Leptin Activation of Corticosterone Production in Hepatocytes May Contribute to the Reversal of Obesity and Hyperglycemia in Leptin-Deficient *ob/ob* Mice

Yanjun Liu,[1,2] Yuichi Nakagawa,[2] Ying Wang,[3] Renshan Li,[2] Xiaojun Li,[4] Takehiko Ohzeki,[2] and Theodore C. Friedman[1]

Glucocorticoids have been implicated as pathophysiological mediators of obesity and insulin resistance and are regulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1). This enzyme regenerates active corticosterone from inactive 11-keto forms. To assess the role of 11β-HSD1–mediated synthesis of active corticosterone in leptin-related obesity and diabetes, we examined the peripheral effect of leptin on 11β-HSD1 activity and gene expression in vivo and in vitro in hepatocytes from *ob/ob* mice and in liver of streptozotocin (STZ)-treated *ob/ob* mice. We observed an inverse relationship between hepatic 11β-HSD1 expression and body weight in *ob/ob* mice and lean littermates. Leptin treatment of *ob/ob* mice markedly increased hepatic 11β-HSD1 activity and mRNA expression. This induction of 11β-HSD1 expression corresponded to reduced levels of circulating corticosterone and weight loss in *ob/ob* mice treated with leptin, indicating that impaired hepatic 11β-HSD1 expression may contribute to the pathogenesis of obesity in *ob/ob* mice. In addition, leptin treatment of STZ-treated *ob/ob* mice caused marked increases in hepatic 11β-HSD1 levels associated with decreased body weight and a significant reduction in hyperglycemia due to pancreatic β-cell damage. Addition of leptin to *ob/ob* mouse primary hepatocytes led to a dose-dependent increase in 11β-HSD1 mRNA expression. In contrast, leptin did not influence 11β-HSD1 expression in primary hepatocytes from *db/db* mice, indicating that leptin regulation of 11β-HSD1 expression is probably mediated by the functional leptin receptor. Thus, leptin appears to be an important metabolic signal that directly activates intrahepatic corticosterone production. These findings suggest that the liver-specific interaction of leptin with 11β-HSD1 is involved in the development of obesity and insulin resistance in *ob/ob* mice. *Diabetes* 52:1409–1416, 2003

From the [1]Division of Endocrinology, Charles R. Drew University of Medicine & Sciences, UCLA School of Medicine, Los Angeles, California; the [2]Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan; the [3]Department of Internal Medicine, Division of Laboratory Research and Development, Charles R. Drew University of Medicine & Sciences, UCLA School of Medicine, Los Angeles, California; and the [4]Department of Cardiology, Cedars-Sinai Medical Center, University of California, Los Angeles, Los Angeles, California.

Address correspondence and reprint requests to Yanjun Liu, Charles R. Drew University of Medicine & Sciences, Division of Endocrinology, 1731 E. 120th St., Los Angeles, CA 90059. E-mail: dryanjunliu@hotmail.com.

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11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; 11β-HSD2, 11β-hydroxysteroid dehydrogenase type 2; DMEM, Dulbecco's modified Eagle's medium; HPA, hypothalamus-pituitary-adrenal; STZ, streptozotocin.

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Obesity is a significant risk factor for health and is thought to constitute a crucial component in the pathogenesis of insulin resistance, type 2 diabetes, hypertension, and cardiovascular disease (1–3). However, the associated mechanisms are poorly understood. One factor that may be important is glucocorticoids. Patients with glucocorticoid excess (Cushing's syndrome) develop reversible obesity and diabetes (4,5), yet in most obese patients, circulating levels of the glucocorticoid cortisol are normal (6). However, careful examination of obese patients has revealed subtle abnormalities in the hypothalamus-pituitary-adrenal (HPA) axis (7,8) that are associated with increased metabolic clearance of cortisol (9,10), suggesting that the pathogenesis of obesity may involve altered peripheral glucocorticoid metabolism. Of importance, tissue metabolism of glucocorticoids is thought to be regulated by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and type 2 (11β-HSD1), which locally interconvert active glucocorticoids (corticosterone in rodents and cortisol in humans) and inert 11-keto forms (11-dehydrocorticosterone and cortisone) (11). The importance of interconversion of glucocorticoids is supported by the renal expression of 11β-HSD2, which inactivates glucocorticoids, thereby conferring aldosterone selectivity at mineralocorticoid receptors (12). 11β-HSD2 is not expressed in most tissues, including liver. In contrast, 11β-HSD1 is expressed principally in glucocorticoid target tissues, such as liver, adipose, and brain, to regenerate active glucocorticoids from the 11-keto forms, and it thereby modulates local glucocorticoid action by regulating ligand supply to the glucocorticoid receptors (13–15). Recent studies have suggested that tissue-specific dysregulation of 11β-HSD1 contributes to the pathogenesis of obesity, hypertension, and insulin resistance (14). Transgenic mice overexpressing 11β-HSD1 in adipose tissue develop visceral obesity when fed a high-fat diet (16). In contrast, patients with obesity have selectively reduced reactivation of cortisone to cortisol, which appears to be secondary to impairment of 11β-HSD1 in liver (17–19). Similar findings of reduced hepatic 11β-HSD1 expression were also reported in obese Zucker rats (20). Reduced intrahepatic regeneration of active corticosterone via impaired hepatic 11β-HSD1 may influence negative feedback regulation of the HPA axis and could contribute to increased glucocorticoid secre-

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tion, which is associated with obesity and insulin resistance (11,19–21). These observations suggest that liver-specific dysregulation of 11β-HSD1 contributes to the development of obesity and diabetes. However, the role of hepatic 11β-HSD1 in obesity and insulin resistance remains unclear.

Mutation of the *ob* gene causes a leptin production defect, resulting in glucocorticoid-dependent obesity and diabetes (22). Treating *ob/ob* mice with recombinant leptin suppresses food intake, reduces body weight, and decreases circulating insulin and corticosterone concentrations (23,24). These studies have shown that interaction of leptin with glucocorticoids may contribute to the development of obesity and diabetes in *ob/ob* mice. However, little is known about tissue-specific changes in leptin-mediated glucocorticoid metabolism in *ob/ob* mice. Whether the interaction of leptin with 11β-HSD1 is associated with the obese phenotype of *ob/ob* mice remains unclear.

To answer these questions, we investigated the role of 11β-HSD1 in leptin-related obesity and diabetes by examining the peripheral effects of leptin on hepatic 11β-HSD1 activity and gene expression in *ob/ob* mice treated with leptin. We also examined the relationship between the antidiabetic effects of leptin and corticosterone metabolism in the liver of streptozotocin (STZ)-treated *ob/ob* mice. Finally, we tested the direct effects of leptin and insulin on 11β-HSD1 expression in primary cultures of hepatocytes from *ob/ob* mice.

