Original Article

Leptin Suppresses Stearoyl-CoA Desaturase 1 by Mechanisms Independent of Insulin and Sterol Regulatory Element–Binding Protein-1c

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Stearoyl-CoA desaturase (SCD)1 catalyzes the rate-limiting reaction of monounsaturated fatty acid (MUFA) synthesis and plays an important role in the development of obesity. SCD1 is suppressed by leptin but induced by insulin. We have used animal models to dissect the effects of these hormones on SCD1. In the first model, leptin-deficient *ob/ob* mice were treated with either leptin alone or with both leptin and insulin to prevent the leptin-mediated fall in insulin. In the second model, mice with a liver-specific knockout of the insulin receptor (LIRKO) and their littermate controls (LOXs) were treated with leptin. As expected, leptin decreased SCD1 transcript, protein, and activity by >60% in *ob/ob* and LOX mice. However, the effects of leptin were not diminished by the continued presence of hyperinsulinemia in *ob/ob* mice treated with both leptin and insulin or the absence of insulin signaling in LIRKO mice. Furthermore, genetic knockout of sterol regulatory element–binding protein (SREBP)-1c, the lipogenic transcription factor that mediates the effects of insulin on SCD1, also had no effect on the ability of leptin to decrease either SCD1 transcript or activity. Thus, the effect of leptin on SCD1 in liver is independent of insulin and SREBP-1c, and leptin, rather than insulin, is the major regulator of hepatic MUFA synthesis in obesity-linked diabetes. *Diabetes* 55: 2032–2041, 2006

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ACC, acetyl-CoA carboxylase; CNS, central nervous system; IRS, insulin receptor substrate; LXR, liver X receptor; MCH, melanin-concentrating hormone; MUFA, monounsaturated fatty acid; SCD, stearoyl-CoA desaturase; SFA, saturated fatty acid; SREBP; sterol regulatory element–binding protein.

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Stearoyl-CoA desaturases (SCDs) catalyze the rate-determining reaction in the synthesis of monounsaturated fatty acids (MUFAs), the introduction of a double bond in the Δ-9 position of acyl-CoAs (rev. in 1). There are four isoforms of SCD in the mouse, which are differentially regulated and vary in tissue distribution (2). SCD1 is the predominant isoform in liver.

SCD1 appears to be a major regulator of energy metabolism. Mouse models of obesity (leptin-deficient *ob/ob* mice), lipoatrophy (transgenic aP2-nSREBP-1c mice), and high-fat feeding have extremely high levels of SCD1, correlating with hepatic steatosis (3,4a). Mice deficient in SCD1, on the other hand, are lean, resistant to diet-induced obesity, and insulin sensitive (5).

Given its effects on energy metabolism, it would seem that SCD1 could be an important therapeutic target for the treatment of obesity, hepatic steatosis, or insulin resistance. In fact, *ob/ob* mice made deficient in SCD1 have decreased obesity, increased insulin sensitivity and energy expenditure, and reversal of their hepatic steatosis (3). Lipoatrophic mice made deficient in SCD1 also have dramatic improvement of their hepatic steatosis and hyperinsulinemia (4).

The mechanism by which SCD exerts its effects on energy metabolism is still not entirely understood. However, recent studies suggest several possibilities (rev. in 1). First, SCD1 activity could decrease lipid oxidation since MUFAs, unlike saturated fatty acids (SFAs), cannot inhibit acetyl-CoA carboxylase (ACC)-1. Disinhibition of ACC activity would lead to an accumulation of malonyl-CoA and repression of carnitine palmitoyl transferase-1, thereby preventing fatty acid oxidation in the mitochondria. Second, by altering the ratio of MUFAs to SFAs, SCD could regulate membrane fluidity; such changes have been linked to obesity, diabetes, and cardiovascular disease. Third, SCD1 might alter expression of the genes of lipid metabolism. SCD1-deficient mice have decreased transcription of sterol regulatory element–binding protein (SREBP)-1c and lipogenic genes but increased transcription of lipid oxidation genes (5). Finally, since SCD1 knockout mice are deficient in cholesteryl esters and triglycerides, even when their diets are supplemented by high levels of MUFA, it appears that decreasing SCD1 activity could decrease lipid accumulation by limiting the supply of endogenously synthesized MUFA (6).

SCD1 is subject to regulation at the levels of transcription, mRNA stability, and enzyme activity. Nutrients such

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TABLE 1
Effects of leptin treatment on *ob/ob* mice

	Control	*ob/ob*			
		Vehicle	Leptin	Insulin	Leptin + insulin
Change in weight (g)		0 ± 0.3	−6.4 ± 1.4*	0.6 ± 0.3	−4.3 ± 0.3†
Food intake ($g \cdot day^{-1} \cdot mouse^{-1}$)	6.2 ± 0.4†	11.3 ± 0.7	1.2 ± 0.3†	9.2 ± 0.4*	2.1 ± 0.4†
Fasting blood glucose (mg/dl)	143 ± 9	268 ± 72	165 ± 21	162 ± 28	92 ± 11‡
Fasting insulin (ng/ml)	1.1 ± 0.4†	7.7 ± 0.4	1.1 ± 0.2†	17.6 ± 2.4*	7.4 ± 1.1‡
Leptin (ng/ml)	3.2 ± 0.5†	0	27.4 ± 4.5*	0	8.4 ± 1.4†‡

Data are means ± SE (n = 4–6). Vehicle-treated *ob/ob* mice and their untreated lean control littermates (Control) were compared with *ob/ob* mice treated with leptin (24 μg/day), insulin, or both for 4 days. The weight change and food intake were measured over the 4 days and the last 24 h, respectively. After 2 days of treatment, mice were fasted for 4 h and insulin and blood glucose were measured. Leptin was measured in the nonfasted state at the end of the experiment. *$P < 0.05$, †$P < 0.005$ vs. untreated *ob/ob*; ‡$P < 0.05$ vs. treatment with leptin alone.

as glucose, fructose, and cholesterol increase SCD expression, whereas polyunsaturated fatty acids decrease it (1). Hormones are also important regulators of SCD. It was discovered >20 years ago that insulin induces SCD activity in rats made diabetic with streptozotocin (7). This induction is due in part to activation of SCD1 transcription by insulin, which occurs in both animal and tissue culture models (8,9).

The effect of insulin on SCD1 appears to be largely mediated by SREBP-1c (10). SREBP-1c is a transcription factor positively regulated by insulin at the transcriptional level. It is upregulated in mouse models with hyperinsulinemia, like leptin deficiency and lipoatrophy, and downregulated in models with insulin deficiency, such as fasting and streptozotocin-induced diabetes (11–14). Mice expressing a constitutively active form of SREBP-1c have increased expression of SCD1 and increased synthesis of MUFAs, while knockout of SREBP-1c leads to a decrease in SCD1 expression (10,15).

Transcriptional profiling of livers of *ob/ob* mice has revealed that leptin also regulates SCD1 expression (3,16). Furthermore, the fact that making *ob/ob* mice deficient in SCD1 reverses much of the leptin-deficient phenotype suggests that SCD1 may be a pivotal mediator of leptin action (3).

While both insulin and leptin regulate SCD1, they also interact in a complex manner with one another. Insulin increases leptin secretion from adipocytes (17). Leptin, on the other hand, increases insulin sensitivity but decreases insulin secretion from pancreatic β-cells, leading to a fall in serum insulin levels (18). Cross talk between insulin and leptin signaling pathways has also been described (19,20). Thus, it is possible that leptin decreases SCD1 expression by decreasing serum insulin levels or by altering insulin signaling.

