THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 277, No. 22, Issue of May 31, pp. 19353–19357, 2002

Printed in U.S.A.

Absence of Sterol Regulatory Element-binding Protein-1 (SREBP-1) Ameliorates Fatty Livers but Not Obesity or Insulin Resistance in *Lep^{ob}/Lep^{ob}* Mice*

Received for publication, February 15, 2002, and in revised form, March 26, 2002
Published, JBC Papers in Press, March 28, 2002, DOI 10.1074/jbc.M201584200

Naoya Yahagi‡, Hitoshi Shimano§¶, Alyssa H. Hasty‡‖, Takashi Matsuzaka§, Tomohiro Ide§, Tomohiro Yoshikawa‡, Michiyo Amemiya-Kudo‡, Sachiko Tomita‡, Hiroaki Okazaki‡, Yoshiaki Tamura‡, Yoko Iizuka‡, Ken Ohashi‡, Jun-ichi Osuga‡, Kenji Harada‡, Takanari Gotoda‡, Ryozo Nagai‡, Shun Ishibashi‡, and Nobuhiro Yamada§

From the ‡Department of Internal Medicine, University of Tokyo Graduate School of Medicine, Tokyo 113-8655 and §Department of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, Ibaraki 305-8575, Japan

Obesity is a common nutritional problem often associated with diabetes, insulin resistance, and fatty liver (excess fat deposition in liver). Leptin-deficient *Lep^{ob}/Lep^{ob}* mice develop obesity and those obesity-related syndromes. Increased lipogenesis in both liver and adipose tissue of these mice has been suggested. We have previously shown that the transcription factor sterol regulatory element-binding protein-1 (SREBP-1) plays a crucial role in the regulation of lipogenesis *in vivo*. To explore the possible involvement of SREBP-1 in the pathogenesis of obesity and its related syndromes, we generated mice deficient in both leptin and SREBP-1. In doubly mutant *Lep$^{ob/ob}$* × *Srebp-1$^{-/-}$* mice, fatty livers were markedly attenuated, but obesity and insulin resistance remained persistent. The mRNA levels of lipogenic enzymes such as fatty acid synthase were proportional to triglyceride accumulation in liver. In contrast, the mRNA abundance of SREBP-1 and lipogenic enzymes in the adipose tissue of *Lep^{ob}/Lep^{ob}* mice was profoundly decreased despite sustained fat, which could explain why the SREBP-1 disruption had little effect on obesity. In conclusion, SREBP-1 regulation of lipogenesis is highly involved in the development of fatty livers but does not seem to be a determinant of obesity in *Lep^{ob}/Lep^{ob}* mice.

Obesity is the most common nutritional problem in the United States, affecting ~33% of adults (1), and is often associated with type 2 diabetes due to insulin resistance (2).

The genetically obese *Lep^{ob}/Lep^{ob}* (*Lep$^{ob/ob}$*) mice develop obesity, glucose intolerance, insulin resistance, and fatty livers (excess fat deposition in liver) due to an inherited deficiency of the appetite-suppressing hormone, leptin (3–7). They present the most severe obesity ever known in both rodents and humans (8), and provide a good model of obesity and its related syndromes including insulin resistance and fatty liver disease.

It has been reported that lipogenesis in both liver and adipose tissue is greater in obese animals than in lean controls (5, 6). The livers of *Lep$^{ob/ob}$* mice have an increase in triglyceride content, probably due to the increased lipogenesis paralleled by elevated mRNA expression and enzymatic activity of several lipogenic enzymes such as fatty acid synthase and ATP citrate lyase (5, 6, 9).

Sterol regulatory element-binding proteins (SREBPs)[1] are transcription factors that belong to the basic helix-loop-helix-leucine zipper family and regulate enzymes responsible for the synthesis of cholesterol, fatty acids, and triglycerides (10, 11). To date, three SREBP isoforms, SREBP-1a, -1c, and -2, have been identified and characterized. SREBP-1a and -1c are transcribed from the same gene, each by a distinct promoter, and the predominant SREBP-1 isoform in liver and adipose tissue is SREBP-1c. Whereas SREBP-2 is relatively selective in transcriptionally activating cholesterol biosynthetic genes, SREBP-1c has a greater role in regulating fatty acid synthesis than cholesterol synthesis (12–15). SREBP-1c was also identified as adipocyte determination and differentiation factor-1 expressed in adipocytes and regulated during determination and differentiation of cultured adipocyte cell lines (16). Thus, the role of SREBP-1 in the regulation of lipogenesis has been established (15, 17–19). Moreover, SREBP-1 now appears to be positioned as a general mediator in the transcriptional action of insulin (20–22).

