-450 4A2; SCAP, SREBP cleavage activating protein; PPARα, peroxisome proliferator-activated receptor α.

each transcription factor is not currently known.

Liver, the principal organ of lipogenesis as well as cholesterogenesis, is responsible for production of triglycerides from excess dietary carbohydrate. A high carbohydrate diet induces mRNA levels for a group of genes designated lipogenic enzymes (reviewed in Refs. 20–22). This transcriptional induction of lipogenic enzymes is most prominently seen during fasting-refeeding treatment to rodents. When mice are fasted, lipogenesis declines. Refeeding fasted animals with a high carbohydrate diet causes marked induction of the mRNA levels of lipogenic enzymes to levels higher than pretreatment, which is often referred to as "overshooting." These lipogenic enzymes include not only genes for fatty acid biosynthesis, such as acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD), but also glycerol-3-phosphate acyltransferase for triglyceride synthesis; glucose-6-phosphate dehydrogenase and malic enzyme, which provide the NADPH for reductive biosynthesis; and ATP citrate lyase, which produces acetyl-CoA in the cytosol and liver-type pyruvate kinase in the glycolytic pathway. Each of these lipogenic genes is known to be regulated at a transcriptional level and has been shown to be activated in the livers of SREBP transgenic mice (16, 18, 23). The distinct regulation of SREBP-1 and -2 has been observed in the physiological response of mouse liver to nutritional change. The amounts of SREBP-1c protein and mRNA, but not those of SREBP-2, dramatically increased after refeeding fasted mice in a similar manner to lipogenic enzyme mRNAs (24). Furthermore, transgenic mice overexpressing SREBP-1c, even when fasted, mimicked the refeeding state of ACC, FAS, and SCD mRNA levels in wild-type animals (24). Taken together, the data suggest that a high level expression of nuclear SREBP-1c in the refed state could be responsible for induction of lipogenic enzymes. However, due to the artificial overexpression of SREBPs in the transgenic mice, we cannot conclude whether SREBP-1 is a physiological regulator for transcription of lipogenic enzymes. The disruption of the SREBP-1 gene caused partial embryonic lethality, with only 10–30% of expected homozygous mice surviving (25). In the initial analysis of the surviving *SREBP-1*$^{-/-}$ mice, there were no significant changes in hepatic mRNA levels of ACC, FAS, and SCD, while genes in the cholesterol biosynthetic pathway were activated (25). However, the study was performed in the nonfasted state of a regular diet, which is not a favorable condition for lipogenesis. In the current study, the SREBP-1 knockout mice were used to re-estimate the effects of the absence of functional SREBP-1 protein on expression of the lipogenic enzymes in an inducible situation (fasting-refeeding treatment or prolonged high carbohydrate diet). After these dietary manipulations, wild-type mice showed marked elevation in all lipogenic genes, while those of *SREBP-1*$^{-/-}$ mice remained suppressed, suggesting that SREBP-1 is crucial for nutritional induction of hepatic lipogenic enzymes.

EXPERIMENTAL PROCEDURES

Animals and Dietary Manipulation—Mice were housed in colony cages with a 12-h light/12-h dark cycle. Mice homozygous for disrupted SREBP-1 gene allele B (*SREBP-1*$^{-/-}$) were prepared as described previously (25) and used for the current studies with age- (30–33 weeks) and sex-matched wild-type animals on the same genetic background (hybrid between C57Black/6J and 129 Sv/Ev). For the fasting and refeeding study, groups of four female *SREBP-1*$^{-/-}$ and wild-type mice were set up. All animals had been fed on a regular chow diet until the fasting and refeeding treatment started. For the refeeding group, animals were fasted 24 h (from 22:00 to 22:00) and then refed with a high carbohydrate/fat-free diet for 12 h (22:00–10:00). This fasting and refeeding cycle was repeated three times at 3-day intervals for the refeeding group to adapt the animals to the dietary manipulation. The final fasting and refeeding cycle was performed after a 1-week interval. The mean percentage increase in the body weights during the refeeding was 8% for wild-type and *SREBP-1*$^{-/-}$ mice. For the fasting group, the animals were fasted for 24 h (10:00–10:00). Both groups were sacrificed between 10:00 and 10:30.

Materials—Regular laboratory diet and high carbohydrate/fat-free diet (70% sucrose and 20% casein) were purchased from Oriental Yeast Co., Ltd. (Osaka, Japan). Rabbit polyclonal antibody against mouse upstream stimulatory factor (USF)-1 and USF-2 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Plasma cholesterol, triglycerides, and nonesterified fatty acids were measured by enzymatic assays using commercial kits (Determiner TC, TG, and NEFA, respectively, Kyowa Medics, Co., Ltd., Tokyo, Japan). The content of cholesterol and triglycerides in liver was measured as described previously (26).

Nuclear Extract Preparation and Immunoblot Analysis—Liver nuclear extracts were prepared as described except for the use of phenylmethylsulfonyl fluoride (1 mM) instead of Pefabloc (27). The samples of 20-μg nuclear protein were subjected to immunoblot analysis with rabbit IgG against mouse SREBP-1 (amino acids 32–250) (18) or against mouse SREBP-2 (amino acids 32–250) (25), followed by horseradish peroxidase-linked goat IgG against rabbit IgG and the ECL kit (Amersham Pharmacia Biotech).

Total RNA Preparation and Blot Hybridization with cDNA Probes—Total RNA was extracted from mouse livers and parametrial adipose tissues using TRIZOL Reagent (Life Technologies, Inc.). Equal aliquots of total RNA from mice in each group were pooled (total 15 μg), subjected to formalin-denatured agarose electrophoresis, and transferred to nylon membrane (Hybond N, Amersham Pharmacia Biotech). Blot hybridization was performed with the cDNA probes labeled with [α-^{32}P]CTP (6000 Ci/mmol) using the Megaprime DNA Labeling System (Amersham Pharmacia Biotech). The cDNA probes for mouse S14, ATP citrate lyase, malic enzyme, liver-type pyruvate kinase, PPARα, acyl-CoA oxidase, cytochrome P-450 4A2 (CYP4A2), and acidic ribosomal phosphoprotein PO (36B4) were prepared by cloning reverse transcriptase-polymerase chain reaction products from mouse liver RNA into TA cloning vectors (Invitrogen). The primers used for polymerase chain reaction were as follows: for rat ATP citrate lyase, 5′ primer was ACATCTGTACCACCTCAGCCATCCAGAA and 3′ primer was GCAGTGGCGTCCACCTTGGCCGCCA (28); for rat liver-type pyruvate kinase, 5′ primer was CCCAGGAGCTGGGCACTGCCTTCTTCCAGC and 3′ primer was AGCCCGTCGTCAATGTAGATGCGGCCCCCCAC (29, 30); for mouse malic enzyme, 5′ primer was CCACCAGCGCGGCTACCTGCTGACGCGGGA and 3′ primer was CCTCTGACTCGCCGGTGCCGCAGCCCGATG (31); for mouse PPARα, 5′ primer was GCCATGGTGGACACGGAAAGCCCACTCTGCCC and 3′ primer was AGATCAGTACATGTCTCTGTAGATCTCTTG (32); for mouse acyl CoA oxidase, 5′ primer was ATGAATCCCGATCTGCGCAAGGAGC and 3′ primer was AAAGGCATGTAACCCGTAGCACTCC (33); for rat CYP4A2, 5′ primer was CTCTGTATTTAGCCCTACAAGATCCCTGGA and 3′ primer was ATGATAGCCTTGGTGTAGGACCTGGAATTT (34); and for mouse 36B4, 5′ primer was ATGATTATCCAAAATGCTTCATTG and 3′ primer was AACAGCATATCCCGAATCTCA (35). Other probes were as described previously (16, 18).