We use three in vivo model systems to better understand the roles of leptin and insulin in the regulation of SCD1. First, leptin-deficient *ob/ob* mice were treated with either leptin alone or both leptin and insulin, preventing the leptin-mediated fall in insulin. This allows us to compare the effects of leptin in the absence or presence of continued hyperinsulinemia. Second, liver insulin receptor knockout (LIRKO) mice, which completely lack hepatic insulin signaling, and their littermate controls (LOXs) were treated with leptin to determine the effects of leptin on SCD1 in the absence or presence of insulin signaling. Finally, SREBP-1c knockout mice and their control subjects were treated with leptin to assess the role of SREBP-1c in mediating SCD1 suppression by leptin.

RESEARCH DESIGN AND METHODS

Leptin treatment of *ob/ob* mice. Male *ob/ob* mice (aged 6 weeks) and their lean *ob* control littermates were purchased from The Jackson Laboratories and acclimated for 2 weeks before study. Mice were implanted with osmotic pumps (1007D; Alzet, Cupertino, CA) containing either PBS or recombinant mouse leptin (24 μg/day; National Hormone and Peptide Program, Harbor-UCLA Medical Center, Torrance, CA) for 4 days. Insulin-treated mice also received four subcutaneous bovine insulin pellets (Linbit; Linshin, Toronto, ON, Canada), a dose found to raise insulin levels in leptin-treated *ob/ob* mice to those found in untreated *ob/ob* mice. Fasting insulin and glucose levels were obtained in a cohort of *ob/ob* mice by subjecting them to a 6-h fast 2 days after initiating treatment with leptin or insulin.

Leptin treatment of LIRKO and SREBP-1c$^{-/-}$ mice. Male LOX (cre^{-}; IR$^{lox/lox}$) and LIRKO (cre^{+};IR$^{lox/lox}$) littermates (aged 6 weeks), generated as described previously (21), were treated with PBS or 30 μg/day leptin, as above. SREBP-1c$^{-/-}$ (15) mice were obtained from The Jackson Laboratories and backcrossed onto the 129SvEV background for six generations. Male knockout or wild-type control mice (aged 12 weeks) were implanted with pumps containing either PBS or leptin (24 μg/day; Amgen, Thousand Oaks, CA). After 4 days of treatment, mice were killed in the random-fed state and serum and liver samples were collected.

Assays. Serum glucose was measured using a glucometer; insulin levels were measured using a radioimmunoassay (*ob/ob* experiment; Linco) or enzyme-linked immunosorbent assays (LIRKO and SREBP-1c knockout experiments; Crystal Chem); leptin levels were measured using an enzyme-linked immunosorbent assay (Crystal Chem).

Real-time PCR. Total RNA was extracted and purified using the RNeasy kit (Qiagen) and used to direct cDNA synthesis using an RT-PCR kit (Clontech). RT-PCR was performed using SYBR green master mix (ABI) and 300 nmol/l of the relevant primers. Isoform-specific primers for SREBP-1c (22) have been described previously. SCD1 primers were 5′-CATCATTCTCATGGTCCTGCT-3′ and 5′-CCCAGTCGTACACGTCATTTT-3′. Expression was calculated as a function of 2^{-Ct} and normalized to TATA-binding protein expression (5′-ACCCTTCACCAATGACTCCTATG-3′ and 5′-TGACTGCAGCAAATCGCTTGG-3′).

SCD enzymatic activity and immunoblotting. Conversion of [1-^{14}C]stearoyl-CoA to [1-^{14}C]oleate was used to measure SCD enzyme activity from liver microsomes prepared from individual mice (3). These microsomes were also subjected to immunoblotting with antibodies against SCD that have been previously described (23). In the *ob/ob* experiment, SCD antibody was used at a 1:5,000 dilution; in the LIRKO experiment, it was used at a 1:500 dilution. Immunoblotting was performed per the Amersham ECL detection system kit protocol.

Hepatic lipid analysis. Hepatic lipid analysis was performed by the Lipid, Lipoprotein and Atherosclerosis Core of the Vanderbilt Mouse Metabolic Phenotyping Centers. Lipids were extracted from liver (24). Phospholipids, triglycerides, and cholesteryl esters were scraped from the plates and methylated using BF_3/methanol, as described by Morrison and Smith (25). The methylated fatty acids were extracted and analyzed by gas chromatography. All gas chromatographic analyses were carried out on an HP 5890 gas chromatograph equipped with flame ionization detectors and an HP 3365 Chemstation. Fatty acid methyl esters were identified by the computer by comparing the retention times to known standards. Inclusion of odd-chain fatty acids as standards permitted the quantitation of the amount of lipid in the sample.

Statistical analysis. Statistical analysis of the data were performed using a two-tailed unpaired t test with unequal variance. Data are presented as the means ± SE unless otherwise indicated.

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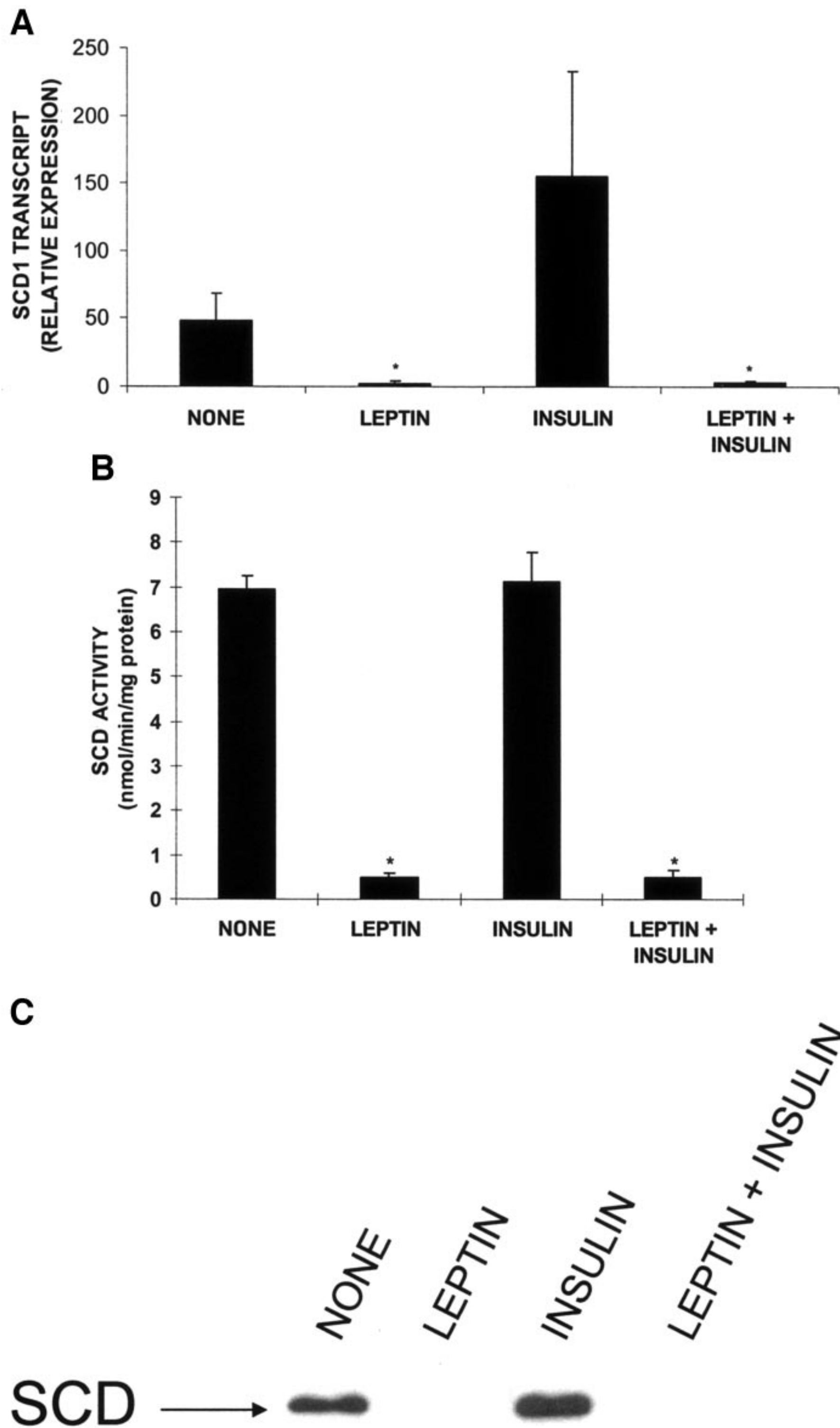