RESEARCH DESIGN AND METHODS

Animals and experimental procedures. Female C57BL/6J obese (*ob/ob*) mice and lean littermates were purchased at 4 weeks of age from Jackson Laboratory and housed in a room illuminated daily from 0700 to 1900 (12:12 h light/dark cycle). All animal protocols were approved by the Charles R. Drew University Humane Care and Use of Laboratory Animal Committee. Temperature was maintained at 21 ± 1°C, and humidity was maintained at 55 ± 5%. Mice were caged individually and allowed free access to tap water and standard laboratory food. Body weight and food intake were recorded daily.

Experiment 1. Two weeks before the experiment, obese mice and lean littermates were divided randomly into three groups: *1*) *ob/ob* mice treated with saline ($n = 9$), *2*) *ob/ob* mice treated with leptin ($n = 8$), and *3*) lean mice treated with saline ($n = 8$). Recombinant murine leptin (Sigma, St. Louis, MO) (1 mg/kg) or the saline vehicle was injected intraperitoneally twice each day (at 0700 and 1900) for 2 weeks. In some mice, at the end of the second week after leptin or saline treatment, an intraperitoneal insulin tolerance test was performed by administration of 1.0 units insulin/kg.

Experiment 2. *ob/ob* mice were treated with a single intraperitoneal injection of STZ (180 mg/kg body wt, freshly dissolved in 10 mmol/l citrate buffer, pH 4.5) (25,26). An equal volume of citrate buffer (pH 4.5) was given to control *ob/ob* mice that were matched for body weight and food consumption. Glucose concentrations in blood samples collected via the tail vein were measured by the glucose oxidase method (Boehringer Mannheim, Mannheim, Germany). Three weeks later, STZ-treated *ob/ob* mice showed hyperglycemia and relatively low insulin levels because of pancreatic β-cell damage. STZ-treated *ob/ob* mice were then subdivided into two groups: STZ-*ob/ob* mice treated with saline ($n = 9$) and STZ-*ob/ob* mice treated with leptin (1 mg/kg i.p.) ($n = 7$), both twice daily for an additional 2 weeks. Non–STZ-treated *ob/ob* mice ($n = 8$) also received vehicle for an additional 2 weeks.

Liver was removed surgically under ether anesthesia and frozen immediately in liquid nitrogen. All tissue samples were stored at −80°C until use. Blood samples were collected from each mouse, kept in an ice bath during processing, and then stored at −80°C until measurement of insulin, corticosterone, and glucose concentrations as described previously (27). Liver corticosterone levels were measured with a corticosterone radioimmunoassay kit for mice (ICN Biomedicals) as reported previously (28).

Primary hepatocyte culture and treatment. Hepatocytes were isolated from 10-week-old female *ob/ob* or *db/db* mice by a two-step collagenase perfusion method (0.5 mg/ml in Hanks' balanced salt solution) as described previously (29). Primary hepatocytes were plated at 1×10^6 cells/dish in 3 ml Dulbecco's modified Eagle's medium (DMEM)/F-12 with 10% fetal bovine serum and incubated at 37°C for 4 h. Cells were then washed with PBS, and the medium was changed to DMEM/F-12-A without fetal bovine serum. After 24 h, hepatocytes were treated with increasing concentrations (10–1,000 ng/ml) of recombinant mouse leptin for 48 h. A leptin concentration occurring in the serum of obese humans (100 ng/ml) (30) was used to treat hepatocytes for different times. In some experiments, insulin (10 nmol/l) was added to *ob/ob* hepatocytes in the absence or presence of leptin (10 ng/ml). The medium was changed daily, and fresh hormones were added. Specific assays for 11β-HSD1 mRNA expression and enzyme activity were then performed.

In vivo assay of 11β-HSD1 activity. The 11β-HSD1 isozyme expresses stable dehydrogenase activity (converting corticosterone [B] to 11-dehydrocorticosterone [A]), which reflects 11β-HSD1 protein levels and reductase activity in vivo (18,20). Thus, we measured 11β-dehydrogenase activity in liver as previously reported (31–33). Briefly, liver was homogenized in Krebs-Ringer buffer solution at 4°C in a Dounce tissue grinder. Protein concentrations of the supernatant were measured with a Bradford assay (Bio-Rad protein assay kit), and the supernatants were diluted to yield uniform protein concentrations. Liver homogenates were incubated with 2 μmol/l [^{3}H]B (specific activity, 90 Ci/mmol; New England Nuclear), 3.4 mmol/l NADP$^+$, and Krebs-Ringer buffer solution (containing 0.2% glucose and BSA, pH 7.4) at 37°C for 10 min in a shaking bath. Steroids were separated by thin-layer chromatography in a chloroform-ethanol (9:1) system. The conversion of [^{3}H]B to [^{3}H]A was calculated from the radioactivity in each fraction.

In vitro assay of 11β-HSD1 activity. 11β-HSD activity was measured in intact isolated hepatocytes in six-well tissue culture dishes as described above by measuring the rate of conversion of [^{3}H]A to [^{3}H]B. [^{3}H]A was synthesized in vitro by incubation of rat kidney NRK-52E cells with 50 μCi [^{3}H]B for 24 h in DMEM at 37°C as previously reported (34). [^{3}H]A was separated from [^{3}H]B by high-performance liquid chromatography with a 24–35% methanol gradient over 55 min at a flow rate of 1.5 ml/min. The medium was then removed and replaced with fresh medium containing 18 nmol/l unlabeled A with 2 nmol/l [^{3}H]A as tracer. An aliquot of medium was removed at intervals between 30 min and 24 h after the addition of steroids. Steroids were separated by thin-layer chromatography, and levels were estimated by scintillation counting. Enzyme activity was expressed as the percent conversion rate of [^{3}H]A to [^{3}H]B. All experiments were carried out on at least three separate occasions in triplicate.

RNA isolation and Northern blot analysis. Total RNA was isolated with a single-step extraction method (RNAzol B) (Invitrogen). The cDNA probe for mouse 11β-HSD1 was provided by Dr. K.E. Chapman (35), and that for PEPCK was provided by Dr. Yoo Warren (36). Probes were labeled with [^{32}P]dCTP by nick translation (Nick Translation System; Life Technologies). Autoradiography was performed by exposure of membranes to X-ray film with an intensifying screen at −70°C for up to 7 days. For densitometric measurements, autoradiographic signals were standardized to signals measured from 18S rRNA (37).

Measurement of 11β-HSD1 mRNA expression in intact cells by real-time quantitative RT-PCR. 11β-HSD1 mRNA levels in primary hepatocytes were measured using quantitative fluorescent real-time PCR. Total RNA (200 ng) was reverse-transcribed with Superscript II RT (Life Technologies). Reactions in which RNA was omitted served as negative controls. Amplification of each target cDNA was then performed with TaqMan PCR reagent kits in the ABI Prism 7700 Sequence Detection System according to the protocols recommended by the manufacturer. The sequences of 11β-HSD1 primers/probe were as follows (38): forward primer 5′-AAGCAGAGCAATGGCAGCAT 3′, reverse primer 5′-GAGCAATCATAGGCT GGGTCAT-3′, and probe 5′-CGTCATCTC CTCCTT GGC TGG GAA-3′. The 18S primer and probe were supplied by PE Biosystems. All reactions were carried out using the following cycling parameters: 55°C for 2 min and 95°C for 10 min, following by 30 cycles of 95°C for 15 s and 60°C for 1 min. Threshold cycle (Ct) readings for each of the unknown samples were then used to calculate the amount of 11β-HSD1 or 18S rRNA relative to the standard.