Recently, it has been reported that both SREBP-1c mRNA and its active nuclear protein are increased in *Lep$^{ob/ob}$* mouse livers (23). It is pathophysiologically intriguing and of clinical relevance to evaluate the possible involvement of SREBP-1 in the development of obesity and its related syndromes, since SREBP-1 could be a potential therapeutic target in these pathological states. These considerations prompted us to investigate the effects of SREBP-1 deletion on the phenotype of *Lep$^{ob/ob}$* mouse by targeted gene disruption.

EXPERIMENTAL PROCEDURES

Animals—Mice deficient in SREBP-1 prepared as previously described (24) were back-crossed six times into the C57BL/6J background and intercrossed with animals heterozygous at the leptin locus (*Lep$^{+/ob}$* C57BL/6J; Jackson Laboratories, Bar Harbor, ME) to generate double heterozygotes. These mice were then interbred to produce *Lep$^{ob/ob}$* mice whose *Srebp-1* genotypes were either wild-type (*Lep$^{ob/ob}$*), heterozygous (*Lep$^{ob/ob}$* × *Srebp-1$^{+/-}$*), or homozygous (*Lep$^{ob/ob}$* × *Srebp-1$^{-/-}$*). The frequency for obtaining the *Lep$^{ob/ob}$* × *Srebp-1$^{-/-}$* progeny was as low as

* This study was supported by the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Dept. of Internal Medicine, Institute of Clinical Medicine, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8575, Japan. Tel./Fax: 81-298-63-2170; E-mail: shimano-tky@umin.ac.jp.

‖ Present address: Division of Cardiovascular Medicine, Vanderbilt University School of Medicine, 383 Preston Research Building, Nashville, TN 37232-6300

[1] The abbreviations used are: SREBP, sterol regulatory element-binding protein; PPARγ, peroxisome proliferator-activated receptor γ; TNFα, tumor necrosis factor α.

TABLE I
Phenotypic characteristics of wild-type, Lep$^{ob/ob}$, and Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ mice

Each value represents the mean ± S.E. Mice used were 12 weeks of age, fed for 12 h after 24-h starvation. *, significance *versus* Lep$^{ob/ob}$ at $p < 0.05$ by Student's *t* test. Other parameters listed above have no significant difference between Lep$^{ob/ob}$ and Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ genotypes. NEFA, nonesterified fatty acid.

Genotype	Wild type	Lep$^{ob/ob}$	Lep$^{ob/ob}$ × Srebp-1$^{-/-}$
Sex	6 males	6 males, 6 females	6 males, 6 females
Body weight (g)	27.1 ± 0.25	43.3 ± 2.4	44.8 ± 1.3
Epididymal fat weight (g)	0.16 ± 0.03	2.7 ± 0.32	3.1 ± 0.28
Liver cholesterol (mg/g)	2.8 ± 0.31	3.2 ± 1.1	4.5 ± 0.84
Plasma cholesterol (mg/dl)	63.0 ± 7.8	118 ± 11	89 ± 4.1*
Plasma triglyceride (mg/dl)	84 ± 9.1	95 ± 6.4	106 ± 14
Plasma NEFA (μmol/l)	192 ± 70	543 ± 53	427 ± 55
Plasma glucose (mg/dl)	160 ± 10	278 ± 19	303 ± 27
Plasma insulin (ng/ml)	2.0 ± 0.41	162 ± 25	141 ± 31

1 in 43 of all pups born, probably due to the partial embryonic lethality of SREBP-1-null mice (24). Genotypes at the SREBP-1 locus were determined by Southern blot analysis with *Bam*HI digestion (24). Genotypes at the leptin locus were determined by a PCR-based restricted fragment length polymorphism analysis; DNA fragments amplified from genomic DNA by PCR using two primers designed on exon 2 of the leptin gene, 5′-TTTGTCCAAGATGGACCAGACT-3′ and 5′-CAGGGAGCAGCTCTTGGA-3′, were digested with *Dde*I restriction endonuclease (New England Biolabs), which cleaves only Lep^{ob} allele-derived products. The digested fragments were separated on a 2.5% NuSieve 3:1 agarose gel (BioWhittaker Molecular Applications, Rockland, ME). PCR protocol was 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min.