FIG. 1. Leptin suppresses SCD1 in both the presence and absence of insulin treatment. As described in RESEARCH DESIGN AND METHODS, RNA and microsomes were prepared from the livers of *ob/ob* mice treated with leptin and/or insulin. Data are presented as means ± SE for *A* and *B* (n = 4–8). *A*: SCD1 mRNA was measured using real-time RT-PCR and primers specific for SCD1. *$P \leq 7 \times 10^{-3}$ vs. untreated *ob/ob* mice. *B*: SCD activity was measured from liver microsome preparations as the production of oleoyl-CoA from [1-C^{14}]stearoyl CoA. *$P < 7 \times 10^{-5}$ vs. untreated *ob/ob* mice. *C*: Liver microsomes (27 μg) were subjected to immunoblotting with an antibody against SCD as described in RESEARCH DESIGN AND METHODS.

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RESULTS

Leptin treatment of *ob/ob* mice

Characterization of leptin-treated* ob/ob *mice. Leptin has been shown to suppress SCD1 expression in *ob/ob* mice (3). However, leptin treatment also decreases serum insulin levels, raising the possibility that the effects of leptin on SCD1 are secondary to the decrease in serum insulin. To test this hypothesis, we treated *ob/ob* mice subcutaneously through an osmotic pump for 4 days with 24 μg/day recombinant leptin (Leptin), implantation of insulin pellets (Insulin), or both (Leptin + Insulin) to maintain hyperinsulinemia in the face of leptin treatment. Untreated *ob/ob* mice (−) and mice treated only with insulin were implanted with vehicle-containing pumps; lean control mice (Control, *ob/+* or +/+) were included for comparison.

Table 1 shows that aside from raising insulin levels approximately twofold, insulin treatment alone did not

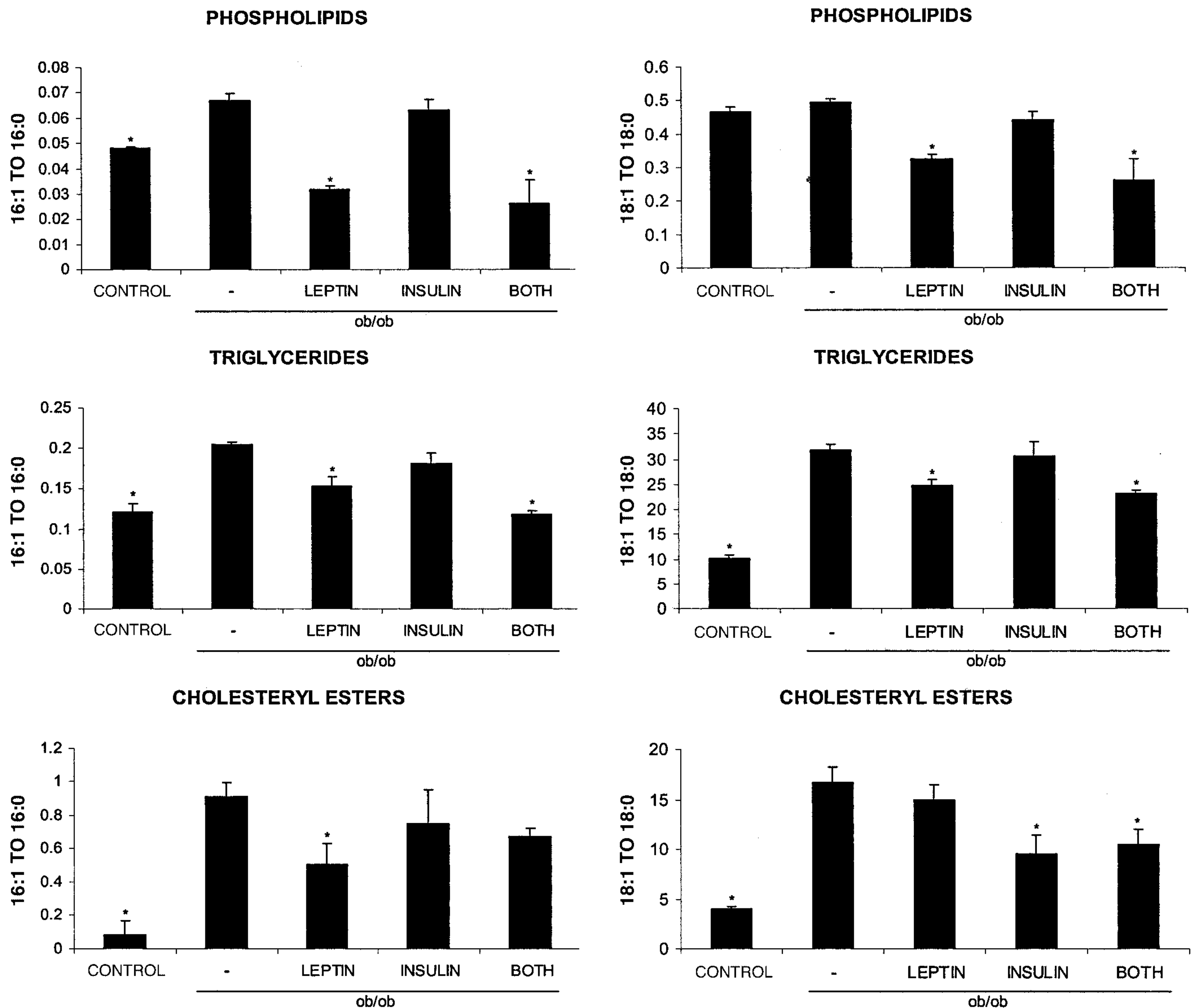

FIG. 2. Leptin decreases the proportion of hepatic MUFAs in *ob/ob* mice. Liver samples from *ob/ob* mice and their lean controls were homogenized in chloroform. Lipids were extracted, separated by thin-layer chromatography, methylated, and analyzed by gas chromatography. The ratios of palmitoleate to palmitate (16:1 to 16:0, *left panels*) and oleate to stearate (18:1 to 18:0, *right panels*) in the three major lipid fractions were calculated for each mouse and expressed as means ± SE (n = 4 for each group). *P < 0.05 compared with untreated *ob/ob*; no significant differences were found between mice treated with leptin alone compared with those treated with both leptin and insulin.

have much effect. In this experiment, insulin-treated mice ate slightly less than untreated *ob/ob* mice, but this was not a reproducible finding. Leptin treatment, on the other hand, caused dramatic weight loss (~6 vs. ~0 g in untreated mice) and decreased food intake by almost 90% compared with untreated *ob/ob* mice. Treatment with leptin also led to complete resolution of hyperinsulinemia with a decrease in serum insulin levels from 7.7 to 1.1 ng/ml, the same level found in lean controls.