Statistical analysis. All data are expressed as the mean ± SE for all of the determinations. Comparisons between the different experimental groups were carried out by an unpaired Student's t test and ANOVA. Values at $P < 0.05$ were considered statistically significant.

RESULTS

Body weight and 11β-HSD1 levels in whole-animal experiments. As shown in Table 1, *ob/ob* mice had very high plasma corticosterone, insulin, and blood glucose levels. The body weight of *ob/ob* mice was significantly higher than that of lean controls ($P < 0.001$). However, the 11β-HSD1 activity in the liver of *ob/ob* mice was markedly

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TABLE 1
Blood glucose, serum insulin, plasma corticosterone, and body weight

	Experiment 1			Experiment 2		
	Lean-saline	*ob/ob*-saline	*ob/ob*-leptin	*ob/ob*-vehicle	*ob/ob*-STZ	*ob/ob*-STZ-leptin
n	8	9	8	8	9	7
Blood glucose (mmol/l)	8.4 ± 0.6	13 ± 0.7*	7.5 ± 0.6†	14 ± 0.9	29 ± 1.2‡	13 ± 0.8§
Plasma insulin (pmol/l)	32 ± 2.7	150 ± 13‖	79 ± 9.8†	155 ± 17	63 ± 7.5¶	55 ± 6.3
Corticosterone (nmol/l)	127 ± 8	402 ± 17‖	177 ± 11#	437 ± 19	690 ± 25**	397 ± 16‡
Body weight (g)	17.9 ± 0.7	43.8 ± 1.7‖	31.8 ± 1.3†	55.5 ± 2.0	41.3 ± 2.0¶	31.1 ± 1.4‡

Data are means ± SE. *$P < 0.05$ vs. saline-lean mice; †$P < 0.01$ vs. saline-*ob/ob* mice; ‡$P < 0.001$ vs. vehicle-*ob/ob* mice; §$P < 0.001$ vs. STZ-*ob/ob* mice; ‖$P < 0.001$ vs. saline-lean mice; ¶$P < 0.01$ vs. vehicle-*ob/ob* mice; #$P < 0.005$ vs. saline-*ob/ob* mice; **$P < 0.05$ vs. vehicle-*ob/ob* mice; ††$P < 0.01$ vs. STZ-*ob/ob* mice.

decreased compared with that of lean littermates (15.34 ± 1.1 vs. 31.7 ± 2.1%, $P < 0.001$) (Fig. 1*A*). When examining both lean and *ob/ob* mice together, there was an inverse correlation between hepatic 11β-HSD1 activity and body weight ($R^2 = 0.958$). The decrease in enzyme activity was paralleled by 11β-HSD1 mRNA expression, which was decreased to 32% that of lean animals (Fig. 1*A* and *B*). In contrast, the level of PEPCK mRNA in liver of *ob/ob* mice was significantly higher than that in lean mice ($P < 0.01$) (Fig. 2).

Treatment of *ob/ob* mice with leptin restored the blood glucose concentration to that of lean mice and reduced serum insulin and corticosterone levels, although the levels were still higher than those in lean mice (Table 1).

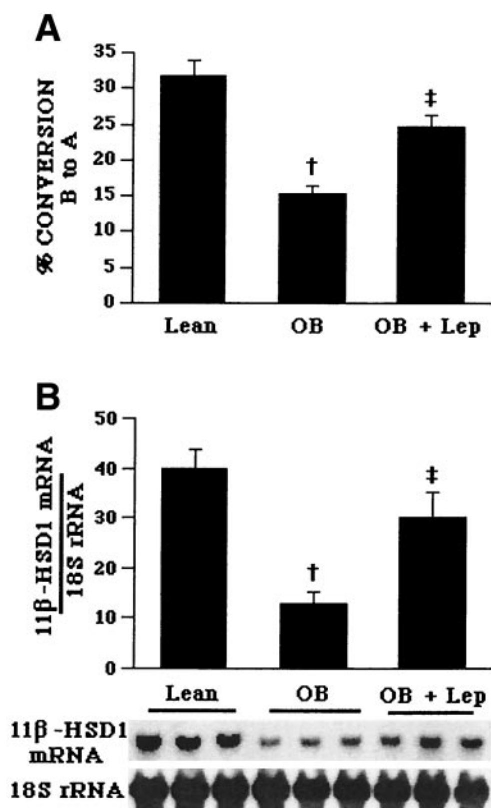

FIG. 1. 11β-HSD1 activity and mRNA expression in the livers of lean mice treated with saline (Lean, $n = 8$), *ob/ob* mice treated with saline (OB, $n = 9$), and *ob/ob* mice treated with leptin (OB + Lep, $n = 8$). Values are means ± SE *A*: Enzyme activity is expressed as the percentage conversion of [^{3}H]B to [^{3}H]A. *B*: Expression and relative quantitation of enzyme mRNA levels expressed relative to the amount of 18S rRNA. †$P < 0.001$ vs. lean controls; ‡$P < 0.01$ vs. *ob/ob* mice.

Body weight loss in *ob/ob* mice after leptin treatment was only 27.6% that of untreated *ob/ob* mice ($P < 0.01$). In contrast, leptin treatment caused a significant increase in 11β-HSD1 activity in liver of *ob/ob* mice (15.34 ± 1.1 to 24.7 ± 1.6%, $P < 0.01$), but the levels of this enzyme were lower than those in lean controls ($P < 0.05$) (Fig. 1*A*). Similarly, leptin treatment induced a twofold increase in 11β-HSD1 mRNA levels in liver of *ob/ob* mice compared with that of untreated *ob/ob* mice ($P < 0.01$), although it did not restore enzyme levels to those of lean controls (Fig. 1*B*) ($P < 0.05$). Northern blot analysis also showed that leptin significantly reduced the hepatic PEPCK mRNA level in *ob/ob* mice compared with that in untreated *ob/ob* mice ($P < 0.01$) (Fig. 2).

Levels of liver corticosterone in lean control, *ob/ob*, and leptin-treated *ob/ob* mice are shown in Fig. 3*A*. Treatment of *ob/ob* mice with leptin significantly reduced the corticosterone level in liver of *ob/ob* mice, but did not restore it to normal ($P < 0.05$).

Results of insulin tolerance tests are shown in Fig. 3*B*. *ob/ob* mice showed significant inhibition of the glucose-lowering effect of insulin compared with that of lean controls at all time points after insulin challenge ($P < 0.01$). Administration of leptin to *ob/ob* mice restored the glucose-lowering effect of insulin (Fig. 3*B*).