The resulting bands were quantified by exposure of the filters to BAS2000 with BAStation software (Fuji Photo Film Co., Ltd., Tokyo, Japan), and the results were normalized to the signal generated from 36B4 mRNA.

Transfections and Luciferase Assays—Human embryonic kidney 293 cells were grown at 37 °C in an atmosphere of 5% CO_2 in Dulbecco's modified Eagle's medium containing 100 units/ml penicillin and 100 μg/ml streptomycin sulfate supplemented with 10% fetal calf serum. Transfection studies were carried out with cells plated on 12-well plates as described previously (16) except that SuperFect (Qiagen) was used for transfection according to the manufacturer's protocol. The indicated amount of each expression plasmid was transfected simultaneously with 0.25 μg of a luciferase reporter plasmid (pFAS-Luc (16)) and 0.5 μg of a control pTKβ-gal reference plasmid (Promega). The total amount of DNA in each transfection was adjusted to 3 μg/well with vector DNA. The amount of luciferase activity in transfectants was normalized to the amount of β-galactosidase activity as measured by a kit (Promega).

RESULTS

To show that SREBP-1 controls transcriptional regulation of hepatic lipogenic enzymes, we hypothesized that the absence of SREBP-1 should cause an impaired response of lipogenic enzyme mRNAs in the liver to refeeding. To test this hypothesis, we performed a fasting and refeeding treatment on *SREBP-1*$^{-/-}$ and wild-type mice. Table I shows the phenotypic charac-

TABLE I

Phenotypic characteristics of wild-type and homozygous (SREBP-1$^{-/-}$) mice for the disrupted SREBP-1 gene

Each value represents the mean ± S.E. Two sets of four female age-matched wild-type and *SREBP-1*$^{-/-}$ mice on the same genetic background had been fed on a regular chow diet. One set of animals were fasted 24 h, and the other set were fasted 24 h and refed with high carbohydrate/fat-free diet 12 h prior to sacrifice at 10:00. *, **, and *** denote significance *versus* fasted wild type at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; #, ##, and ### denote significance *versus* refed wild type at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively; and $, $$, and $$$ denote significance *versus* fasted *SREBP-1*$^{-/-}$ at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Genotype	Fasted		Refed	
	Wild type	*SREBP-1*$^{-/-}$	Wild type	*SREBP-1*$^{-/-}$
Body weight (g)	24.8 ± 0.90	26.6 ± 0.81	28.5 ± 2.6	22.3 ± 0.46#$
Liver/body weight	0.036 ± 0.002	0.036 ± 0.002	0.064 ± 0.006**	0.055 ± 0.007$$
Liver cholesterol (mg/g)	2.0 ± 0.037	3.8 ± 0.39*	3.4 ± 0.39	8.4 ± 0.62###$$
Liver triglyceride (mg/g)	43.5 ± 6.5	61.0 ± 1.8	48.3 ± 8.7	30.0 ± 5.1$$
Plasma cholesterol (mg/dl)	60.7 ± 3.5	41.0 ± 4.6*	63.0 ± 7.8	40.3 ± 3.3##
Plasma triglyceride (mg/dl)	59.0 ± 4.7	46.9 ± 4.9	84.4 ± 9.1*	36.0 ± 2.0###
Plasma NEFA[a] (mg/dl)	618 ± 100	534 ± 20.0***	192 ± 70	50.5 ± 11$$$
Blood sugar (mg/dl)	72.0 ± 9.5	84.0 ± 4.5	146 ± 1.7**	145 ± 11$$

[a] Nonesterified fatty acid.

teristics of both groups in the study. The mean percentage increase in body weight during the refeeding was the same (8%) between refed wild-type and *SREBP-1*$^{-/-}$ mice. The livers from both refed groups were significantly enlarged, which is customarily observed in refed rodents. Cholesterol content in the liver was significantly higher in *SREBP-1*$^{-/-}$ mice than in wild-type mice in both fasted and refed states, which is consistent with the previous report on the nonfasted *SREBP-1*$^{-/-}$ mice (25). The fasting-refeeding treatment decreased liver triglyceride content in *SREBP-1*$^{-/-}$ mice, while no change was detected in the livers of wild-type mice. In the wild-type animals, plasma nonesterified fatty acid levels were elevated by fasting and suppressed by refeeding. In the *SREBP-1*$^{-/-}$ mice, the suppression of plasma NEFA by refeeding seemed more pronounced than that observed in wild-type mice. There was no significant difference between *SREBP-1*$^{-/-}$ and wild-type mice in blood sugar levels in either nutritional state.

Fig. 1 shows immunoblot analysis of SREBPs in liver nuclear extracts. In the fasted state, the mature form of SREBP-1 in the liver nuclear extracts pooled from wild-type mice was barely detectable, while refeeding dramatically increased the mature protein level. The aberrant protein of ~40 kDa derived from the disrupted SREBP-1 gene in the knockout mice (indicated by an *asterisk* in Fig. 1) was also markedly enhanced by refeeding. This aberrant protein consists of the N-terminal part of the native SREBP-1 and lacks the helix-loop-helix region, leading to a lack of any transactivity for SRE-containing promoters (25). The amount of the SREBP-2 protein in the nuclear extracts from *SREBP-1*$^{-/-}$ mice was approximately 2-fold increased compared with that of wild-type mice in both fasted and refed states.