Mice were housed in a temperature-controlled environment with a 12-h light/dark cycle and free access to water and a standard chow diet (Oriental MF, Oriental Yeast, Tokyo, Japan) containing 60% carbohydrate, 13% fat, and 27% protein on a caloric basis. All experiments were performed with 12-week-old mice. For the Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ experiment, mice were refed for 12 h following a 24-h fast prior to sacrifice in order to minimize the variation in dietary conditions. For the fasting and refeeding study, mice were fasted for 24 h or refed for 12 h after 24-h starvation. All animals were sacrificed in an early phase of the light cycle under anesthesia with diethyl ether.

Blood Chemistries and Liver Lipid Analyses—Enzymatic assay kits were used for the determination of cholesterol (Determiner TC; cholesterol oxidase method; Kyowa Medex, Tokyo, Japan), triglycerides (TG LH; lipoprotein lipase method; Wako Chemicals, Tokyo, Japan), and nonesterified fatty acids (nonesterified fatty acid C; acyl-CoA oxidase method; Wako Chemicals). Plasma glucose was measured by ANTSENSE II (Bayer Medical, Tokyo, Japan) based on the immobilized glucose oxidase membrane/hydrogen peroxide electrode method. Plasma insulin was determined by the mouse insulin enzyme-linked immunosorbent assay kit (Wako Chemicals). The content of cholesterol and triglycerides in liver was measured as described previously (25).

RNA Isolation and Northern Blotting—Total RNA from liver and epididymal fat pad was isolated with Trizol reagent (Invitrogen), and 10-μg RNA samples equally pooled from six mice of each genotype were run on a 1% agarose gel containing formaldehyde and transferred to a nylon membrane. The cDNA probes used were cloned as previously described (15, 26). The probes were labeled with [α-^{32}P]dCTP using the Megaprime DNA labeling system kit (Amersham Biosciences). The membranes were hybridized with the radiolabeled probe in Rapid-hyb Buffer (Amersham Biosciences) at 65 °C with the exception of TNFα, for which ULTRAhyb hybridization buffer (Ambion) was used at 42 °C. The membranes were washed in 0.1× SSC, 0.1% SDS at 65 °C. Blots were exposed to Eastman Kodak Co. XAR-5 film and the BAS imaging plate for the BAS2000 BIO IMAGING ANALYZER (Fuji Photo Film, Tokyo, Japan). The quantification results obtained with the BAS2000 system were normalized to the signal generated from 36B4 (acidic ribosomal phosphoprotein P0) mRNA.

RESULTS

Effects of SREBP-1 Absence on Obesity, Insulin Resistance, and Fatty Liver in Lep$^{ob/ob}$ Mice—To assess the potential effects of SREBP-1 deficiency on obesity and its related syndromes, we intercrossed Lep$^{ob/ob}$ and SREBP-1-null mice and obtained six male and six female mice deficient in both leptin and SREBP-1 (Lep$^{ob/ob}$ × Srebp-1$^{-/-}$) in the C57BL/6J background. The frequency for obtaining the Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ progeny was 3-fold lower than expected, presumably due to the partial embryonic lethality of SREBP-1-null mice (24). This lower proportion of Srebp-1$^{-/-}$ homozygotes was similar at all *Lep* genotypes, indicating that there were no lethal interactions between the two gene deficiencies. These Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ mice showed no significant difference in body weight throughout the

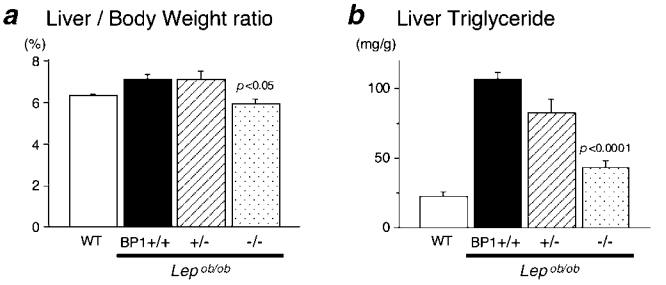

FIG. 1. **Effects of SREBP-1 disruption on fatty livers in Lep$^{ob/ob}$ mice.** Liver weight/body weight ratio (*a*) and liver triglyceride content (*b*) in wild-type (□) C57BL/6J mice, Lep$^{ob/ob}$ (■), Lep$^{ob/ob}$ × Srebp-1$^{+/-}$ (▨), and Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ (▩) mice. *Bars*, S.E. for each group. *p* values (Student's *t* test) indicate differences between Lep$^{ob/ob}$ ($n = 6$) and Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ mice ($n = 6$).