Hyperglycemia and 11β-HSD1 expression in STZ-treated *ob/ob* mice. Blood glucose levels in STZ-treated *ob/ob* mice were significantly higher than those of untreated *ob/ob* mice ($P < 0.001$, Table 1). As expected,

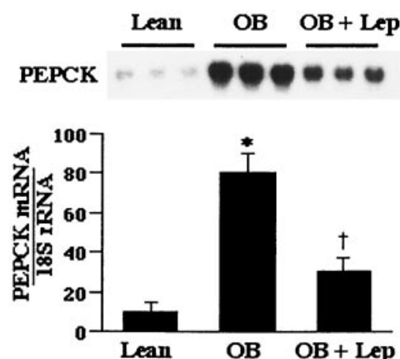

FIG. 2. Northern blot hybridization demonstrating the alterations of PEPCK mRNA expression and relative quantitation of PEPCK mRNA levels in livers of lean littermates treated with saline (Lean, $n = 8$), *ob/ob* mice treated with saline (OB, $n = 9$), and *ob/ob* mice treated with leptin (OB + Lep, $n = 8$). Values are means ± SE for the ratio of PEPCK mRNA to 18S rRNA. *$P < 0.001$ vs. lean controls; †$P < 0.01$ vs. saline *ob/ob* mice.

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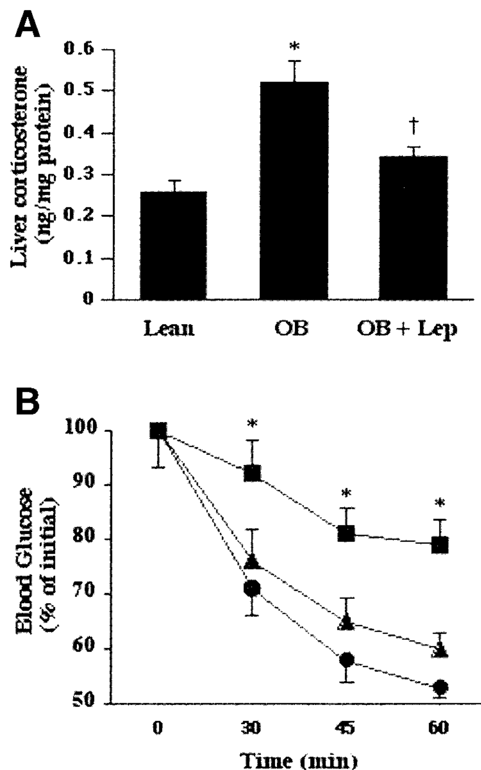

FIG. 3. ***A*: Corticosterone levels in the livers of lean littermates treated with saline (Lean, *n* = 8), *ob/ob* mice treated with saline (OB, *n* = 9), and *ob/ob* mice treated with leptin (OB + Lep, *n* = 8). Values are means ± SE. *$P < 0.01$ vs. lean mice; †$P < 0.05$ vs. *ob/ob* mice. *B*: Insulin tolerance test in lean mice treated with saline (●), *ob/ob* mice treated with saline (■), and *ob/ob* mice treated with leptin (▲). *n* = 4–5 mice/group. *$P < 0.01$ vs. leptin-treated *ob/ob* mice. Blood samples were collected from the tail at the indicated time, and glucose levels were measured.**

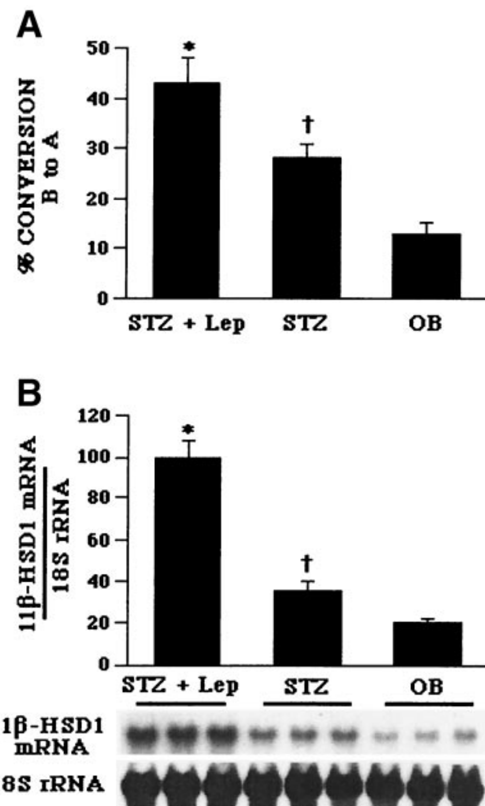

FIG. 4. Comparison of hepatic 11β-HSD1 activity and mRNA levels in *ob/ob* controls treated with vehicle (OB, *n* = 8), STZ-*ob/ob* mice treated with saline (STZ, *n* = 9), and STZ-*ob/ob* mice treated with leptin (STZ + Lep, *n* = 7). Values are means ± SE *A*: Enzyme activity expressed as percentage conversion of [^{3}H]B to [^{3}H]A. *B*: Expression and relative quantitation of 11β-HSD1 mRNA levels are expressed relative to the amount of 18S rRNA. *$P < 0.001$ vs. STZ-*ob/ob* mice; †$P < 0.01$ vs. *ob/ob* controls.

serum insulin concentrations in STZ-treated *ob/ob* mice were reduced by 41% that of *ob/ob* controls ($P < 0.01$). The body weight of STZ-treated *ob/ob* mice was significantly lower than that of untreated *ob/ob* mice ($P < 0.01$). In contrast, STZ-treated *ob/ob* mice had plasma corticosterone concentrations that were higher than those of controls ($P < 0.01$). Moreover, 11β-HSD1 activity in liver of STZ-treated *ob/ob* mice increased significantly by 37% that of vehicle-treated *ob/ob* mice ($P < 0.01$) (Fig. 4*A*). Enzyme activity correlated with the increased levels of enzyme mRNA shown in Fig. 4*A* and *B*. Leptin treatment of STZ-treated *ob/ob* mice reversed the elevated blood glucose and plasma corticosterone concentrations to those of controls (Table 1). However, leptin treatment did not significantly reduce serum insulin levels, but it did induce a loss of body weight to a level 26% lower than that in STZ-treated *ob/ob* mice ($P < 0.01$, Table 1). In addition, with leptin, hepatic levels of 11β-HSD1 activity and mRNA were markedly higher than levels observed in STZ-treated *ob/ob* animals (Fig. 4).

Primary cultures of hepatocytes. In primary culture, hepatocytes from *ob/ob* mice showed only minimal dehydrogenase activity (<5% conversion of [^{3}H]B to [^{3}H]A in intact cells, even after 24-h incubations). However, 11β-reductase activity was clearly detected with 40–60% conversion of [^{3}H]A to [^{3}H]B. Thus, 11β-HSD function is primarily expressed as a reductase in intact *ob/ob* mouse hepatocytes. As shown in Fig. 5*A*, treatment of primary cultures of *ob/ob* hepatocytes with increasing doses of leptin led to a dose-dependent induction of 11β-HSD1 activity from 20.2 ± 1.1% (control) to 28.4 ± 2.1% at 10 ng/ml leptin, 37.8 ± 2.9% at 100 ng/ml leptin, and 49.3 ± 3.5% at 1,000 ng/ml leptin. At a concentration of leptin that occurs in obese patients in vivo (100 ng/ml), 11β-HSD1 activity in primary cultures of *ob/ob* hepatocytes increased significantly after treatment with leptin for periods ranging from 18 to 72 h (Fig. 6*A*). However, in primary cultures of *db/db* hepatocytes, leptin had no significant effect on 11β-HSD1 activity (Figs. 5*C* and 6*C*).