Figs. 2–5 compare mRNA levels for various genes in the livers of wild-type and *SREBP-1*$^{-/-}$ mice that were fasted, or fasted and refed. Fig. 2 shows the results for SREBP-1 and -2, SREBP cleavage activating protein (SCAP), and HMG CoA synthase and reductase. In accordance with the nuclear protein data, SREBP-1 mRNA level in the fasted wild-type animals was very low and markedly induced by refeeding. The aberrant SREBP-1 transcript from the disrupted gene was also elevated in the refed liver. In contrast, there was no significant change in mRNA levels of SREBP-2 in the fasted and refed wild-type mice and in fasted SREBP−/− mice. Refed *SREBP-1*$^{-/-}$ mice showed only a slight increase in SREBP-2 mRNA level as compared with other groups. The mRNA of SCAP did not change markedly except that refed *SREBP-1*$^{-/-}$ mice had a slightly decreased level, the biological significance of which is currently unknown. The mRNA levels for both hydroxymethylglutaryl-CoA synthase and reductase, rate-limiting enzymes in the cholesterol synthetic pathway, were significantly increased only in refed SREBP−/− mice.

FIG. 1. **Immunoblot analysis of SREBP-1 and SREBP-2 in nuclear extracts from livers of fasted or refed wild-type (*WT*) and SREBP-1$^{-/-}$ (*KO*) mice.** For each group, livers from four mice shown in Table I were pooled, and aliquots (30 μg of protein) of nuclear extracts were subjected to immunoblot analysis with rabbit anti-mouse SREBP-1 or SREBP-2 IgG as the primary antibody and horseradish peroxidase-labeled donkey anti-rabbit IgG (Amersham Pharmacia Biotech) as the secondary antibody. In the nuclear extracts of *SREBP-1*$^{-/-}$ mice, aberrant SREBP-1 proteins derived from disrupted allele of the SREBP-1 gene were detected at ~40 kDa (25) as indicated by an *asterisk* for comparison with a native mature protein of 68 kDa from the wild-type SREBP-1 gene.

Fig. 3 shows changes in mRNA levels in the genes involved in fatty acid biosynthesis. The acetyl-CoA carboxylase, fatty acid synthase, and stearoyl-CoA desaturase 1 are lipogenic enzymes that play a central role in the production of major long chain monounsaturated fatty acids in mammals by conversion of acetyl-CoA to palmitoleate (C16:1) and oleate (C18:1). The mRNA levels of these enzymes were suppressed in livers of fasted wild-type mice and required longer exposure for signal detection (data not shown; see Table II for reference). They were markedly elevated in the normal livers by refeeding (Fig. 3). The mRNA levels for acetyl-CoA carboxylase and fatty acid synthase in the fasted *SREBP-1*$^{-/-}$ mice were low and essentially similar to those of fasted normal mice. However, the *SREBP-1*$^{-/-}$ mice had a blunted induction of these enzymes upon refeeding compared with wild-type mice (Fig. 3 and Table II). The absence of SREBP-1 caused significant decreases in SCD1 mRNA levels in both fasted and refed states. The most dramatic differences in the hepatic mRNA levels of refed

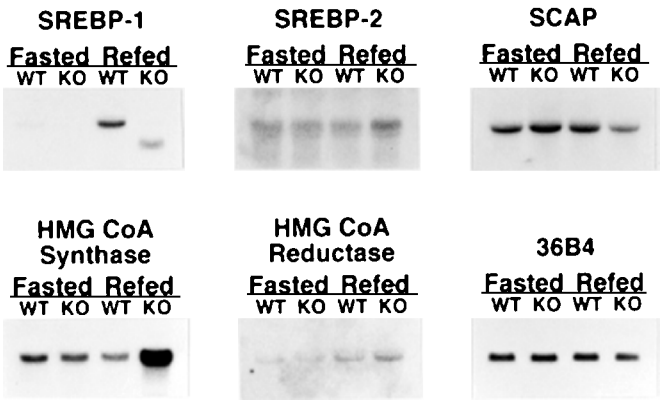

FIG. 2. **Quantification of mRNA levels of SREBP-1 and -2, SCAP, hydroxymethylglutaryl-CoA synthase, and reductase in livers from fasted or refed wild-type (*WT*) and SREBP-1$^{-/-}$ (*KO*) mice as measured by Northern blot analysis.** Total RNA was extracted from the livers of the mice in each treatment group (Table I). Equal aliquots of 15 μg were pooled and subjected to electrophoresis and blot hybridization with the indicated ^{32}P-labeled cDNA probe. The shorter band observed in *SREBP-1*$^{-/-}$ mice with SREBP-1 probe is the aberrant transcript derived from the disrupted SREBP-1 gene (25). The relative ratio of the signal from each group *versus* fasted wild-type mice was calculated after correction by the corresponding signal from 36B4 using the BAS system (Fuji) and is shown in Table II.

FIG. 3. **Quantification of mRNA levels of the genes involved in fatty acid synthesis in livers from fasted or refed wild-type (*WT*) and SREBP-1$^{-/-}$ (*KO*) mice as measured by Northern blot analysis.** The RNA filters prepared as described in the Fig. 2 legend were hybridized with ^{32}P-labeled cDNA probe of mouse acetyl-CoA carboxylase, fatty acid synthase, or stearoyl-CoA desaturase. The results of quantification by the BAS system were shown in Table II.

SREBP-1$^{-/-}$ mice were observed in other lipogenic enzymes. As depicted in Fig. 4, the genes for glycerol-3-phosphate acyltransferase, ATP citrate lyase, glucose-6-phosphate dehydrogenase, and S14 were markedly activated by refeeding in the wild-type mice (Fig. 4 and Table II). In contrast, these refeeding responses were severely reduced in *SREBP-1*$^{-/-}$ mice. Notably, the mRNA levels of glucose-6-phosphate dehydrogenase, glycerol-3-phosphate acyltransferase, and S14 in the refed *SREBP-1*$^{-/-}$ mice remained barely detectable, similar to their fasting level. The mRNA level of ATP citrate lyase in the refed *SREBP-1*$^{-/-}$ livers was also markedly lower than that in refed wild-type mice. The refeeding response of malic enzyme in wild type mice was relatively small (6-fold) as compared with other lipogenic enzymes. This induction of malic enzyme was also attenuated in SREBP-1−/− mice (Fig. 4 and Table II). These data suggested that SREBP-1 dominates transcriptional regulation for those lipogenic enzymes in the liver.

To confirm the validity of the fasting-refeeding protocol in our studies, we measured mRNA levels for genes that were known to be regulated by fasting and refeeding but in a different fashion from lipogenic enzymes. Acyl-CoA oxidase and CYP4A2 are involved in peroxisomal and microsomal oxidation of fatty acids, respectively. Induction of these enzymes has been shown to be mediated through PPARα and its ligands (36) and has also been reported to be induced by fasting and suppressed by refeeding (37). As shown in Fig. 5, both the acyl-CoA oxidase and CYP4A2 mRNAs were 4-fold higher in fasted livers than in refed livers from both wild-type and *SREBP-1*$^{-/-}$ mice, confirming the previous report (37). These data indicate that, as expected, SREBP-1 has no effect on the message levels of fatty acid oxidation genes during a fasting-refeeding cycle. The mRNAs of PPARα were also found to be regulated in a similar manner to its down stream genes, suggesting that the transcriptional change of PPARα could partially contribute to the nutritional regulation of acyl-CoA oxidase and CYP4A2 genes.