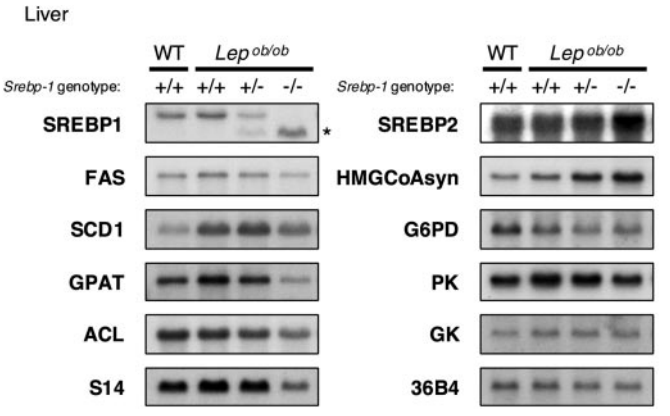

FIG. 2. **Effects of SREBP-1 deletion on the hepatic gene expression in Lep$^{ob/ob}$ mice.** Northern blot analysis of various genes including lipogenic and glycolytic enzymes in liver is shown. *Lanes* (*left* to *right*) show wild type (*WT*), Lep$^{ob/ob}$, Lep$^{ob/ob}$ × Srebp-1$^{+/-}$, and Lep$^{ob/ob}$ × Srebp-1$^{-/-}$. Total RNA (10 μg) pooled equally from six mice was subjected to Northern blotting, followed by hybridization with the indicated cDNA probes. *FAS*, fatty acid synthase; *SCD*, stearoyl-CoA desaturase; *GPAT*, glycerol-3-phosphate acyltransferase; *ACL*, ATP citrate lyase; *S14*, Spot 14; *HMGCoAsyn*, hydroxymethylglutaryl-CoA synthase; *G6PD*, glucose-6-phosphate dehydrogenase; *PK*, pyruvate kinase; *GK*, glucokinase. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading. *, the aberrant messenger RNA from the disrupted *Srebp-1* allele that encodes a truncated protein that is null for transcriptional activity. The results of quantification by the BAS imaging plate are shown in Table II.

TABLE II

Quantification of gene expression levels in livers from wild-type, Lep$^{ob/ob}$, Lep$^{ob/ob}$ × Srebp-1$^{+/-}$, and Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ mice by Northern blot analysis shown in Fig. 2

Values are the relative ratio of each signal *versus* the corresponding *Lep$^{ob/ob}$* mice corrected for the signal from 36B4 (acidic ribosomal phosphoprotein P0) as loading control. * and **, significance *versus Lep$^{ob/ob}$* at $p < 0.05$ and $p < 0.01$ (Student's t test), respectively, determined by another Northern blot analysis quantifying gene expression levels in individual mice. ND, not detectable. FAS, fatty acid synthase; SCD1, stearoyl-CoA desaturase-1; GPAT, glycerol-3-phosphate acyltransferase; ACL, ATP citrate lyase; S14, Spot 14; HMGCoAsyn, hydroxymethylglutaryl-CoA synthase; G6PD, glucose-6-phosphate dehydrogenase; PK, pyruvate kinase; GK, glucokinase.

Genotype	Wild type	*Lep$^{ob/ob}$*	*Lep$^{ob/ob}$ × Srebp-1$^{+/-}$*	*Lep$^{ob/ob}$ × Srebp-1$^{-/-}$*
SREBP1	0.83	1.00	0.53**	ND
FAS	0.77	1.00	0.90	0.72**
SCD1	0.29*	1.00	1.34	0.82*
GPAT	0.63	1.00	0.86	0.31**
ACL	1.06	1.00	0.98	0.70*
S14	0.70	1.00	0.92	0.44**
SREBP2	0.94	1.00	1.30	1.98**
HMGCoAsyn	0.73	1.00	2.06*	2.63**
G6PD	1.40	1.00	0.83	1.11
PK	0.74	1.00	1.16	0.80
GK	0.65	1.00	1.05	1.08