The results of real-time quantitative PCR for 11β-HSD1 mRNA expression in primary hepatocytes treated with 10–1,000 ng/ml leptin are shown in Figs. 5*B* and 6*B*. After leptin treatment of *ob/ob* hepatocytes, the 11β-HSD1 mRNA levels increased two- to sixfold above control levels (Fig. 5*B*). Leptin (100 ng/ml) treatment for 18–72 h resulted in a one- to fourfold increase in 11β-HSD1 gene expression in *ob/ob* hepatocytes compared with control levels (Fig. 6*B*). In contrast, leptin treatment did not have a significant effect on 11β-HSD1 mRNA expression in *db/db* primary hepatocytes (Figs. 5*D* and 6*D*).

In addition, real-time quantitative PCR analysis also demonstrated that insulin (10 nmol/l) reduced 11β-HSD1 mRNA levels in hepatocytes ($P < 0.05$, Fig. 7). Cotreatment with insulin and leptin attenuated the leptin-mediated changes in 11β-HSD1 mRNA levels in primary hepatocytes ($P < 0.01$, Fig. 7).

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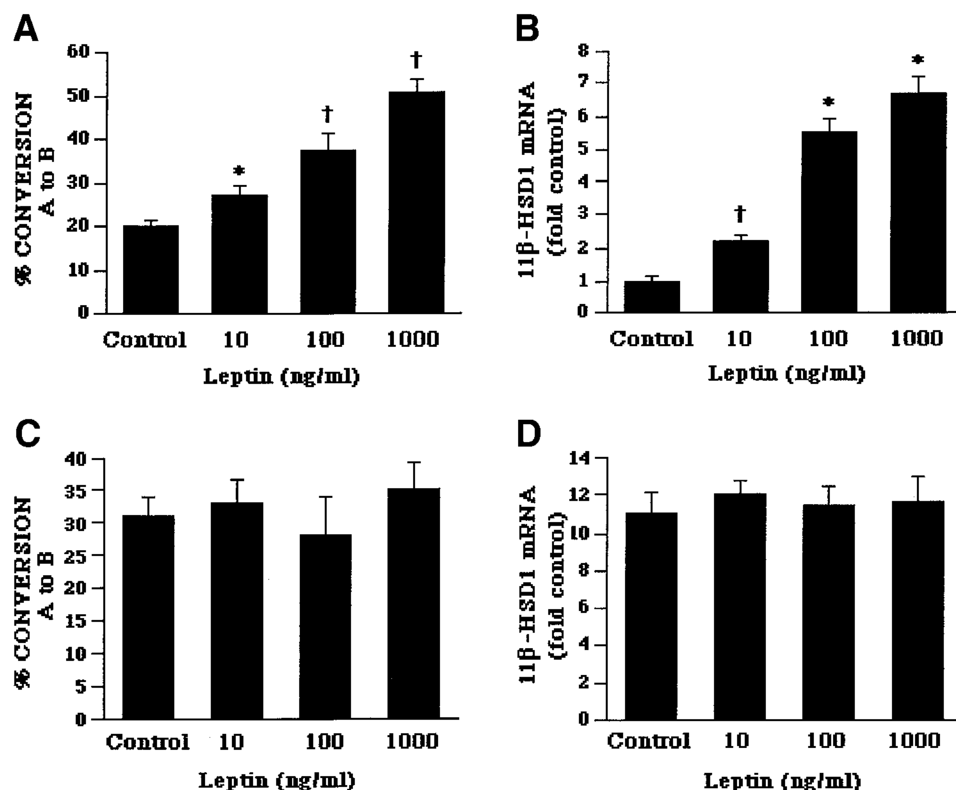

FIG. 5. Effects of leptin on 11β-HSD1 activities and mRNA levels in primary cultures of hepatocytes from both *ob/ob* (*A* and *B*) and *db/db* (*C* and *D*) mice. Hepatocytes were incubated in media with indicated concentrations of mouse leptin (10–1,000 ng/ml) for 48 h. Values are means ± SE from three to five separate culture preparations. *A* and *C*: 11β-HSD1 activity was expressed as the percent conversion of [^{3}H]A to [^{3}H]B in medium from hepatocytes after 30 min. **P* < 0.05 vs. controls; †*P* < 0.01 vs. control. *B* and *D*: The levels of 11β-HSD1 mRNA expression were determined by quantitative real-time PCR and are expressed relative to the amount of mRNA found in controls. †*P* < 0.01 vs. controls; **P* < 0.001 vs. controls.

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DISCUSSION

Accumulating evidence has shown that glucocorticoids play a fundamental role in the development of obesity and insulin resistance. In *ob/ob* mice, removal of adrenal steroids reverses the obese phenotype, and corticosterone replacement results in reappearance of the obesity syndrome (39,40), highlighting the crucial role of glucocorticoids in the pathogenesis of obesity and insulin resistance. Importantly, the role of glucocorticoids in obesity depends not only on circulating glucocorticoid levels, but also on intracellular pre-receptor metabolism, which is regulated by 11β-HSD1. Enhanced expression of 11β-HSD1 in adipose tissue promotes central obesity in obese humans and other obese animals (17–21). However, obese patients have increased metabolic clearance of cortisol and enhanced ACTH-cortisol secretion (9,10), which is believed to be due to impaired 11β-HSD1 activity in liver. Similarly, obese animals also have impaired hepatic 11β-HSD1 activity that may stimulate glucocorticoid-induced obesity and insulin resistance (20,21). These data demonstrate that obesity might be correlated with a selective inhibition of 11β-HSD1 in the liver. Consistent with these findings, we observed that obesity in *ob/ob* mice was associated with an impairment of hepatic 11β-HSD1 expression. 11β-HSD1 activity in hepatocytes from *ob/ob* mice was significantly lower than that in hepatocytes from lean mice. This reduction in 11β-HSD1 activity corresponded to elevated serum corticosterone concentrations and body weight, suggesting that impairment of hepatic 11β-HSD1 expression contributes to the development of obesity in *ob/ob* mice.