In addition to liver, adipose tissue is another organ in which lipogenic enzymes are thought to respond to fasting-refeeding at the transcription level. As shown in Fig. 6, fasting-refeeding changes in mRNA levels of lipogenic enzymes from adipose tissues of wild-type mice were less dramatic than those observed in the liver. Notably, stearoyl-CoA desaturase, glucose-6-P dehydrogenase, and pyruvate kinase had little response to refeeding. SREBP-1−/− adipose tissues exhibited impaired induction of other lipogenic enzymes, although the reduction was generally less pronounced than that observed in the livers with exception of malic enzyme in which the refeeding pattern was similar in both tissues. Quantification of the relative amount of mRNA is summarized in Table II.

Lipogenic enzymes in the liver were also induced when animals were put on a high carbohydrate diet for a prolonged period of a time. The promoter regions of lipogenic enzymes such as fatty acid synthase, liver-type pyruvate kinase, and S14 have been extensively analyzed and shown to contain cis-acting elements, identified as carbohydrate (glucose or insulin)-response elements (38–40). To determine the long term effect of a high carbohydrate diet on hepatic mRNA levels of lipogenic enzymes, two sets of wild-type and SREBP-1−/− mice were fed a high sucrose diet and a normal chow diet for 2 weeks. The mice in this series of experiments were sacrificed in a nonfasted state. The hepatic mRNA levels for lipogenic genes in the four groups of animals are shown in Fig. 7. All of the lipogenic genes were robustly induced by a high carbohydrate diet in livers of wild type mice as compared with those on a normal diet. The carbohydrate induction of acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase, ATP citrate lyase, and pyruvate kinase was partially blunted in SREBP−/− mice. In contrast, it was almost abolished in glycerol-3-phosphate acyltransferase, glucose-6-phosphate dehydrogenase, malic enzyme, and S14. Therefore, the amounts of these lipogenic enzyme mRNAs after a high carbohydrate diet were profoundly lower in SREBP-1−/− mice compared with controls, while differences between groups on a normal chow diet were not striking for any of lipogenic enzymes. This pattern is very similar to that observed in the refeeding response.

The USFs have been shown to play an important role in the transcription of fatty acid synthase, S14, and liver pyruvate kinase. To examine the roles of USFs in conjunction with the deletion of the SREBP-1 gene, we determined the amounts of USF-1 and -2 in wild-type and *SREBP-1*$^{-/-}$ mice by immunoblot analysis (Fig. 8). There was no significant difference in the amount of USF-1 or -2 protein in the liver nuclear extracts from *SREBP-1*$^{-/-}$ and wild-type mice in either fasted or refed state.

The *SREBP-1*$^{-/-}$ mice produce an aberrant mRNA and the resulting shortened protein from the disrupted SREBP-1 gene, which has been shown to be inactive for SRE-containing promoters (25). To exclude a minor possibility that this aberrant protein might have dominant negative effects on transactivity of lipogenic enzymes, causing reduction in their mRNA levels, we investigated the effect of overexpression of the aberrant SREBP-1 protein on fatty acid synthase promoter activity by luciferase reporter assays (Fig. 9). When the expression vector containing the cDNA encoding aberrant SREBP-1 protein (25)

TABLE II

Quantification of lipogenic enzyme mRNAs in livers and adipose tissues from fasted or refed wild-type and SREBP-1$^{-/-}$ mice

Values are the relative ratio of each signal *versus* the corresponding fasted wild type corrected for the signal from 36B4 as loading control.

Gene[a]	Liver				Adipose tissue			
	Fasted		Refed		Fasted		Refed	
	Wild-type	SREBP-1$^{-/-}$	Wild-type	SREBP-1$^{-/-}$	Wild-type	SREBP-1$^{-/-}$	Wild-type	SREBP-1$^{-/-}$
SREBP-1	1.0	0.6	8.5	5.3[b]	1.0	0.50	4.2	9.6[b]
SREBP-2	1.0	0.9	0.9	1.4	1.0	1.0	0.9	1.0
Lipogenic enzymes								
ACC	1.0	1.5	20	9.3	1.0	0.50	2.3	1.7
FAS	1.0	2.0	90	21	1.0	0.37	2.5	1.3
SCD	1.0	0.26	16	3.8	1.0	0.61	1.5	0.7
GPAT	1.0	0.93	8.0	0.53	1.0	1.2	3.6	2.4
ACL	1.0	3.7	103	17	1.0	0.58	29	9.5
G6PD	1.0	0.4	14	1.6	1.0	0.85	1.8	0.72
ME	1.0	1.3	5.9	1.6	1.0	0.53	5.8	1.2
LPK	1.0	2.0	19	14	1.0	0.68	1.3	0.9
S_{14}	1.0	2.2	150	13	1.0	0.66	8.5	5.1
Cholesterogenic enzymes								
HMG CoA Syn	1.0	0.92	0.80	4.0	1.0	1.2	1.7	3.4
HMG CoA Red	1.0	0.7	1.8	4.1	1.0	0.5	1.7	3.8
PPARα-inducible genes								
PPARα	1.0	1.4	0.3	0.3	ND[c]	ND	ND	ND
AOX	1.0	0.9	0.4	0.2	ND	ND	ND	ND
CYP4A2	1.0	1.1	0.22	0.16	ND	ND	ND	ND

[a] GPAT, glycerol-3-phosphate acyltransferase; ACL, ATP citrate lyase; ME, malic enzyme; Syn, synthase; Red, reductase; AOX, acyl-CoA oxidase.

[b] The signal of aberrant SREBP-1 mRNA derived from the disrupted SREBP-1 gene.

[c] ND, not determined.

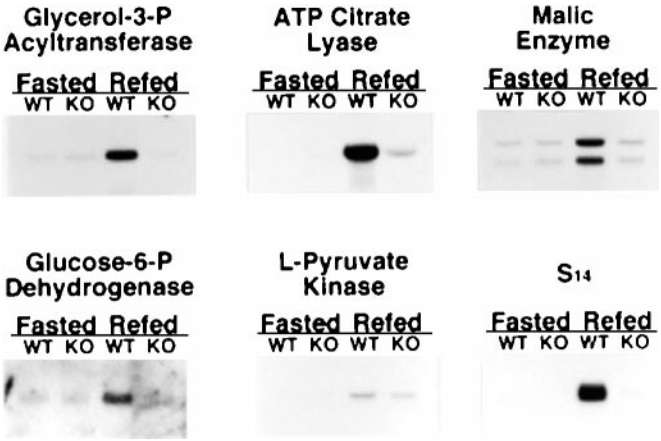

FIG. 4. **Quantification of mRNA levels of various genes supporting lipogenesis in livers from fasted or refed wild-type (*WT*) and SREBP-1$^{-/-}$ (*KO*) mice as measured by Northern blot analysis.** The RNA filters prepared as described in the Fig. 2 legend were hybridized with the indicated ^{32}P-labeled cDNA probe. The results of quantification by the BAS system were shown in Table II.