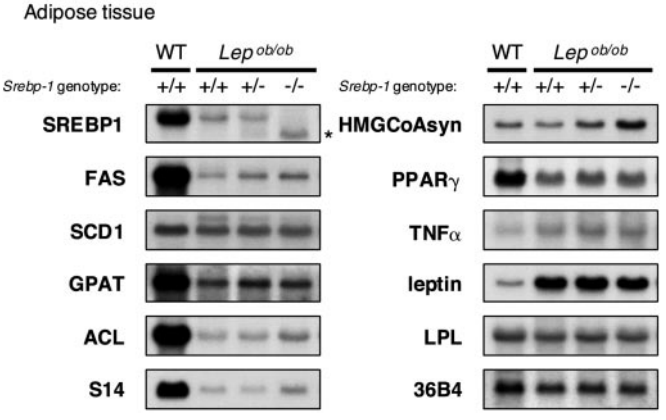

FIG. 3. **Effects of SREBP-1 absence on the mRNA expression of various genes in adipose tissue from *Lep$^{ob/ob}$* mice.** Northern blot analysis of various genes including lipogenic enzymes in adipose tissue is shown. *Lanes* (*left* to *right*) show wild type (*WT*), *Lep$^{ob/ob}$*, *Lep$^{ob/ob}$ × Srebp-1$^{+/-}$*, and *Lep$^{ob/ob}$ × Srebp-1$^{-/-}$*. Total RNA (10 μg) pooled equally from six mice was subjected to Northern blotting, followed by hybridization with the indicated cDNA probes. *FAS*, fatty acid synthase; *SCD*, stearoyl-CoA desaturase; *GPAT*, glycerol-3-phosphate acyltransferase; *ACL*, ATP citrate lyase; *S14*, Spot 14; *HMGCoAsyn*, hydroxymethylglutaryl-CoA synthase; *LPL*, lipoprotein lipase. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading. *, the aberrant messenger RNA from the disrupted *Srebp-1* allele that encodes a truncated protein. The results of quantification by the BAS imaging plate are shown in Table III.

study period (until 12 weeks of age) or in epididymal or parametrial fat pad weight compared with *Lep$^{ob/ob}$* mice of either sex (Table I). In addition, the plasma glucose and insulin concentration of *Lep$^{ob/ob}$ × Srebp-1$^{-/-}$* mice were elevated to similar levels as those of *Lep$^{ob/ob}$* controls, suggesting that they had an equal level of insulin resistance irrespective of their *Srebp-1* genotype. These results indicated that SREBP-1 absence had little influence on the development of obesity and insulin resistance originating from leptin deficiency. Histological examination of the adipose tissue from *Lep$^{ob/ob}$ × Srebp-1$^{-/-}$* mice revealed no change in adipocyte hypertrophy as compared with *Lep$^{ob/ob}$* mice (data not shown). Plasma cholesterol levels of double homozygotes were significantly lower than those of *Lep$^{ob/ob}$* mice. Neither plasma triglyceride nor nonesterified fatty acid was significantly altered by SREBP-1 disruption.

In contrast, the adiposity of *Lep$^{ob/ob}$* mouse liver was greatly influenced by the *Srebp-1* genotype. The triglyceride content in the livers of *Lep$^{ob/ob}$ × Srebp-1$^{-/-}$* mice was less than one-half that of *Lep$^{ob/ob}$* mice, and the *Srebp-1* heterozygotes showed an intermediate value (Fig. 1*b*). The total liver weight of *Lep$^{ob/ob}$ × Srebp-1$^{-/-}$* mice was also decreased in comparison with *Lep$^{ob/ob}$* controls (Fig. 1*a*), partly due to the diminished triglyceride accumulation. Cholesterol content in the livers of *Lep$^{ob/ob}$ × Srebp-1$^{-/-}$* mice was higher than in *Lep$^{ob/ob}$* mice, although no statistical significance was observed.

We concluded from these findings that the absence of SREBP-1 had no effect on the obesity and insulin resistance of *Lep$^{ob/ob}$* mice; however, it did ameliorate triglyceride accumulation in liver.

Mechanisms by Which SREBP-1 Disruption Attenuates Fatty Liver in Lep$^{ob/ob}$ Mice—To elucidate the underlying mechanisms for the amelioration of fatty livers by SREBP-1 knockout in *Lep$^{ob/ob}$* mice, we evaluated the hepatic mRNA expression of various lipogenic enzymes by Northern blot analysis (Fig. 2, Table II). Total RNA was extracted from the livers of six mice of each genotype in a fed state. The mRNA abundance of SREBP-1 was reduced in *Lep$^{ob/ob}$ × Srebp-1$^{+/-}$* and was completely abolished in *Lep$^{ob/ob}$ × Srebp-1$^{-/-}$* mice. The protein product of the aberrant mRNA (denoted by *asterisk* in Figs. 2–4) from the disrupted allele was previously reported to be inactive as a transcription factor (15, 24). By Northern blot analysis, we were able to show that the mRNA levels of various lipogenic enzymes such as fatty acid synthase, stearoyl-CoA desaturase 1, glycerol-3-phosphate acyltransferase, ATP citrate lyase, and spot 14 were decreased by SREBP-1 deletion in *Lep$^{ob/ob}$* mice livers. Meanwhile, mRNA levels for hydroxymethylglutaryl-CoA synthase, a key enzyme of cholesterol biosynthesis, and its controlling transcription factor, SREBP-2, were reciprocally increased in *Lep$^{ob/ob}$ × Srebp-1$^{-/-}$* mice. These differences were confirmed to be statistically significant by another Northern blot analysis quantifying gene expression levels in individual animals. The expression levels of glucokinase and pyruvate kinase, major glycolytic enzymes, were not altered by SREBP-1 disruption.