In *ob/ob* mice, genetic leptin deficiency results in glucocorticoid-dependent obesity and diabetes. Treating *ob/ob* mice with recombinant leptin normalizes circulating corticosterone levels and reverses the obese phenotype (23,24). The present study showed that physiological concentrations of leptin increase 11β-HSD1 activity and mRNA expression at the transcriptional levels in isolated *ob/ob* mouse hepatocytes through a direct action. However, *db/db* mice are insensitive to leptin because of a mutation in the leptin receptor (41). Leptin did not influence 11β-HSD1 expression in primary hepatocytes from *db/db* mice, demonstrating that the effects of leptin on hepatic 11β-HSD1 are mediated by the leptin receptor. Moreover, we also found that treatment of *ob/ob* mice with leptin restores levels of hepatic 11β-HSD1 expression to normal levels and reverses the increase in plasma corticosterone levels and body weight. Therefore, we conclude that decreased hepatic 11β-HSD1 activity and mRNA expression play crucial roles in the obesity observed in *ob/ob* mice. The reversal or attenuation of the obese phenotype of *ob/ob* mice by leptin may be mediated, at least in part, by activation of hepatic 11β-HSD1 expression. Hepatic induction of 11β-HSD1 corresponded to reduced levels of circulating glucocorticoids and body weight, indicating that hepatic 11β-HSD1 may be involved in the response of the glucocorticoid system to exogenous leptin in modulating the obese phenotype of *ob/ob* mice. The reduced synthesis of intracellular corticosterone via impaired hepatic 11β-HSD1 expression in *ob/ob* mice may contribute to the leptin deficiency-induced activation of the HPA axis, which is associated with elevated circulating glucocorticoid levels and body weight. This is consistent with several studies that showed that hepatic 11β-HSD1 mediates the negative feedback regulation of the HPA axis by endogenous glucocorticoids (11,21). Lack of hepatic 11β-HSD1 expression in null mice enhances the response of the adrenal glands to ACTH and elevates circulating

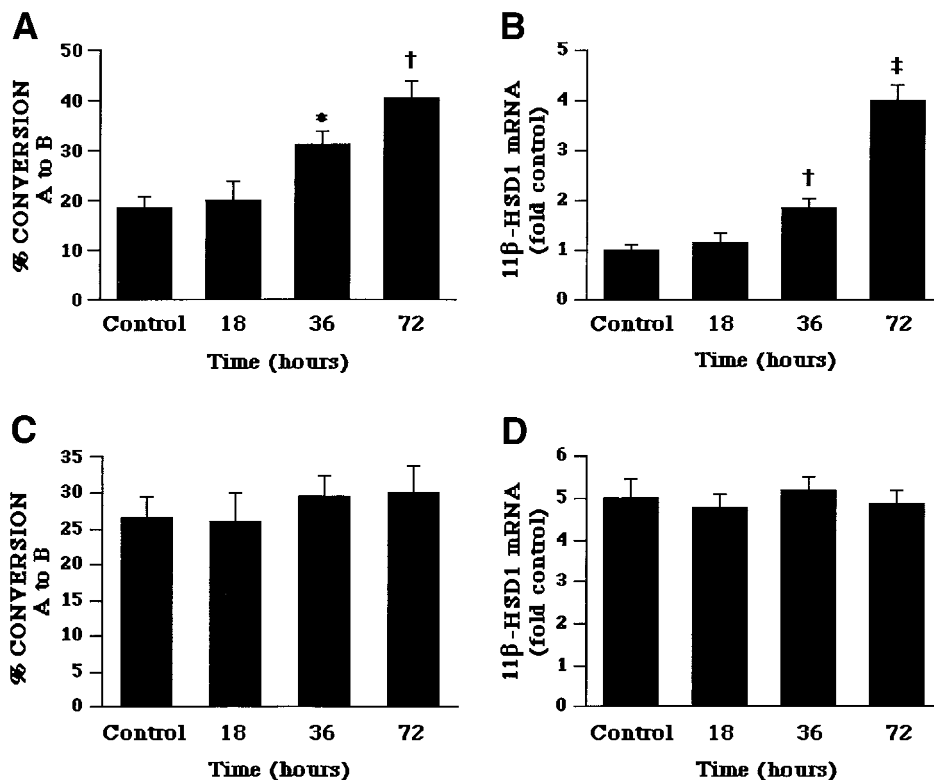

FIG. 6. Effects of leptin on 11β-HSD1 activities and mRNA levels in primary cultures of hepatocytes from both *ob/ob* (*A* and *B*) and *db/db* (*C* and *D*) mice. Hepatocytes were incubated with leptin (100 ng/ml) for different times. Values are means ± SE from three separate culture preparations. *A* and *C*: 11β-HSD1 activity expressed as the percent conversion of [^{3}H]A to [^{3}H]B in medium from hepatocytes after 30 min. *P < 0.05 vs. controls; †P < 0.01 vs. controls. *B* and *D*: The levels of 11β-HSD1 mRNA expression were determined by quantitative real-time PCR and are expressed relative to the amount of mRNA found in controls. †P < 0.05 vs. controls; ‡P < 0.01 vs. controls.

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corticosterone levels (11,33). Similarly, a recent study of obese Zucker rats also indicated that inhibition of hepatic 11β-HSD1 might stimulate the HPA axis to increase circulating glucocorticoids and body weight (20,21). Moreover, activation of the HPA axis in obese patients is thought to be associated with impaired hepatic 11β-HSD1 activity (16–19). These important findings may clarify how the impairment of hepatic 11β-HSD1 expression contributes to glucocorticoid secretion and ultimately the obese phenotype of *ob/ob* mice. The present study provides experimental evidence that alteration of hepatic 11β-HSD1 activity and gene expression may be an important component in the development of obesity and diabetes. In addition, enhanced 11β-HSD1 expression in adipose tissue is observed in *ob/ob* mice (data not shown). This is consistent with studies in obese rats where elevated circulating glucocorticoid levels induce adipose 11β-HSD1 activity (21). Thus, the possibility that facilitation of local glucocorticoid action in adipose tissue is involved in the development of obesity in *ob/ob* mice must also be considered.

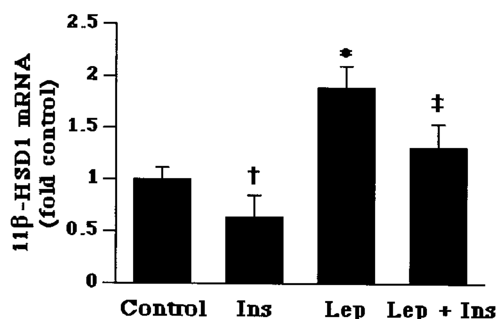

FIG. 7. The relative quantitations of 11β-HSD1 mRNA levels in primary culture hepatocytes from *ob/ob* mice. The level of 11β-HSD1 mRNA expression was analyzed by quantitative real-time PCR and are expressed relative to the amount of mRNA found in the untreated hepatocytes. Values are means ± SE from three separate culture preparations. The *ob/ob* mouse hepatocytes were treated with insulin (10 nmol/l) in the absence or presence of leptin (10 ng/ml) for 48 h. †P < 0.05 vs. controls; *P < 0.001 vs. controls; ‡P < 0.01 vs. leptin-treated hepatocytes. Ins, insulin; Lep, leptin.