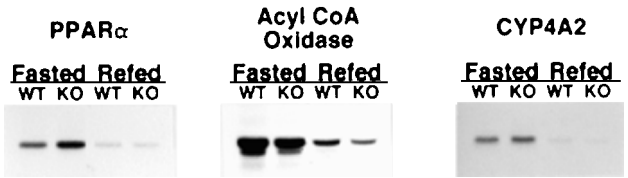

FIG. 5. **Quantification of mRNA levels of PPARα, acyl-CoA oxidase, and CYP4A2 genes in livers from fasted or refed wild-type (*WT*) and SREBP-1$^{-/-}$ (*KO*) mice as measured by Northern blot analysis.** The RNA filters prepared as described in the Fig. 2 legend were hybridized with the indicated ^{32}P-labeled cDNA probe. The results of quantification on the BAS imaging plate were shown in Table II.

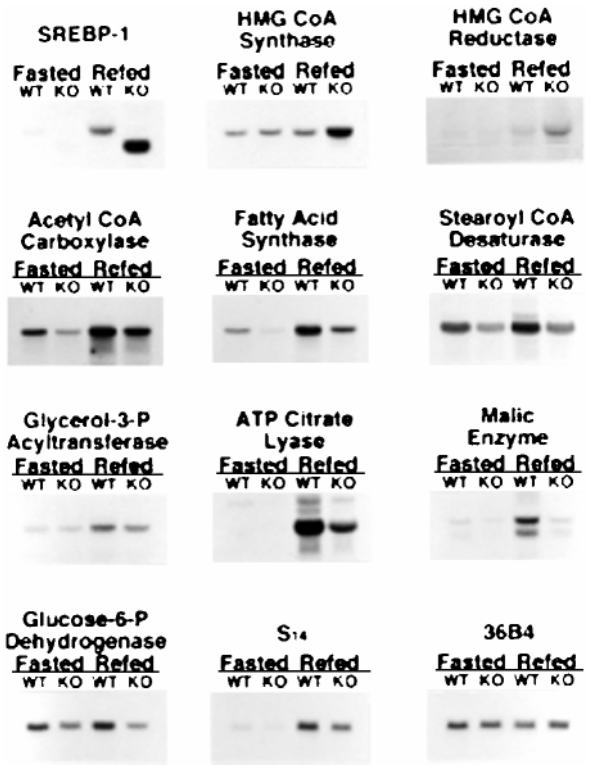

FIG. 6. **Quantification of mRNA levels of various genes in adipose tissue from fasted or refed wild-type (*WT*) and SREBP-1$^{-/-}$ (*KO*) mice as measured by Northern blot analysis.** Total RNA was isolated from parametrial adipose tissue of each treatment group (Table I). The RNA filters were prepared as described for the livers in the Fig. 2 legend and hybridized with the indicated ^{32}P-labeled cDNA probes. The shorter band observed in *SREBP-1$^{-/-}$* mice with SREBP-1 probe was the aberrant transcript derived from the disrupted SREBP-1 gene (25). The results of quantification by the BAS system were shown in Table II.

was transfected into 293 cells, there was no significant change in the luciferase activity derived from the reporter gene to which the promoter of fatty acid synthase was fused (pFAS-Luc) (25). Meanwhile, an expression vector for an authentic dominant negative version of SREBP-1 that lacks its N-terminal transactivation domain (41) suppressed the activity of FAS-luc gene in a dose-dependent manner. These data suggest that the aberrant SREBP-1 protein does not influence the promoter of the fatty acid synthase gene in either a negative or positive fashion.

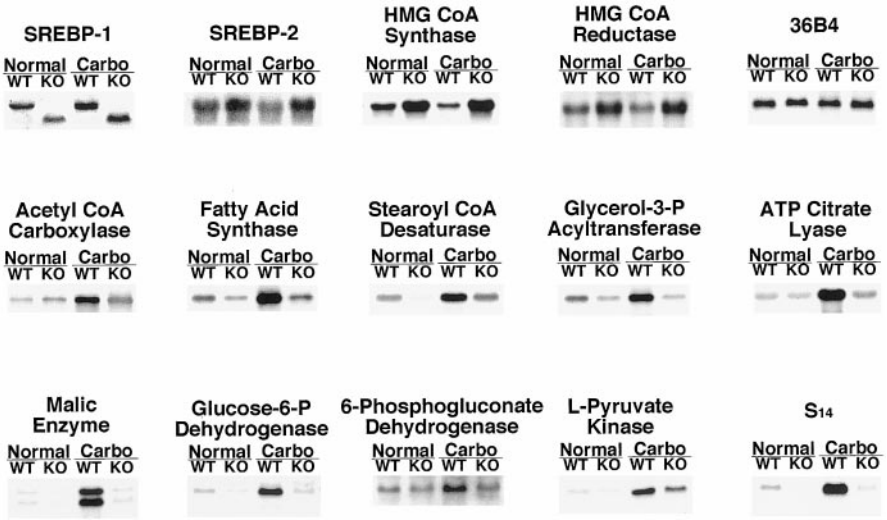

FIG. 7. **Amounts of various mRNAs in livers from wild-type (*WT*) and SREBP-1$^{-/-}$ (*KO*) mice fed a normal chow (*Normal*) and a high carbohydrate/fat-free (*Carbo*) diet for 2 weeks.** Four male wild-type and SREBP-1$^{-/-}$ mice were fed a high carbohydrate/fat-free diet or a normal regular diet for 2 weeks and sacrificed in a nonfasted state. Total RNA was prepared from the livers of each group. Equal aliquots of 15 μg were subjected to hybridization with the indicated ^{32}P-labeled cDNA probes. *GPAT*, glycerol-3-phosphate acyltransferase; *ACL*, ATP citrate lyase; *G6PD*, glucose-6-phosphate dehydrogenase; *6PG*, 6-phosphogluconate dehydrogenase; *ME*, malic enzyme; *PK*, liver pyruvate kinase.