When mice were treated with insulin, in addition to

TABLE 2
Effects of leptin treatment on LIRKO mice

	LOX	LOX + leptin	LIRKO	LIRKO + leptin
Change in weight (g)	0.8 ± 0.2	−0.2 ± 0.2*	1.4 ± 0.3	0.3 ± 0.3†
Food intake (g · day^{-1} · mouse^{-1})	3.5 ± 0.1	2.4 ± 0.1*	4.0 ± 0.3	2.3 ± 0.1†
Blood glucose (mg/dl)	146 ± 7	128 ± 8	179 ± 17	125 ± 10†
Insulin (ng/ml)	0.30 ± 0.06	0.17 ± 0.17	4.3 ± 0.85‡	2.0 ± 0.44†
Leptin (ng/ml)	6.6 ± 0.9	59 ± 12*	34 ± 7.7‡	229 ± 21†

Data are means ± SE (n = 4–8). LOX and LIRKO mice were treated with vehicle or leptin (30 μg/day) for 4 days. The change in weight and food intake were measured over the 4 days. Blood glucose, insulin, and leptin were measured in the nonfasted state at the end of the experiment, just prior to killing. *P < 0.05, leptin treatment of LOX; †P < 0.05, leptin treatment of LIRKO; ‡P < 0.05, LOX vs. LIRKO (untreated).

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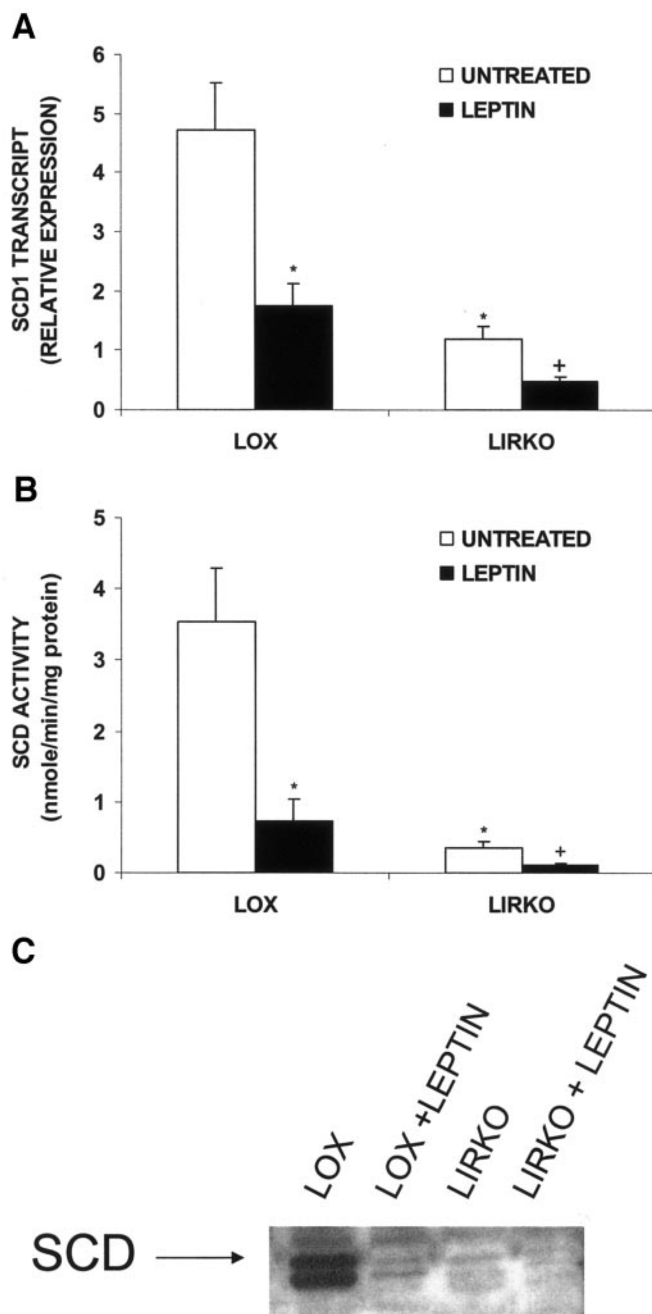

FIG. 3. Leptin decreases SCD1 in LIRKO livers. Livers were harvested from the mice (described in Table 2) and used to prepare RNA for real-time RT-PCR analysis and microsomes for assay of SCD activity and immunoblotting. Data are presented as means ± SE for *A* and *B* (n = 4–8). *A*: Real-time PCR analysis using primers specific for SCD1. *$P \leq 0.01$ vs. untreated LOX mice; +$P \leq 0.01$ for leptin treatment of LIRKO mice. *B*: SCD1 activity was measured as the production of oleoyl-CoA from [1-^{14}]stearoyl CoA. *$P < 0.03$ vs. untreated LOX mice; +$P < 0.03$ for leptin treatment of LIRKO mice. *C*: Liver microsomes (150 μg) were subjected to immunoblotting with antibodies against SCD. SCD protein was not detectable in LIRKO mice even after prolonged exposure.

leptin, they remained hyperinsulinemic with fasting insulin levels similar to those found in untreated *ob/ob* mice (7.4 vs. 7.7 ng/ml in untreated mice). Interestingly, the same dose of insulin that produced a serum insulin level of 17.6 ng/ml in the absence of leptin produced a serum insulin level of only 7.4 ng/ml in the presence of leptin. Similarly, leptin levels were more than threefold lower in mice treated with both leptin and insulin than in mice treated with leptin alone. In both experiments, insulin and leptin were derived largely, or completely, from an exogenous source at a fixed rate. Thus, insulin appeared to stimulate the clearance of leptin, and, conversely, leptin appeared to stimulate the clearance of insulin. The basis of these changes in clearance is not known but is currently under investigation. As a correlate to these hormone levels, *ob/ob* mice treated with insulin, in addition to leptin, appeared to lose less weight and eat more than mice treated with leptin alone, although they had a more dramatic improvement in blood glucose control.

SCD1 mRNA, protein, and activity in leptin-treated* ob/ob *mice. Using real-time RT-PCR, we measured SCD1 transcript in the livers of these mice (Fig. 1*A*). We found that leptin treatment of *ob/ob* mice suppressed SCD1 mRNA, and the degree of suppression was the same in either the presence or absence of exogenous insulin treatment. SCD protein paralleled mRNA levels and was undetectable in leptin-treated mice, even when they were simultaneously treated with insulin (Fig. 1*C*). SCD activity, which was measured as conversion of [1-^{14}C]stearoyl-CoA to [1-^{14}C]oleoyl-CoA by liver microsomes, also paralleled mRNA levels with a similar 10-fold suppression by leptin in the absence or presence of continued hyperinsulinemia (Fig. 1*B*). Insulin treatment, on the other hand, did not significantly alter SCD1 transcript or activity by itself, or in conjunction with, leptin treatment.

