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

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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

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 cortisol to cortisone, 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-
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 11β-HSD1 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/J6 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 used for the 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 (Sigma) and then were plated in 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 nmol/l) of recombinant mouse leptin. Amino acids 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 liver assay 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). 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 [32P]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 10 min. For densitometric measurements, autoradiographic signals were standardized to signals measured from 18S rRNA (37).

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

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<thead>
<tr>
<th>Experiment 1</th>
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<td>saline</td>
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<tr>
<td>Blood glucose (mmol/l)</td>
<td>8.4 ± 0.6</td>
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<tr>
<td>Plasma insulin (pmol/l)</td>
<td>32 ± 2.7</td>
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<tr>
<td>Corticosterone (nmol/l)</td>
<td>127 ± 8</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>17.9 ± 0.7</td>
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Data are means ± SE. *P < 0.05 vs. saline-lean mice; †P < 0.01 vs. saline-ob/ob mice; ‡P < 0.001 vs. STZ-ob/ob mice; ††P < 0.001 vs. STZ-ob/ob mice; †‡P < 0.001 vs. saline-ob/ob mice; †§P < 0.001 vs. saline-ob/ob mice; †††P < 0.001 vs. vehicle-ob/ob mice; ††‡P < 0.001 vs. vehicle-ob/ob mice; ††††P < 0.001 vs. vehicle-ob/ob mice.

decreased compared with that of lean littermates (15.34 ± 1.1 vs. 31.7 ± 2.1%, P < 0.001) (Fig. 1A). When examining both lean and ob/ob mice together, there was an inverse correlation between hepatic 11β-HSD1 activity and body weight (R² = 0.958). The decrease in enzyme activity was paralleled by 11β-HSD1 mRNA expression, which was decreased to 32% of that of lean animals (Fig. 1A 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).

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. 1A). 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. 1B) (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. 3A. 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. 3B. 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. 3B).

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,
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. 4A). Enzyme activity correlated with the increased levels of enzyme mRNA shown in Fig. 4A 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 [3H]B to [3H]A in intact cells, even after 24-h incubations). However, 11β-reductase activity was clearly detected with 40–60% conversion of [3H]A to [3H]B. Thus, 11β-HSD function is primarily expressed as a reductase in intact ob/ob mouse hepatocytes. As shown in Fig. 5A, 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. 6A). However, in primary cultures of db/db hepatocytes, leptin had no significant effect on 11β-HSD1 activity (Figs. 5C and 6C).

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. 5B and 6B. After leptin treatment of ob/ob hepatocytes, the 11β-HSD1 mRNA levels increased two- to sixfold above control levels (Fig. 5B). 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. 6B). In contrast, leptin treatment did not have a significant effect on 11β-HSD1 mRNA expression in db/db primary hepatocytes (Figs. 5D and 6D).

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). Co-treatment 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. 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 [3H]B to [3H]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.
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 glucocorticoids.
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.

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

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 culture preparations. A and C: 11β-HSD1 activity expressed as the percent conversion of [3H]A to [3H]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.

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.
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-dehydrocorticoestone (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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