Leptin treatment of *ob/ob* mice also reversed the elevated serum insulin levels and induction of hepatic 11β-HSD1 expression. Indeed, early studies observed that insulin reduced 11β-HSD1 activity in skin fibroblasts and liver cells (13,42). We recently reported that low circulating levels of insulin induce renal 11β-HSD1 activity and mRNA expression in STZ-treated diabetic rats (37). In the present study, we observed that insulin reduces 11β-HSD1 activity and attenuates the leptin-induced expression of 11β-HSD1 in primary cultures of hepatocytes. Our observation that leptin has a direct effect on 11β-HSD1 indicates not only that leptin has insulin-independent actions, but also that the elevated serum insulin levels in *ob/ob* mice may be involved in the impairment of hepatic 11β-HSD1. In contrast, reduced serum insulin levels after leptin treatment might contribute to the activation of this enzyme in the liver of *ob/ob* animals. Our conclusion of both the insulin-independent and insulin-dependent effect of leptin on hepatic 11β-HSD1 is consistent with several studies suggesting that insulin antagonizes the actions of leptin and that some effects of leptin in peripheral tissues can be partly obtained in the setting of reduced circulating insulin levels (43,44).

It has become clear that leptin can improve glucose metabolism in both normal and obese animals and that leptin-induced reduction of blood glucose levels in these animals does not require increased secretion of insulin (43–45). Such a concept is supported by data from studies in which leptin acted independently of insulin to restore euglycemia and normalize glucose turnover in insulin-deficient STZ-induced diabetic rats (46,47). In the present

study, we observed that leptin treatment of *ob/ob* and STZ-treated *ob/ob* mice causes marked elevation of hepatic 11β-HSD1 expression, reduction of body weight and circulating corticosterone levels, and reversal of hyperglycemia within 2 weeks without the use of insulin. Moreover, we also observed that leptin treatment of *ob/ob* mice increased insulin sensitivity and reduced the expression of PEPCK, a gluconeogenic enzyme that is correlated with the acceleration of gluconeogenic pathways in the *ob/ob* mouse liver (48). These results provide new evidence that leptin plays insulin-independent roles in the control of glucose metabolism and that modulation of hepatic 11β-HSD1 expression may be involved in the glucose-lowering effects of leptin in obese mice. This is consistent with recent reports that insulin resistance is associated with decreased intracellular cortisol production via impaired hepatic 11β-HSD1 activity and that expression of 11β-HSD1 in liver facilitates the maintenance of glucose homeostasis in obese humans (17,18). Leptin correction of impaired hepatic 11β-HSD1 expression may restore the physiological levels of intracellular corticosterone and thereby promote the restoration of circulating glucocorticoids, which is correlated with the reduction of hepatic corticosterone levels and improvement of insulin resistance in leptin-treated *ob/ob* mice. Although overexpressing 11β-HSD1 may increase local corticosterone levels, maintaining the physiological activity of intracellular 11β-HSD1 may not elevate tissue glucocorticoid levels, because it reflects the metabolic balance between active corticosterone and inactive 11-dehydrocorticosterone (11,28). This result is supported by recent findings that lean rats have high expression of hepatic 11β-HSD1 compared with obese rats, yet have normal metabolic features, including glucocorticoid homeostasis, insulin sensitivity, and hepatic glucose production (21).

In summary, we demonstrated that impairment of 11β-HSD1 expression by hepatocytes is associated with development of the obese phenotype of *ob/ob* mice. We also found that physiological concentrations of leptin increase hepatic 11β-HSD1 activity and mRNA expression and that these actions are mediated by the leptin receptor. The liver-specific association of leptin with 11β-HSD1 expression may be involved in the modulation of obesity and insulin resistance in *ob/ob* mice. This suggests that selective enzyme induction within the liver is a new pathway for understanding the importance of tissue-specific dysregulation of 11β-HSD1 in the development of obesity and diabetes.

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REFERENCES

1. Glass AR: Endocrine aspects of obesity. *Med Clin North Am* 73:139–160, 1989
2. Bjorntorp P: Visceral fat accumulation: the missing link between psychosocial factors and cardiovascular disease? *J Intern Med* 230:195–201, 1991
3. Bouchard C, Despres JP, Mauriege P: Genetic and nongenetic determinants of regional fat distribution. *Endocr Rev* 14:72–93, 1993
4. Bjorntorp P, Rosmond R: Obesity and cortisol. *Nutrition* 16:924–936, 2000
5. Andrews RC, Walker BR: Glucocorticoids and insulin resistance: old hormones, new targets. *Clin Sci (Lond)* 96:513–523, 1999
6. Marin P, Darin N, Amemiya T, Andersson B, Jern S, Bjorntorp P: Cortisol secretion in relation to body fat distribution in obese premenopausal women. *Metabolism* 41:882–886, 1992
7. Wajchenberg BL: Subcutaneous and visceral adipose tissue: their relation to the metabolic syndrome. *Endocr Rev* 21:697–738, 2000
8. Rosmond R, Dallman MF, Bjorntorp P: Stress-related cortisol secretion in men: relationships with abdominal obesity and endocrine, metabolic and hemodynamic abnormalities. *J Clin Endocrinol Metab* 83:1853–1859, 1998
9. Dunklemann SS, Fairhurst B, Plager J, Waterhouse C: Cortisol metabolism in obesity. *J Clin Endocrinol Metab* 24:832–841, 1964
10. Migeon CJ, Green OC, Eckert JP: Study of adrenocortical function in obesity. *Metabolism* 12:718–739, 1963
11. Seckl JR, Walker BR: Minireview: 11beta-hydroxysteroid dehydrogenase type: a tissue-specific amplifier of glucocorticoid action. *Endocrinology* 142:1371–1376, 2001
12. Funder JW, Pearce PT, Smith R, Smith AI: Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. *Science* 242:583–585, 1988
13. Voice MW, Seckl JR, Edwards CR, Chapman KE: 11 beta-hydroxysteroid dehydrogenase type 1 expression in 2S FAZA hepatoma cells is hormonally regulated: a model system for the study of hepatic glucocorticoid metabolism. *Biochem J* 317:621–625, 1996
14. Bujalska IJ, Kumar S, Stewart PM: Does central obesity reflect "Cushing's disease of the omentum"? *Lancet* 349:1210–1213, 1997
15. Rajan V, Edwards CR, Seckl JR: 11 Beta-hydroxysteroid dehydrogenase in cultured hippocampal cells reactivates inert 11-dehydrocorticosterone, potentiating neurotoxicity. *J Neurosci* 16:65–70, 1996
16. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294:2166–2170, 2001
17. Stewart PM, Boulton A, Kumar S, Clark PM, Shackleton CH: Cortisol metabolism in human obesity: impaired cortisone → cortisol conversion in subjects with central adiposity. *J Clin Endocrinol Metab* 84:1022–1027, 1999
18. Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DE, Johnson O, Walker BR: Tissue-specific dysregulation of cortisol metabolism in human obesity. *J Clin Endocrinol Metab* 86:1418–1421, 2001
19. Rask E, Walker BR, Soderberg S, Livingstone DE, Eliasson M, Johnson O, Andrew R, Olsson T: Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11beta-hydroxysteroid dehydrogenase type 1 activity. *J Clin Endocrinol Metab* 87:3330–3336, 2002
20. Livingstone DE, Jones GC, Smith K, Jamieson PM, Andrew R, Kenyon CJ, Walker BR: Understanding the role of glucocorticoids in obesity: tissue-specific alterations of corticosterone metabolism in obese Zucker rats. *Endocrinology* 141:560–563, 2000
21. Livingstone DE, Kenyon CJ, Walker BR: Mechanisms of dysregulation of 11 beta-hydroxysteroid dehydrogenase type 1 in obese Zucker rats. *J Endocrinol* 167:533–539, 2000
22. Meister B, Arvidsson U: The hormone leptin reduces body weight: a mutant gene makes the mouse obese. *Lakartidningen* 93:247–251, 1996
23. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, Lallone RL, Burley SK, Friedman JM: Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 269:543–546, 1995
24. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F: Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 269:540–543, 1995
25. Le Marchand Y, Loten EG, Assimacopoulos-Jeannet F, Forgue ME, Freychet P, Jeanrenaud B: Effect of fasting and streptozotocin in the obese-hyperglycemic (ob/ob) mouse: apparent lack of a direct relationship between insulin binding and insulin effects. *Diabetes* 26:582–590, 1977
26. Dubuc PU: Interactions between insulin and glucocorticoids in the maintenance of genetic obesity. *Am J Physiol* 263:E550–E555, 1992
27. Liu YJ, Nakagawa Y, Toya K, Wang Y, Saegusa H, Nakanishi T, Ohzeki T: Effects of spironolactone on systolic blood pressure in experimental diabetic rats. *Kidney Int* 57:2064–2071, 2000
28. Yau JL, Noble J, Kenyon CJ, Hibberd C, Kotelevtsev Y, Mullins JJ, Seckl JR: Lack of tissue glucocorticoid reactivation in 11beta-hydroxysteroid dehydrogenase type 1 knockout mice ameliorates age-related learning impairments. *Proc Natl Acad Sci U S A* 12:3656–3660, 2001
29. Honkakoski P, Negishi M: Protein serine/threonine phosphatase inhibitors