Changes in fatty acid composition in leptin-treated* ob/ob *mice. To determine whether the changes in SCD activity were reflected in the proportion of MUFAs and SFAs, hepatic lipids were extracted and the relative contribution of the major fatty acids was quantitated using gas chromatography. The ratio of palmitoleate to palmitate (16:1 to 16:0) and oleate to stearate (18:1 to 18:0) could then be calculated for each of the major lipid fractions (Fig. 2).

Treatment with insulin alone had no significant effect on the ratio of MUFAs to SFAs in the phospholipid and triglyceride fractions. Leptin, however, decreased the ratios of palmitoleate to palmitate and oleate to stearate by 30–50% in the phospholipid and triglyceride fractions with more variable effects in the cholesteryl ester fraction. Additional treatment with insulin did not diminish this effect. While leptin decreased the ratio of MUFAs to SFAs, the degree to which it restored these ratios to the normal values seen in the lean controls varied among the lipid fractions. After leptin treatment, the ratio of MUFAs to SFAs dropped below normal in the phospholipid fraction but remained high in the triglyceride and cholesteryl ester fractions.

Leptin treatment of LIRKO mice

Characterization of leptin-treated LIRKO mice. LIRKO mice lack hepatic insulin signaling entirely. This leads to unsuppressed hepatic gluconeogenesis, elevated serum insulin levels (due to both an increase in insulin secretion and a decrease in insulin clearance), and mild hyperglycemia (21). Surprisingly, they are also hyperleptinemic, with a dramatic increase in leptin-binding protein (soluble receptor) in the serum (S.E. Cohen, E. Kokkotou, S.B.B., T. Kondo, J. Kratzsch, C.S. Mantzoros, C.R.K., unpublished observations). Thus, untreated LIRKO mice had slightly higher blood glucose values, almost 15-fold higher insulin levels, and 5-fold higher leptin levels (Table 2). Despite their hyperleptinemia, LIRKO mice responded in a manner similar to LOX mice when treated with 30

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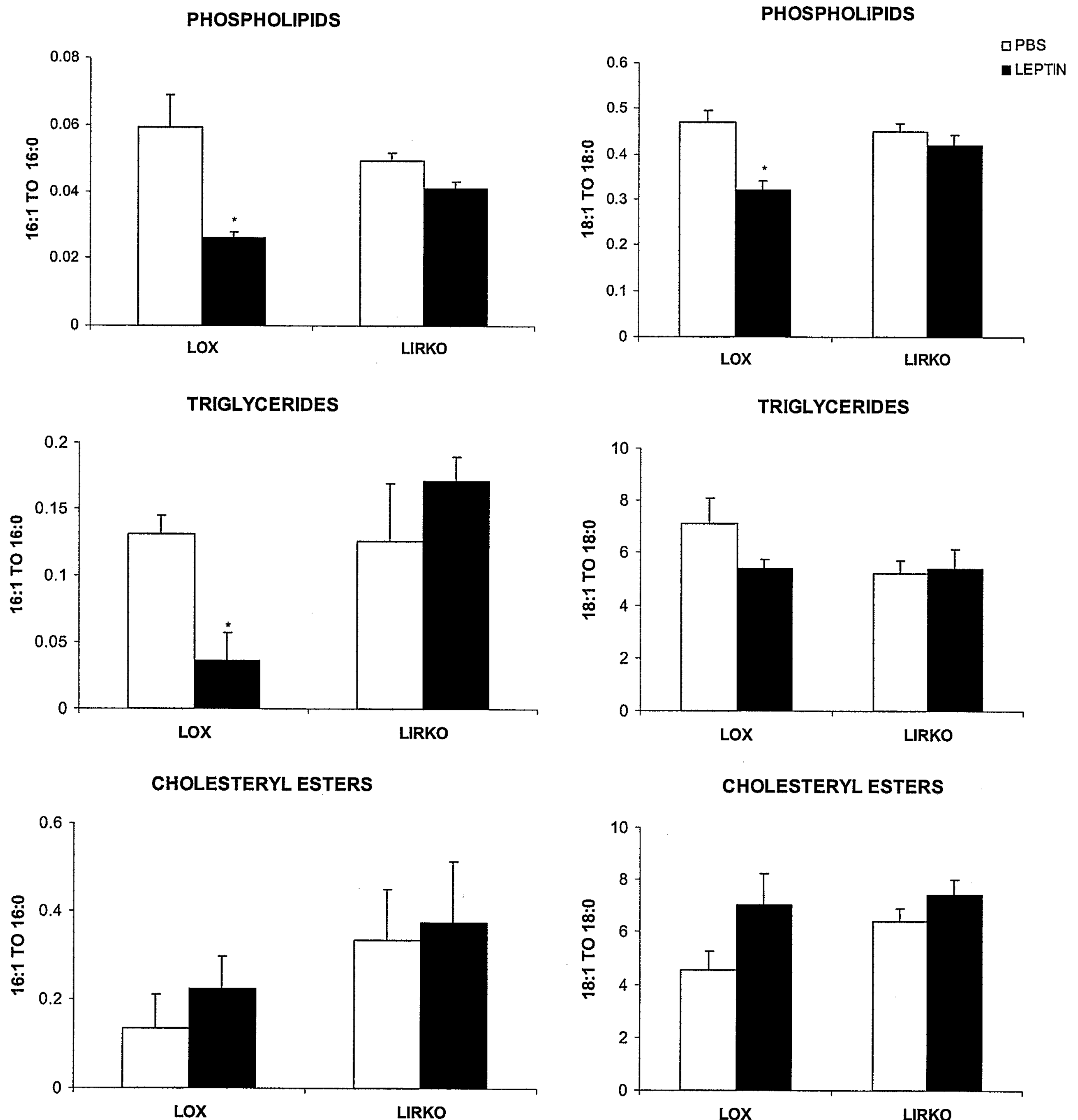

FIG. 4. Leptin does not decrease MUFA content in LIRKO mice. Lipids derived from the mice (described in Table 2) were analyzed by gas chromatography, as in Fig. 2. The ratios of palmitoleate to palmitate (16:1 to 16:0, *left panels*) and oleate to stearate (18:1 to 18:0, *right panels*) in the three major lipid fractions were calculated for each mouse and expressed as means ± SE (n = 4 for each group). *P < 0.05 vs. untreated LOX mice.

μg/day leptin through an osmotic subcutaneous pump. LOX and LIRKO mice had a similar decrease in weight gain, a 40% decrease in food intake, a 50% decrease in nonfasted insulin levels, and a 7- to 10-fold increase in serum leptin levels. However, the leptin levels achieved in the LIRKO mice were almost four times higher than those achieved in LOX mice treated with the same dose of leptin, consistent with a change in leptin clearance in the LIRKO mouse.