Distinct Influence of SREBP-1 Absence on Lipogenesis in Adipose Tissue—While the adiposity of liver was significantly decreased in the *Lep$^{ob/ob}$×Srebp-1$^{-/-}$* mice, adipose tissue mass was unchanged. To investigate the mechanism by which this occurs, we evaluated the mRNA levels of various lipogenic genes in the adipose tissue of mice from each group by Northern blot analysis (Fig. 3, Table III). Quite unexpectedly, the mRNA abundance of lipogenic enzymes was markedly suppressed in adipose tissue of *Lep$^{ob/ob}$* mice compared with wild-type. Consistently, the mRNA level of SREBP-1 was also profoundly reduced in *Lep$^{ob/ob}$* mouse adipose tissue. The disruption of SREBP-1 did not further decrease the lipogenic gene expression in adipose tissue of *Lep$^{ob/ob}$* mice at all.

TABLE III

Quantification of gene expression levels in adipose tissue from wild-type, Lep$^{ob/ob}$, Lep$^{ob/ob}$ × Srebp-1$^{+/-}$, and Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ mice by Northern blot analysis shown in Fig. 3

Values are the relative ratio of each signal *versus* the corresponding Lep$^{ob/ob}$ mice corrected for the signal from 36B4 (acidic ribosomal phosphoprotein P0) as loading control. * and **, significance *versus* Lep$^{ob/ob}$ at $p < 0.05$ and $p < 0.01$ (Student's t test), respectively, determined by another Northern blot analysis quantifying gene expression levels in individual mice. ND, not detectable. FAS, fatty acid synthase; SCD, stearoyl-CoA desaturase-1; GPAT, glycerol-3-phosphate acyltransferase; ACL, ATP citrate lyase; S14, Spot 14; HMGCoAsyn, hydroxymethylglutaryl-CoA synthase; G6PD, glucose-6-phosphate dehydrogenase; PK, pyruvate kinase; GK, glucokinase.

Genotype	Wild type	Lep$^{ob/ob}$	Lep$^{ob/ob}$ × Srebp-1$^{+/-}$	Lep$^{ob/ob}$ × Srebp-1$^{-/-}$
SREBP1	4.04**	1.00	0.71**	ND
FAS	10.83**	1.00	1.18	1.55
SCD1	0.86	1.00	0.95	0.96
GPAT	3.49**	1.00	1.21	1.10
ACL	10.32**	1.00	1.03	1.63
S14	6.49**	1.00	0.76	1.57
HMGCoAsyn	0.75	1.00	1.51*	2.49**
PPARγ	1.53*	1.00	1.07	1.14
TNFα	0.39*	1.00	1.10	1.08
Leptin	0.11**	1.00	1.07	0.91
LPL	0.70	1.00	1.03	0.98

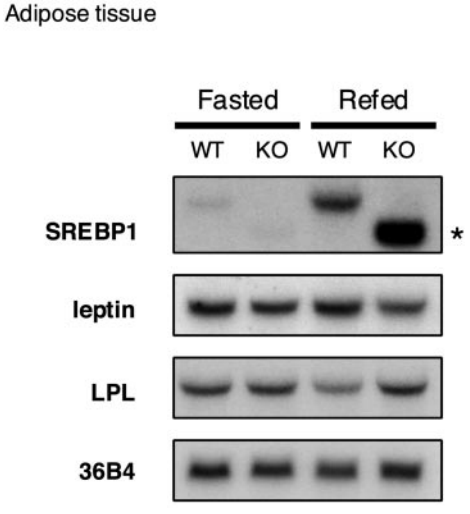

FIG. 4. **Effects of SREBP-1 absence on mRNA levels of leptin and lipoprotein lipase genes in the adipose tissue of lean mice.** Northern blot analysis of leptin and lipoprotein lipase (*LPL*) genes in adipose tissue from wild-type (*WT*) and *Srebp-1*$^{-/-}$ (*KO*) mice (four male mice each) was conducted in a fasted (*left two lanes*) or refed state in the *Lep*$^{+}$/*Lep*$^{+}$ background. Total RNA (10 μg) pooled equally from four mice was run in each lane. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading. *, the aberrant messenger RNA from the disrupted *Srebp-1* allele that encodes a truncated protein.