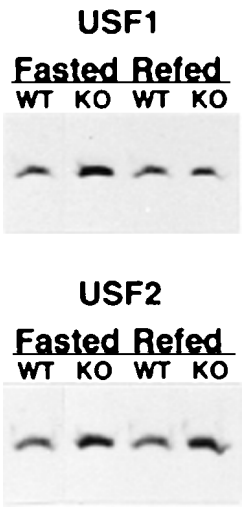

FIG. 8. **Immunoblot analysis of USF-1 and -2 in nuclear extracts from livers of wild-type (*WT*) and SREBP-1$^{-/-}$ (*KO*) mice that were fasted and refed.** The filter of nuclear extract proteins was prepared as described in Fig. 1 legend. Immunoblot analysis was carried out with rabbit anti-mouse USF-1 or -2 IgG as the primary antibody.

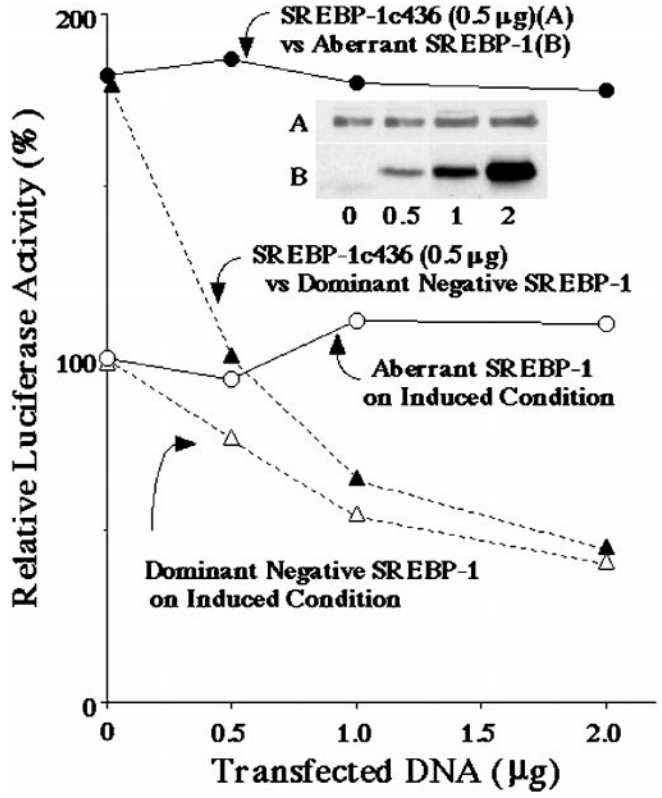

FIG. 9. **Luciferase reporter gene assay to determine the effect of the aberrant SREBP-1 protein from SREBP-1$^{-/-}$ mice on fatty acid synthase promoter activities.** The 293 cells were transfected with the indicated amount of pTK-aberrant SREBP-1c (25) (*open* or *closed circle*) or pTK-SREBP-1DN (dominant negative version of SREBP-1 under the thymidine kinase promoter) (*open* or *closed triangle*) with or without 0.5 μg of pTK-SREBP-1c463 (expression plasmid for a truncated version of SREBP-1c (25)). A reporter plasmid, the fatty acid synthase promoter fused to the luciferase gene (FAS-luc (25)), and a reference plasmid of TK-β-galactosidase gene were cotransfected. After transfection, the cells were cultured for 16 h in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 1 μg/ml 25-hydroxycholesterol, and 10 μg/ml cholesterol to suppress endogenous SREBP activity in the presence of TK-SREBP-1c463 (*closed circle* or *triangle*) or supplemented with 10% lipoprotein-deficient serum and 10 mM pravastatin to induce endogenous SREBP activities in the absence of TK-SREBP-1c463 (*open circle* or *triangle*). Luciferase activity was measured and normalized to β-galactosidase activity. The values were plotted as percentages of the endogenous activity from the cells in an induced condition. Expression of the truncated (dominant positive) and aberrant SREBP-1 proteins in the nuclei of transfected cells was confirmed by immunoblot analysis (*insets A* and *B*, respectively).

DISCUSSION

The current study clearly demonstrates that SREBP-1 plays a key role in hepatic transcriptional regulation of lipogenic enzymes. Deletion of the SREBP-1 gene markedly suppressed expression of an entire class of lipogenic enzymes in the refed state where lipogenesis should be fully induced. The similar results from *SREBP-1*$^{-/-}$ mice fed a high carbohydrate diet for 2 weeks suggest that SREBP-1 is important for long term as well as short term induction of lipogenic enzymes. In contrast, in a fasted state in which lipogenesis should be suppressed, the amount of SREBP-1 in wild-type liver was substantially low at both mRNA and protein levels. Consequently, there was no marked difference in the fasting mRNA level of each lipogenic enzyme between wild-type and *SREBP-1*$^{-/-}$ mice. Thus, SREBP-1 might not be involved in maintaining the fasting level of minimal transcription for lipogenic genes. This also partially explains the lack of a clear reduction in hepatic mRNA levels of fatty acid synthetic genes in *SREBP-1*$^{-/-}$ mice in the initial study where the animals were sacrificed in a partially fasted state due to their normal feeding pattern.

The intensity of impairment in refeeding response due to the deletion of SREBP-1 varies among lipogenic genes in the liver.

The most dramatic suppression in the refed liver was observed in glucose-6-phosphate dehydrogenase, glycerol-3-phosphate acyltransferase, and malic enzyme, for which induction of mRNA levels by the refeeding was completely abolished. SREBP-1 seems to dominate transcriptional regulation for those enzymes in the liver. The other hepatic lipogenic enzymes were also grossly suppressed, but they partially retained a refeeding response in *SREBP-1*$^{-/-}$ mice. This residual refeeding response in *SREBP-1*$^{-/-}$ mice was more prominent in the adipose tissue. These results suggest that other factors, presumably helix-loop-helix proteins, could also be involved in normal refeeding response.

The USFs have been extensively explored as possible transcriptional factors that could be responsible for the nutritional regulation of lipogenic enzymes such as fatty acid synthase, S14, and L-pyruvate kinase through binding to the carbohydrate or insulin-response elements. Both USF-1 and USF-2 homodimers and the heterodimer of the two, which is presumably the physiological form, have been shown to bind to and activate the promoter of those genes (40, 42–44). However, the role of USFs in nutritional responsiveness of lipogenic enzymes has been controversial (45, 46). It should be noted that USFs are relatively abundant proteins and are able to bind the c-Myc E-box. This binding has been thought to contribute to the difficulties encountered in analyzing DNA binding of Myc/Max/Mad network complexes (47). Recently, gene knockout mice for USF-1 and USF-2 have been generated and reported (48, 49). The mRNA levels for fatty acid synthase in the livers of refed mice deficient for USF-1 or -2 were markedly reduced, suggesting that USFs are required for fatty acid synthase gene expression (50). In our study, we showed that the amounts of USF proteins in the nuclear extracts were not changed during fasting and refeeding, whereas the nuclear active mature form of SREBP-1 was markedly suppressed in fasting and induced upon refeeding. It is likely that USFs are essential factors that are required to maintain transcriptional levels of fatty acid synthase irrespective of nutritional state, while SREBP-1 is nutritionally regulatable and is responsible for induced production of fatty acids when excess energy is available. USFs and SREBPs do not appear to compensate for each other in the knockout studies. In addition, *in vitro* data including gel shift assay have implicated that both USFs and SREBPs bind to the fatty acid synthase promoter independently, and there is no evidence for synergistic action (51). However, these observations supporting mutual independence of USFs from SREBP-1 cannot explain the fact that deficiency of either USFs or SREBP-1 caused profound suppression (70–80%) of fatty acid synthase mRNA upon refeeding. Further investigation of possible mutual interaction of SREBP-1 and USFs is needed to elucidate the molecular mechanism by which SREBP-1 and USFs are involved in activation of the fatty acid synthase gene.