SCD1 transcript, protein, and activity in leptin-treated LIRKO mice. Real-time RT-PCR analysis showed that in the absence of leptin treatment, LIRKO mice had an ~80% reduction in the level of SCD1 expression compared with LOX mice (Fig. 3*A*). Nonetheless, leptin treatment decreased SCD1 mRNA by 60% in both LIRKO and LOX mice. This decrease was reflected in SCD protein levels in LOX mice. In LIRKO mice, SCD protein levels were below the threshold of detection, even in the absence of leptin,

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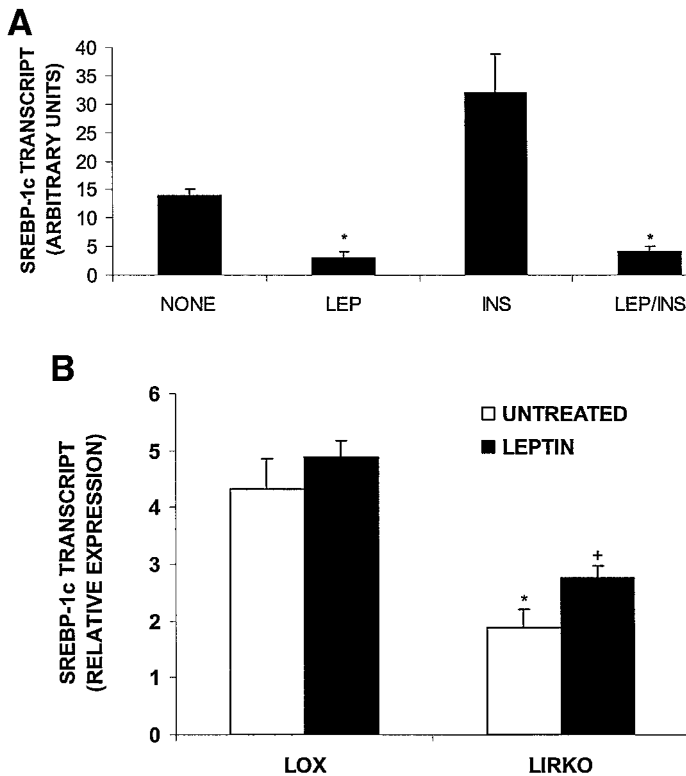

FIG. 5. Leptin decreases SREBP-1c in *ob/ob* mice but not LOX or LIRKO mice. Livers from the mice (described in Tables 1 and 2) were used to generate RNA for real-time RT-PCR analysis with primers specific for SREBP-1c or IRS-2. Data are presented as means ± SE (n = 6–8 for each group). *A*: Leptin (LEP) treatment of *ob/ob* mice decreases SREBP-1c in the presence or absence of insulin (INS). *$P < 10^{-4}$ compared with untreated *ob/ob* mice. *B*: Leptin treatment does not decrease SREBP-1c in LOX or LIRKO mice. *P = 0.002 vs. untreated LOX mice; #P < 0.05 for leptin treatment of LIRKO mice.

precluding us from determining whether leptin treatment of these mice resulted in further suppression (Fig. 3*C*). SCD activity in liver microsomes from LIRKO mice paralleled SCD1 transcript levels and was only 10% of that observed in LOX mice (Fig. 3*B*). As with SCD1 mRNA, both LOX and LIRKO mice had a 70–80% suppression of SCD activity by leptin. Thus, leptin can effectively suppress SCD transcript and activity in the liver, even in the complete absence of insulin signaling.

Changes in fatty acid composition in leptin-treated LIRKO mice. Since leptin treatment decreased SCD transcript and activity in LOX and LIRKO mice, we predicted that it would also decrease the ratio of MUFAs to SFAs in the liver. In LOX mice, leptin decreased the ratios of palmitoleate to palmitate (16:0 to 16:1) by 50–75% and oleate to stearate (18:1 to 18:0) by 30% in the phospholipid and triglyceride fractions (Fig. 4). In contrast, despite lower levels of SCD activity, untreated LIRKO mice did not have significantly lower ratios of MUFAs to SFAs when compared with LOX mice. Moreover, leptin treatment of LIRKO mice had no effect on the ratio of MUFA to SFA in any of the lipid fractions. Thus, insulin signaling, in addition to leptin, may be required to alter the composition of hepatic lipids.

Expression of SREBP-1c mRNA in leptin-treated *ob/ob* and LIRKO mice. The SREBPs are a family of nuclear transcription factors that are known to regulate lipid and cholesterol synthesis (rev. in 26). SREBP-1c normally regulates SCD1 and is therefore important in the synthesis of MUFAs (10). SREBP-1c is upregulated in *ob/ob* mice, lipoatrophic mice, and insulin receptor substrate (IRS)-2 knockout mice and is decreased by leptin treatment in each model (12,27). In the *ob/ob* liver, leptin effectively suppressed SREBP-1c even in the presence of insulin treatment (Fig. 5*A*). In contrast, leptin did not change SREBP-1c in LOX mice and increased SREBP-1c in LIRKO mice (Fig. 5*B*).

Leptin treatment of SREBP-1c knockout mice. To further assess the role of SREBP-1c in leptin-mediated suppression of SCD1, we infused leptin into SREBP-1c knockout mice. We found that leptin decreased body weight and food intake to the same extent in SREBP-1c knockout mice as in wild-type mice (online appendix [available at http://diabetes.diabetesjournals.org]). Leptin decreased SCD1 transcript by 40% and SCD1 activity by 65% in the knockout mice, which was comparable to the changes seen in wild-type mice (Fig. 6*A* and *B*). Leptin did not alter SREBP-1c or SREBP-1a mRNA in either wild-type or knockout mice (data not shown). Therefore, leptin can reduce SCD1 expression and activity even in absence of the transcription factor SREBP-1c.

DISCUSSION

The fact that knockout of SCD1 in mice causes increased energy expenditure, increased insulin sensitivity, and resistance to diet-induced obesity suggests that SCD1 may be a global regulator of energy metabolism (5). Since leptin suppresses SCD1 transcript and activity, it has been proposed that SCD1 may mediate many of leptin's effects on energy metabolism (3). However, leptin decreases insulin secretion (18) and may also interfere with insulin action (20), raising the possibility that some of the effects of leptin on SCD1 may be secondary to changes in insulin levels or insulin action. In the current study, we used three different genetic models in which individual components of the system had been deleted, showing that leptin suppresses SCD1 through an insulin-independent pathway that does not require SREBP-1c.

In one experimental paradigm, we treated leptin-deficient *ob/ob* mice with leptin alone or leptin and insulin. We found that leptin suppresses SCD1 transcript, SCD protein, SCD activity, and the ratio of MUFAs to SFAs in *ob/ob* mice. The presence of continued hyperinsulinemia using exogenous insulin does not impair these effects. Thus, leptin suppression of SCD1 does not require a fall in insulin levels.

In the second experiment, we treated LOX and LIRKO mice with leptin to assess the effects of leptin in the complete absence of insulin signaling. We found that leptin suppresses SCD1 transcript, protein, and activity to similar levels in the presence or absence of insulin signaling. Interestingly, however, the effect of leptin on hepatic fatty acid composition appeared to require insulin signaling, as leptin decreased the ratio of MUFAs to SFAs in the LOX mice but not in the LIRKO mice, which lack hepatic insulin action. Although the exact role of insulin in determining the fatty acid composition of the liver is not known, insulin may act by altering fatty acid uptake, synthesis, and/or clearance.