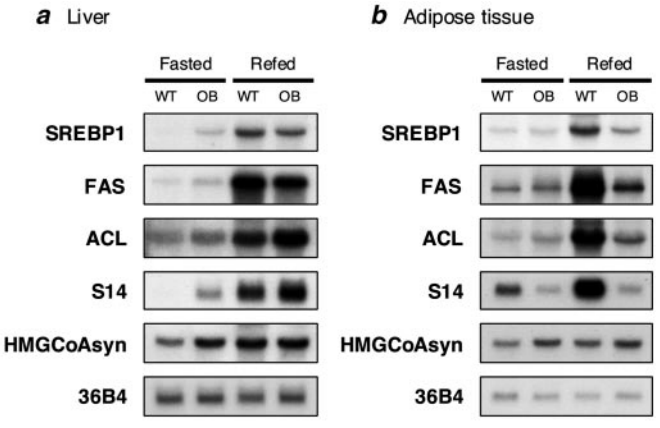

FIG. 5. **Comparison of refeeding responses in lipogenic genes between liver and adipose tissue from Lep$^{ob/ob}$ mice.** Northern blot analysis of lipogenic enzymes in liver (*a*) and adipose tissue (*b*) is shown. Wild-type (*WT*) and Lep$^{ob/ob}$ (*OB*) mice (six male mice each) are compared in a fasted (*left two lanes*) or refed state. Total RNA (10 μg) pooled equally from six mice was run in each lane. *FAS*, fatty acid synthase; *ACL*, ATP citrate lyase; *S14*, Spot 14; *HMGCoAsyn*, hydroxymethylglutaryl-CoA synthase. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

Effects of SREBP-1 Disruption on Markers for Insulin Resistance—Several adipocyte-derived genes such as tumor necrosis factor α (TNFα) and peroxisome proliferator-activated receptor γ (PPARγ) have been implicated to be related to insulin resistance in adipose tissue (27), and their mRNA levels were also examined in our study. We demonstrated that the expression of PPARγ, a nuclear receptor for thiazolidinedions, insulin-sensitizing drugs, was decreased, whereas TNFα, which has been suggested to be a causal cytokine for insulin resistance, was increased in the adipose tissue of Lep$^{ob/ob}$ mice compared with wild type (Fig. 3, Table III), both of which reflect the insulin-resistant state of Lep$^{ob/ob}$ mice. However, the comparison of Lep$^{ob/ob}$ and Lep$^{ob/ob}$ × Srebp-1$^{-/-}$ mice revealed that neither PPARγ nor TNFα was altered by the absence of SREBP-1 in the Lep$^{ob/ob}$ mouse adipose tissue. These data provided further evidence that SREBP-1 is not involved in the insulin resistance of Lep$^{ob/ob}$ mice. The expression levels of lipoprotein lipase (17, 28) and leptin (29) that had been reported to be regulated by SREBP-1 were also independent of *Srebp-1* genotype, which held true again in the Lep^{+}/Lep^{+} background (Fig. 4).

Defective Responses of Lep$^{ob/ob}$ Lipogenic Enzymes in Adipose Tissue to Dietary Manipulation—Lipogenic enzymes are known to be markedly induced in liver and adipose tissue when animals are refed after starvation. To further study the unexpected suppression of lipogenic genes in Lep$^{ob/ob}$ mouse adipose tissue, we evaluated the refeeding response in the adipose tissue of wild-type and Lep$^{ob/ob}$ mice (Fig. 5). Livers from refed wild-type and Lep$^{ob/ob}$ mice displayed a similar extent of induction of all lipogenic genes including the *Srebp-1* gene. In contrast, the mRNA levels of SREBP-1 and lipogenic enzymes in the adipose tissue stayed markedly repressed in the Lep$^{ob/ob}$ mice even after refeeding, whereas wild-type adipose tissue showed robust refeeding responses. These data demonstrated that the adipose tissue of Lep$^{ob/ob}$ mice had dysregulation of lipogenic gene expression in a fed state, whereas the liver of Lep$^{ob/ob}$ mice was normal in the refeeding responses.

DISCUSSION

The current study clearly demonstrates that SREBP-1 plays a crucial role in the development of fatty livers in Lep$^{ob/ob}$ mice. The disruption of SREBP-1 caused a significant reduction in hepatic expression of a battery of lipogenic genes and prevented fatty livers in Lep$^{ob/ob}$ mice, indicating that SREBP-1 controls triglyceride accumulation in the liver by regulating the

expression levels of lipogenic enzymes. These data are in accordance with previous reports that the overexpression of SREBP-1 induced lipogenic enzymes and resulted in fatty livers in several mouse models including SREBP-1a and -1c transgenic mice (12, 23, 26). It can be concluded, therefore, that SREBP-1 is a key transcription factor that nutritionally regulates hepatic gene expression of lipogenic enzymes and triglyceride deposition in the liver.