Hasegawa and Uyeda *et al.* (52) recently identified a novel factor designated glucose response element-binding protein from rat liver nuclear extracts, which binds to glucose response elements of liver pyruvate kinase and fatty acid synthase genes. Estimation of the physiological significance for glucose response element-binding protein awaits its molecular cloning.

Another interesting observation was the regulation of SREBP-1 mRNA itself in the fasting-refeeding treatment. SREBP-1 mRNA of wild-type mice was decreased during the fasting and markedly induced during refeeding in the same manner as other lipogenic enzymes. Therefore, SREBP-1 is regulated in a lipogenic fashion at both mRNA and protein levels and could belong to the same family of lipogenic enzymes. The data from transgenic mice demonstrated that SREBP-1 could transactivate the SREBP-1 gene itself, which might explain the overshooting phenomenon of lipogenesis at refeeding by a positive feedback system in SREBP-1 transcriptional regulation. However, in the refed *SREBP-1*$^{-/-}$ mice, an aberrant transcript derived from the intact promoter of disrupted SREBP-1 gene was also induced in the same way as native SREBP-1 in the wild-type mice, while the downstream lipogenic mRNAs were entirely suppressed in the absence of functional SREBP-1. These data implicate the following intriguing possibility. Although SREBP-1 directly regulates and its absence impairs lipogenic enzyme transcription, there may be an upstream factor or mechanism which controls transcription of SREBP-1. The primary response to refeeding at this level was normal as demonstrated by the induction of the aberrant SREBP-1 mRNA.

It is surprising that a single transcriptional factor, SREBP-1, as observed in the current study, can induce such a wide range of genes. Some of the genes analyzed in the current study such as acetyl-CoA carboxylase, fatty acid synthase, stearoyl-CoA desaturase, and glycerol-3-phosphate acyltransferase, have been proven to be a direct target of SREBPs by promoter analysis (3, 10–13). The direct binding of SREBP-1 has yet to be shown for other lipogenic enzyme genes, such as ATP citrate lyase, glucose-6-phosphate dehydrogenase, malic enzyme, pyruvate kinase, and S14. An extensive promoter analysis of these genes might unveil a previously undefined mechanism for the dual binding specificity of SREBPs to SRE and E-box sequences (3) or lead to the discovery of new versions of SRE- or E-box-related sequences.

Impaired nutritional induction of lipogenesis in *SREBP-1*$^{-/-}$ mice provides further evidence that SREBP-1 is more specific to fatty acid synthesis and that SREBP-2 is more specific to cholesterol synthesis. This is demonstrated by the fact that activated SREBP-2 in the refed *SREBP-1*$^{-/-}$ mice overshot cholesterogenic genes but was not sufficient to compensate for the lack of SREBP-1 in the induction of lipogenic enzymes. As expected from the high degree of homology between SREBP-1 and -2, both molecules have the ability to bind to and activate the promoters of all cholesterogenic and lipogenic enzymes studied to date (17–19, 23). The distinct specificities of SREBP-1 and -2 for lipogenic and cholesterogenic genes *in vivo* might be reflective of nutritional activation of SREBP-1 and -2 at different levels. SREBP-2 regulates cholesterol synthetic genes through the cleavage of its precursor form to an active nuclear form in a process of interaction with SCAP and proteases in a sterol-dependent manner. Although it still requires the cleavage steps, SREBP-1 seems to regulate lipogenesis through its own mRNA level. Further studies are needed to clarify the nutritional regulation of glucose and fatty acid metabolism in lipogenic organs and to understand how insulin and/or glucose or its metabolites could signal to the transcriptional regulation of lipogenic genes presumably through SREBP-1. This information is clinically relevant to understanding the link between glucose and fatty acid metabolism, because SREBP-1 seems to be involved in insulin resistance as well as differentiation of adipocytes (53, 54).

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REFERENCES

1. Hillgartner, F. B., Salati, L. M., and Goodridge, A. G. (1995) *Physiol. Rev.* **75,** 47–76
2. Brown, M. S., and Goldstein, J. L. (1997) *Cell* **89,** 331–340
3. Kim, J. B., Spotts, G. D., Halvorsen, Y. D., Shih, H. M., Ellenberger, T., Towle, H. C., and Spiegelman, B. M. (1995) *Mol. Cell. Biol.* **15,** 2582–2588
4. Parraga, A., Bellsolell, L., Ferre-D'Amare, A. R., and Burley, S. K. (1998) *Structure* **6,** 661–672
5. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., Goldstein,