In contrast to *ob/ob* mice, leptin did not decrease SREBP-1c mRNA in either LIRKO or LOX mice, suggesting that leptin may act through an SREBP-1c–independent pathway to suppress SCD1. This is confirmed by the finding that leptin treatment decreases SCD1 transcript

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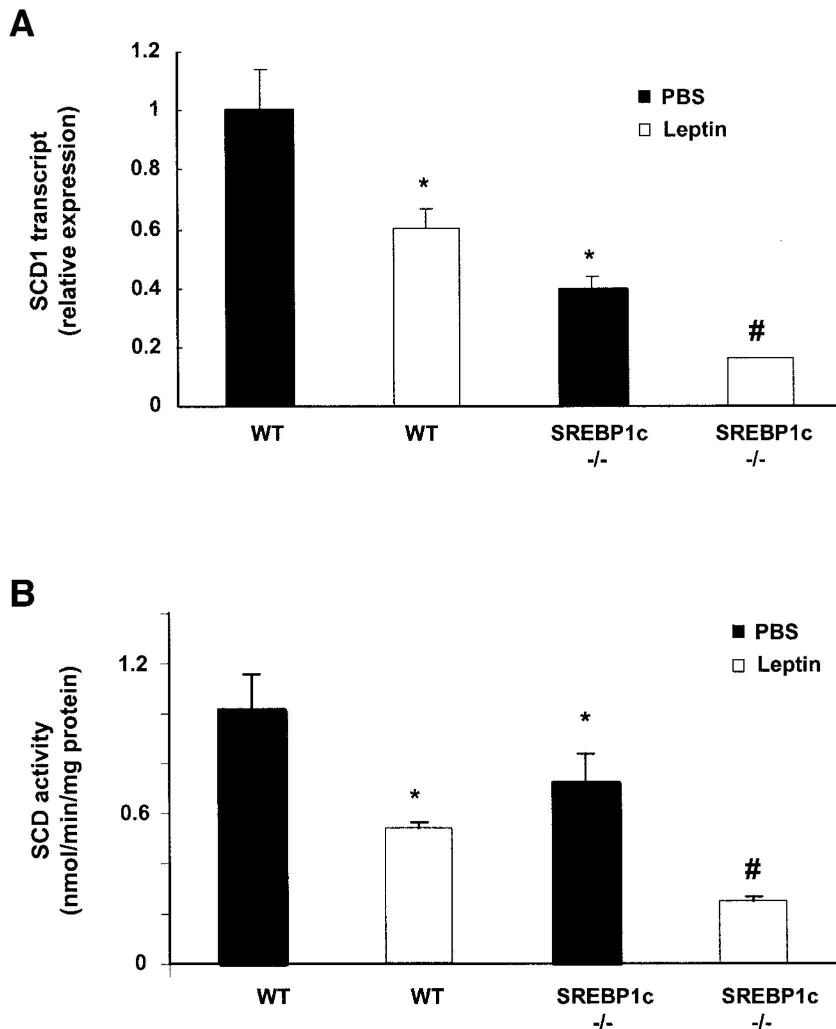

FIG. 6. Leptin decreases SCD1 in SREBP-1c$^{-/-}$ mice. Livers were used to prepare RNA for real-time RT-PCR analysis (*A*) and microsomes to assay SCD activity (*B*). Data are presented as means ± SE (*n* = 6–8 for each group). *$P < 0.01$ vs. untreated wild-type (WT) mice; #$P < 0.001$ vs. untreated SREBP-1c$^{-/-}$ mice.

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and activity to a similar extent in both SREBP-1c knockout and wild-type mice. Thus, leptin does not require SREBP-1c to suppress SCD1.

In these experiments, the role of insulin in regulating SCD1 is clearly subordinate to that of leptin. However, it is possible that in states of low or absent leptin, insulin becomes an important regulator of SCD1 transcription. Thus, in *ob/ob* mice, hyperinsulinemia in the absence of leptin drives high expression of SCD1. Reducing serum insulin in these mice, by pair feeding, for example, decreases SCD1 levels (3). Similarly, streptozotocin-induced diabetic animals, which have low levels of leptin, also have very low levels of SCD1 that are restored with insulin treatment (8).

Both insulin and leptin, in addition to other factors such as glucose, alter the expression of SREBP-1c (10,28). Insulin activates the liver X receptor (LXR), which interacts with LXR binding sites in the SREBP-1c promoter and increases expression of SREBP-1c (29), which in turn upregulates transcription of SCD1 (30). Therefore, in the refed state, the induction of SCD1 expression by insulin is abolished by knockout of SREBP-1c (15). Leptin, on the other hand, has been shown to decrease SREBP-1c in *ob/ob* mice, IRS-2 knockout mice, and lipoatrophic mice, as well as wild-type rats made hyperleptinemic by adenoviral gene transfer (12,27,31). In contrast to insulin, the effects of leptin on SCD1 are not abolished by knockout of SREBP-1c. Interestingly, fructose and, to a lesser extent, LXR agonists are also able to regulate SCD1 independently of SREBP-1c (15,32).

Several studies suggest that the central nervous system (CNS), rather than the liver, is the primary site of leptin action. First, leptin treatment of hepatoma cell lines and primary hepatocytes does not suppress SCD1 mRNA (data not shown). Second, liver-specific knockout of the leptin receptor does not produce the hepatic steatosis, hyperinsulinemia, or obesity characteristic of *ob/ob* mice (33). Knockout of the leptin receptor in neurons, on the other hand, recapitulates the phenotype of the global leptin receptor deficiency of *db/db* mice, with hyperphagia, obesity, and hepatic steatosis (33). Finally, intracerebroventricular infusion of a very small amount of leptin is able to suppress SCD1 transcription and activity without increasing serum leptin levels (4).

In the CNS, leptin impacts several neurotransmitters, including neuropeptide Y, agouti-related protein, cocaine- and amphetamine-regulated transcript, proopiomelanocortin, and melanin-concentrating hormone (MCH). Recent studies have suggested that manipulation of the neural circuitry can alter hepatic lipogenesis. Intracerebroventricular infusions of neuropeptide Y can decrease hepatic ACC activity and lipogenic gene transcription (34). Intracerebroventricular injection of a melanocortin receptor agonist decreases

hepatic SCD1 expression (35). On an *ob/ob* background, knockout of MCH lowers hepatic SCD1 transcript (36).

There are several mechanisms by which leptin signaling in the CNS might be transmitted to the liver. First, leptin may decrease hepatic SCD by acting through other hormones such as corticosterone, which is known to enhance Δ-9 desaturase activity in hepatoma cells (37). Interestingly, both corticosterone and SCD1 are high in *ob/ob* mice but decreased by knockout of MCH (36). Alternatively, leptin may act through autonomic innervation of the liver to alter SCD activity. Finally, leptin may act by altering the availability of nutrients such as cholesterol or polyunsaturated fatty acids. In this regard, it is worth noting that the proportion of linoleic acid (18:2), arachidonic acid (20:4), or both were increased by leptin treatment of LOX and *ob/ob* mice (data not shown).

In summary, SCD1 is a promising target for therapeutic interventions but its regulation is complex. While insulin and leptin are important regulators of SCD1 expression and activity, we show here that the role of insulin is subordinate to that of leptin. However, insulin may have effects on SCD1 under some circumstances and play a role in the accumulation of hepatic MUFAs. Decreasing SCD1 activity in insulin-resistant states may be beneficial in a number of diseases. Our data suggest that increasing leptin action rather than decreasing insulin action may be more important in regulating SCD1.