The observations from adipose tissue showed a remarkable contrast to those from liver. We demonstrated from the comparison of wild-type and *Lep$^{ob/ob}$* mice that the mRNA levels of lipogenic enzymes and adipose tissue mass were not correlated, suggesting that lipogenesis in adipose tissue is not the primary cause of obesity in *Lep$^{ob/ob}$* mice. The lower expression of SREBP-1 and lipogenic enzymes in the *Lep$^{ob/ob}$* mouse adipose tissue as compared with those in wild-type has also been described elsewhere as a part of the microarray analysis (30, 31). The difference was more pronounced in our experiments that were performed in a refed state (Fig. 5). It has been shown by enzymatic assay experiments that lipogenesis in *Lep$^{ob/ob}$* mouse adipose tissue is elevated in the early dynamic phase until 7–8 weeks of age and thereafter suppressed in the late static phase when insulin resistance becomes evident (32, 33). In contrast, hepatic lipogenesis in *Lep$^{ob/ob}$* mice remains consistently higher than in wild-type animals. Therefore, lipogenesis is not likely to be important in the sustained hypertrophy of adipocytes in older *Lep$^{ob/ob}$* mice. These results can be explained by the fact that adipose mass is related not only to *de novo* fatty acid synthesis but also to fatty acid intake mediated through lipoprotein lipase (34). In our data, the expression of lipoprotein lipase was not affected by SREBP-1 absence, which could be another reason for sustained obesity in the doubly mutant mice. Adipocyte hypertrophy can be also influenced by lipolysis through the action of hormone-sensitive lipase (35).

We conclude from these results that the inherent suppression of SREBP-1 in *Lep$^{ob/ob}$* mouse adipose tissue was the primary cause of the ineffectiveness of SREBP-1 disruption on obesity. Another factor that might explain the phenotypic discrepancy between liver and adipose tissue in the *Lep$^{ob/ob}$* × *Srebp-1$^{-/-}$* mice is the different contribution of SREBP-1 to the regulation of lipogenesis in the two organs (15); the disruption of SREBP-1 strongly suppressed the refeeding responses of lipogenic enzymes in the liver, whereas their expression was less influenced by SREBP-1 levels in the adipose tissue. It is possible that there are other specific transcription factors that regulate lipogenesis in adipocytes and that SREBP-1 contribution is of minor importance in this tissue (15).

We demonstrated that the adipose tissue of older *Lep$^{ob/ob}$* mice could not respond fully to the refeeding manipulation, whereas the livers responded normally at least with respect to lipogenesis. Given that the refeeding response of lipogenesis highly depends upon insulin action, it could be said that hyperinsulinemia in *Lep$^{ob/ob}$* mice could overcome insulin resistance to lipogenesis in the liver but could not in the adipose tissue. This is consistent with previous reports that the adipose tissue was more affected in an insulin-resistant state of *Lep$^{ob/ob}$* mice than the liver where lipid synthesis remained sensitive to insulin stimulation throughout life (6).

It has been reported that nonalcoholic fatty liver disease is associated with insulin resistance and hyperinsulinemia even in lean subjects with normal glucose tolerance (36). Fatty liver disease and insulin resistance in these patients might represent an initial stage of the metabolic syndrome X (37, 38). In this study, we were able to segregate fatty liver disease from insulin resistance syndromes by the disruption of SREBP-1, an indication that excess triglyceride accumulation in the liver is not a cause but rather the result of insulin resistance and hyperinsulinemia.

In summary, we demonstrated that the absence of SREBP-1 attenuated fatty livers but not obesity or insulin resistance in *Lep$^{ob/ob}$* mice. It was revealed that SREBP-1 plays a crucial role in the regulation of lipogenic gene expression and triglyceride accumulation in the liver. The ineffectiveness of SREBP-1 disruption on obesity presumably resulted from the decreased expression of SREBP-1 in *Lep$^{ob/ob}$* mouse adipose tissue. The data also suggested that lipogenesis is not a determinant of obesity in *Lep$^{ob/ob}$* mice.

Acknowledgments—We are grateful to Drs. Michael S. Brown and Joseph L. Goldstein for continuous support for the project. We appreciate Dr. K. Komeda for support in maintaining mouse colonies.

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