J. L., and Brown, M. S. (1993) *Cell* **75,** 187–197
6. Hua, X., Yokoyama, C., Wu, J., Briggs, M. R., Brown, M. S., Goldstein, J. L., and Wang, X. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 11603–11607
7. Ericsson, J., Jackson, S. M., Lee, B. C., and Edwards, P. A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 945–950
8. Guan, G., Jiang, G., Koch, R. L., and Shechter, I. (1995) *J. Biol. Chem.* **270,** 21958–21965
9. Sato, R., Inoue, J., Kawabe, Y., Kodama, T., Takano, T., and Maeda, M. (1996) *J. Biol. Chem.* **271,** 26461–26464
10. Kim, J. B., and Spiegelman, B. M. (1996) *Genes Dev.* **10,** 1096–1107
11. Lopez, J. M., Bennett, M. K., Sanchez, H. B., Rosenfeld, J. M., and Osborne, T. E. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 1049–1053
12. Tabor, D. E., Kim, J. B., Spiegelman, B. M., and Edwards, P. A. (1998) *J. Biol. Chem.* **273,** 22052–22058
13. Ericsson, J., Jackson, S. M., Kim, J. B., Spiegelman, B. M., and Edwards, P. A. (1997) *J. Biol. Chem.* **272,** 7298–7305
14. Tontonoz, P., Kim, J. B., Graves, R. A., and Spiegelman, B. M. (1993) *Mol. Cell. Biol.* **13,** 4753–4759
15. Shimomura, I., Shimano, H., Horton, J. D., Goldstein, J. L., and Brown, M. S. (1997) *J. Clin. Invest.* **99,** 838–845
16. Shimano, H., Horton, J. D., Shimomura, I., Hammer, R. E., Brown, M. S., and Goldstein, J. L. (1997) *J. Clin. Invest.* **99,** 846–854
17. Pai, J., Guryev, O., Brown, M. S., and Goldstein, J. L. (1998) *J. Biol. Chem.* **273,** 26138–26148
18. Shimano, H., Horton, J. D., Hammer, R. E., Shimomura, I., Brown, M. S., and Goldstein, J. L. (1996) *J. Clin. Invest.* **98,** 1575–1584
19. Horton, J. D., Shimomura, I., Brown, M. S., Hammer, R. E., Goldstein, J. L., and Shimano, H. (1998) *J. Clin. Invest.* **101,** 2331–2339
20. Towle, H. C. (1995) *J. Biol. Chem.* **270,** 23235–23238
21. Granner, D., and Pilkis, S. (1990) *J. Biol. Chem.* **265,** 10173–10176
22. Goodridge, A. G. (1987) *Annu. Rev. Nutr.* **7,** 157–185
23. Shimomura, I., Shimano, H., Korn, B. S., Bashmakov, Y., and Horton, J. D. (1998) *J. Biol. Chem.* **273,** 35299–35306
24. Horton, J. D., Bashmakov, Y., Shimomura, I., and Shimano, H. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95,** 5987–5992
25. Shimano, H., Shimomura, I., Hammer, R. E., Herz, J., Goldstein, J. L., Brown, M. S., and Horton, J. D. (1997) *J. Clin. Invest.* **100,** 2115–2124
26. Yokode, M., Hammer, R. E., Ishibashi, S., Brown, M. S., and Goldstein, J. L. (1990) *Science* **250,** 1273–1275
27. Sheng, Z., Otani, H., Brown, M. S., and Goldstein, J. L. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 935–938
28. Elshourbagy, N. A., Near, J. C., Kmetz, P. J., Sathe, G. M., Southan, C., Strickler, J. E., Gross, M., Young, J. F., Wells, T. N., and Groot, P. H. (1990) *J. Biol. Chem.* **265,** 1430–1435
29. Noguchi, T., Inoue, H., Chen, H. L., Matsubara, K., and Tanaka, T. (1983) *J. Biol. Chem.* **258,** 15220–15223
30. Simon, M. P., Besmond, C., Cottreau, D., Weber, A., Chaumet-Riffaud, P., Dreyfus, J. C., Trepat, J. S., Marie, J., and Kahn, A. (1983) *J. Biol. Chem.* **258,** 14576–14584
31. Sul, H. S., Wise, L. S., Brown, M. L., and Rubin, C. S. (1984) *J. Biol. Chem.* **259,** 555–559
32. Amri, E. Z., Bonino, F., Ailhaud, G., Abumrad, N. A., and Grimaldi, P. A. (1995) *J. Biol. Chem.* **270,** 2367–2371
33. Miyazawa, S., Hayashi, H., Hijikata, M., Ishii, N., Furuta, S., Kagamiyama, H., Osumi, T., and Hashimoto, T. (1987) *J. Biol. Chem.* **262,** 8131–8137
34. Heng, Y. M., Kuo, C. S., Jones, P. S., Savory, R., Schulz, R. M., Tomlinson, S. R., Gray, T. J., and Bell, D. R. (1997) *Biochem. J.* **325,** 741–749
35. Krowczynska, A. M., Coutts, M., Makrides, S., Brawerman, G. (1989) *Nucleic Acids Res.* **17,** 6408
36. Ren, B., Thelen, A. P., Peters, J. M., Gonzalez, F. J., and Jump, D. B. (1997) *J. Biol. Chem.* **272,** 26827–26832
37. Kroetz, D. L., Yook, P., Costet, P., Bianchi, P., and Pineau, T. (1998) *J. Biol. Chem.* **273,** 31581–31589
38. Moustaid, N., Beyer, R. S., and Sul, H. S. (1994) *J. Biol. Chem.* **269,** 5629–5634
39. Thompson, K. S., and Towle, H. C. (1991) *J. Biol. Chem.* **266,** 8679–8682
40. Shih, H., and Towle, H. C. (1994) *J. Biol. Chem.* **269,** 9380–9387
41. Sato, R., Yang, J., Wang, X., Evans, M. J., Ho, Y. K., Goldstein, J. L., and Brown, M. S. (1994) *J. Biol. Chem.* **269,** 17267–17273
42. Wang, D., and Sul, H. S. (1995) *J. Biol. Chem.* **270,** 28716–28722
43. Liu, Z., Thompson, K. S., and Towle, H. C. (1993) *J. Biol. Chem.* **268,** 12787–12795
44. Shih, H. M., Liu, Z., and Towle, H. C. (1995) *J. Biol. Chem.* **270,** 21991–21997
45. Shih, H. M., and Towle, H. C. (1992) *J. Biol. Chem.* **267,** 13222–13228
46. Kaytor, E. N., Shih, H., and Towle, H. C. (1997) *J. Biol. Chem.* **272,** 7525–7531
47. Sommer, A., Bousset, K., Kremmer, E., Austen, M., and Luscher, B. (1998) *J. Biol. Chem.* **273,** 6632–6642
48. Vallet, V. S., Henrion, A. A., Bucchini, D., Casado, M., Raymondjean, M., Kahn, A., and Vaulont, S. (1997) *J. Biol. Chem.* **272,** 21944–21949
49. Vallet, V. S., Casado, M., Henrion, A. A., Bucchini, D., Raymondjean, M., Kahn, A., and Vaulont, S. (1998) *J. Biol. Chem.* **273,** 20175–20179
50. Casado, M., Vallet, V. S., Kahn, A., and Vaulont, S. (1999) *J. Biol. Chem.* **274,** 2009–2013
51. Wang, D., and Sul, H. S. (1997) *J. Biol. Chem.* **272,** 26367–26374
52. Hasegawa, J., Osatomi, K., Wu, R. F., and Uyeda, K. (1999) *J. Biol. Chem.* **274,** 1100–1107
53. Kim, J. B., Sarraf, P., Wright, M., Yao, K. M., Mueller, E., Solanes, G., Lowell, B. B., and Spiegelman, B. M. (1998) *J. Clin. Invest.* **101,** 1–9
54. Shimomura, I., Hammer, R. E., Richardson, J. A., Ikemoto, S., Bashmakov, Y., Goldstein, J. L., and Brown, M. S. (1998) *Genes Dev.* **12,** 3182–3194