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REFERENCES

1. Ntambi JM, Miyazaki M: Regulation of stearoyl-CoA desaturases and role in metabolism. *Prog Lipid Res* 43:91–104, 2004
2. Miyazaki M, Jacobson MJ, Man WC, Cohen P, Asilmaz E, Friedman JM, Ntambi JM: Identification and characterization of murine SCD4, a novel heart-specific stearoyl-CoA desaturase isoform regulated by leptin and dietary factors. *J Biol Chem* 278:33904–33911, 2003
3. Cohen P, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, Sharma R, Hudgins LC, Ntambi JM, Friedman JM: Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science* 297:240–243, 2002
4. Asilmaz E, Cohen P, Miyazaki M, Dobrzyn P, Ueki K, Fayzikhodjaeva G, Soukas AA, Kahn CR, Ntambi JM, Socci ND, Friedman JM: Site and mechanism of leptin action in a rodent form of congenital lipodystrophy. *J Clin Invest* 113:414–424, 2004

4a. Biddinger SB, Almind K, Miyazaki M, Kokkotou E, Ntambi JM, Kahn CR: Effects of diet and genetic background on sterol regulatory element–binding protein-1c, stearoyl-Coa desaturase 1, and the development of the metabolic syndrome. *Diabetes* 54:1314–1323, 2005

5. Ntambi JM, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, Song Y, Cohen P, Friedman JM, Attie AD: Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci U S A* 99:11482–11486, 2002
6. Miyazaki M, Kim YC, Ntambi JM: A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. *J Lipid Res* 42:1018–1024, 2001
7. Prasad MR, Joshi VC: Regulation of rat hepatic stearoyl coenzyme A desaturase: the roles of insulin and carbohydrate. *J Biol Chem* 254:997–999, 1979
8. Waters KM, Ntambi JM: Insulin and dietary fructose induce stearoyl-CoA desaturase 1 gene expression of diabetic mice. *J Biol Chem* 269:27773–27777, 1994
9. Weiner FR, Smith PJ, Wertheimer S, Rubin CS: Regulation of gene expression by insulin and tumor necrosis factor alpha in 3T3–L1 cells: modulation of the transcription of genes encoding acyl-CoA synthetase and stearoyl-CoA desaturase-1. *J Biol Chem* 266:23525–23528, 1991
10. Shimomura I, Shimano H, Korn BS, Bashmakov Y, Horton JD: Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J Biol Chem* 273:35299–35306, 1998
11. Shimomura I, Bashmakov Y, Horton JD: Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *J Biol Chem* 274:30028–30032, 1999
12. Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL: Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. *Mol Cell* 6:77–86, 2000
13. Horton JD, Bashmakov Y, Shimomura I, Shimano H: Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc Natl Acad Sci U S A* 95:5987–5992, 1998
14. Shimomura I, Bashmakov Y, Ikemoto S, Horton JD, Brown MS, Goldstein JL: Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc Natl Acad Sci U S A* 96:13656–13661, 1999
15. Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, Brown MS: Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem* 277:9520–9528, 2002
16. Liang CP, Tall AR: Transcriptional profiling reveals global defects in energy metabolism, lipoprotein, and bile acid synthesis and transport with reversal by leptin treatment in ob/ob mouse liver. *J Biol Chem* 276:49066–49076, 2001
17. Bradley RL, Cheatham B: Regulation of *ob* gene expression and leptin secretion by insulin and dexamethasone in rat adipocytes. *Diabetes* 48:272–278, 1999
18. Kulkarni RN, Wang ZL, Wang RM, Hurley JD, Smith DM, Ghatei MA, Withers DJ, Gardiner JV, Bailey CJ, Bloom SR: Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, in vivo, in mice. *J Clin Invest* 100:2729–2736, 1997
19. Szanto I, Kahn CR: Selective interaction between leptin and insulin signaling pathways in a hepatic cell line. *Proc Natl Acad Sci U S A* 97:2355–2360, 2000
20. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. *Science* 274:1185–1188, 1996
21. Michael MD, Kulkarni RN, Postic C, Previs SF, Shulman GI, Magnuson MA, Kahn CR: Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. *Mol Cell* 6:87–97, 2000
22. Yang J, Goldstein JL, Hammer RE, Moon YA, Brown MS, Horton JD: Decreased lipid synthesis in livers of mice with disrupted site-1 protease gene. *Proc Natl Acad Sci U S A* 98:13607–13612, 2001
23. Heinemann FS, Ozols J: Degradation of stearoyl-coenzyme A desaturase: endoproteolytic cleavage by an integral membrane protease. *Mol Biol Cell* 9:3445–3453, 1998
24. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226:497–509, 1957
25. Morrison WR, Smith LM: Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron flouride-methanol. *J Lipid Res* 53:600–608, 1964
26. Horton JD, Goldstein JL, Brown MS: SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 109:1125–1131, 2002
27. Tobe K, Suzuki R, Aoyama M, Yamauchi T, Kamon J, Kubota N, Terauchi Y, Matsui J, Akanuma Y, Kimura S, Tanaka J, Abe M, Ohsumi J, Nagai R, Kadowaki T: Increased expression of the sterol regulatory element-binding protein-1 gene in insulin receptor substrate-2(-/-) mouse liver. *J Biol Chem* 276:38337–38340, 2001
28. Matsuzaka T, Shimano H, Yahagi N, Amemiya-Kudo M, Okazaki H, Tamura Y, Iizuka Y, Ohashi K, Tomita S, Sekiya M, Hasty A, Nakagawa Y, Sone H, Toyoshima H, Ishibashi S, Osuga J, Yamada N: Insulin-independent induction of sterol regulatory element–binding protein-1c expression in the livers of streptozotocin-treated mice. *Diabetes* 53:560–569, 2004
29. Chen G, Liang G, Ou J, Goldstein JL, Brown MS: Central role for liver X receptor in insulin-mediated activation of Srebp-1c transcription and

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stimulation of fatty acid synthesis in liver. *Proc Natl Acad Sci U S A* 101:11245–11250, 2004

30. Tabor DE, Kim JB, Spiegelman BM, Edwards PA: Identification of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. *J Biol Chem* 274:20603–20610, 1999
31. Kakuma T, Lee Y, Higa M, Wang Z, Pan W, Shimomura I, Unger RH: Leptin, troglitazone, and the expression of sterol regulatory element binding proteins in liver and pancreatic islets. *Proc Natl Acad Sci U S A* 97:8536–8541, 2000
32. Miyazaki M, Dobrzyn A, Man WC, Chu K, Sampath H, Kim HJ, Ntambi JM: Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms. *J Biol Chem* 279:25164–25171, 2004
33. Cohen P, Zhao C, Cai X, Montez JM, Rohani SC, Feinstein P, Mombaerts P, Friedman JM: Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 108:1113–1121, 2001
34. Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B: Chronic intracerebroventricular neuropeptide Y administration to normal rats mimics hormonal and metabolic changes of obesity. *Endocrinology* 133:1753–1758, 1993
35. Lin J, Choi YH, Hartzell DL, Li C, Della-Fera MA, Baile CA: CNS melanocortin and leptin effects on stearoyl-CoA desaturase-1 and resistin expression. *Biochem Biophys Res Commun* 311:324–328, 2003
36. Segal-Lieberman G, Bradley RL, Kokkotou E, Carlson M, Trombly DJ, Wang X, Bates S, Myers MG Jr, Flier JS, Maratos-Flier E: Melanin-concentrating hormone is a critical mediator of the leptin-deficient phenotype. *Proc Natl Acad Sci U S A* 100:10085–10090, 2003
37. Marra CA, de Alaniz MJ: Regulatory effect of various steroid hormones on the incorporation and metabolism of [14C]stearate in rat hepatoma cells in culture. *Mol Cell Biochem* 145:1–9, 1995

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