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suppress phenobarbital-induced Cyp2b10 gene transcription in mouse primary hepatocytes. *Biochem J* 330:889–895, 1998
30. Bornstein SR, Uhlmann K, Haidan A, Ehrhart-Bornstein M, Scherbaum WA: Evidence for a novel peripheral action of leptin as a metabolic signal to the adrenal gland: leptin inhibits cortisol release directly. *Diabetes* 46:1235–1238, 1997
31. Liu YJ, Nakagawa Y, Toya K, Ozeki T: Sex-specific effects of growth hormone on hepatic 11beta-hydroxysteroid dehydrogenase activity and gene expression in hypothyroid rats. *Life Sci* 61:325–334, 1997
32. Lakshmi V, Monder C: Purification and characterization of the corticosteroid 11 beta-dehydrogenase component of the rat liver 11 beta-hydroxysteroid dehydrogenase complex. *Endocrinology* 123:2390–2398, 1988
33. Kotelevtsev Y, Holmes MC, Burchell A, Houston PM, Schmoll D, Jamieson P, Best R, Brown R, Edwards CR, Seckl JR, Mullins JJ: 11Beta-hydroxysteroid dehydrogenase type 1 knockout mice show attenuated glucocorticoid-inducible responses and resist hyperglycemia on obesity or stress. *Proc Natl Acad Sci U S A* 94:14924–14929, 1997
34. Whorwood CB, Ricketts ML, Stewart PM: Epithelial cell localization of type 2 11 beta-hydroxysteroid dehydrogenase in rat and human colon. *Endocrinology* 135:2533–2541, 1994
35. Rajan V, Chapman KE, Lyons V, Jamieson P, Mullins JJ, Edwards CR, Seckl JR: Cloning, sequencing and tissue-distribution of mouse 11 beta-hydroxysteroid dehydrogenase-1 cDNA. *J Steroid Biochem Mol Biol* 52:141–147, 1995
36. Yoo-Warren H, Monahan JE, Short J, Short H, Bruzel A, Wynshaw-Boris A, Meisner HM, Samols D, Hanson RW: Isolation and characterization of the gene coding for cytosolic phosphoenolpyruvate carboxykinase (GTP) from the rat. *Proc Natl Acad Sci U S A* 12: 3656–3660, 1983
37. Liu YJ, Nakagawa Y, Ohzeki T: Gene expression of 11beta-hydroxysteroid dehydrogenase type 1 and type 2 in the kidneys of insulin-dependent diabetic rats. *Hypertension* 31:885–889, 1998
38. Berger J, Tanen M, Elbrecht A, Hermanowski-Vosatka A, Moller DE, Wright SD, Thieringer R: Peroxisome proliferator-activated receptor-gamma ligands inhibit adipocyte 11beta-hydroxysteroid dehydrogenase type 1 expression and activity. *J Biol Chem* 276:12629–12635, 2001
39. Ohshima K, Shargill NS, Chan TM, Bray GA: Adrenalectomy reverses insulin resistance in muscle from obese (ob/ob) mice. *Am J Physiol* 246:E193–E197, 1984
40. Freedman MR, Horwitz BA, Stern JS: Effect of adrenalectomy and glucocorticoid replacement on development of obesity. *Am J Physiol* 250: R595–R607, 1986
41. Ghilardi N, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC: Defective STAT signaling by the leptin receptor in diabetic mice. *Proc Natl Acad Sci U S A* 13:6231–6235, 1996
42. Hammami MM, Siiteri PK: Regulation of 11 beta-hydroxysteroid dehydrogenase activity in human skin fibroblasts: enzymatic modulation of glucocorticoid action. *J Clin Endocrinol Metab* 73:326–334, 1991
43. Cohen B, Novick D, Rubinstein M: Modulation of insulin activities by leptin. *Science* 274:1185–1188, 1996
44. Barzilai N, Wang J, Massilon D, Vuguin P, Hawkins M, Rossetti L: Leptin selectively decreases visceral adiposity and enhances insulin action. *J Clin Invest* 100:3105–3110, 1997
45. Sivitz WI, Walsh SA, Morgan DA, Thomas MJ, Haynes WG: Effects of leptin on insulin sensitivity in normal rats. *Endocrinology* 138:3395–3401, 1997
46. Chinookoswong N, Wang JL, Shi ZQ: Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat. *Diabetes* 48:1487–1492, 1999
47. Sindelar DK, Havel PJ, Seeley RJ, Wilkinson CW, Woods SC, Schwartz MW: Low plasma leptin levels contribute to diabetic hyperphagia in rats. *Diabetes* 48:1275–1280, 1999
48. Reul BA, Becker DJ, Ongemba LN, Bailey CJ, Henquin JC, Brichard SM: Improvement of glucose homeostasis and hepatic insulin resistance in *ob/ob* mice given oral molybdate. *J Endocrinol* 155:55–64, 1997

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