Pharmacological Evaluation of Adipose Dysfunction via 11\(\beta\)-Hydroxysteroid Dehydrogenase Type 1 in the Development of Diabetes in Diet-Induced Obese Mice with Cortisone Pellet Implantation

Nobuteru Akiyama, Yuko Akiyama, Hideaki Kato, Takayuki Kuroda, Takashi Ono, Keiichi Imagawa, Kenji Asakura, Toshihiro Shinosaki, Toshihiko Murayama, and Kohji Hanasaki

Medicinal Research Laboratories, Shionogi & Co., Ltd., Osaka, Japan (N.A., Y.A., H.K., T.K., T.O., K.I., T.S., K.H.); and Laboratory of Chemical Pharmacology, Graduate School of Pharmaceutical Sciences, Chiba University, Chiba, Japan (N.A., T.M.)

Received December 4, 2013; accepted February 6, 2014

ABSTRACT

Signals from intracellular glucocorticoids (GCs) via 11\(\beta\)-hydroxysteroid dehydrogenase type 1 (11\(\beta\)-HSD1) in adipose tissues have been reported to serve as amplifiers leading to deterioration of glucose metabolism associated with obesity. To elucidate adipose dysfunction via 11\(\beta\)-HSD1 activation in the development of obesity-related diabetes, we established novel diabetic mice by implanting a cortisone pellet (CP) in diet-induced obesity (DIO) mice. Cortisone pellet–implanted DIO mice (DIO/CP mice) showed hyperglycemia, insulin resistance, hyperlipidemia, and ectopic fat accumulation, whereas cortisone pellet implantation in lean mice did not induce hyperglycemia. In DIO/CP mice, indexes of lipolysis such as plasma glycerol and nonesterified fatty acids (NEFAs) increased before hyperglycemia appeared. Furthermore, the adipose mRNA level of 11\(\beta\)-HSD1 was up-regulated in DIO/CP mice compared with sham-operated DIO mice. RU486 (mifepristone, 11\(\beta\)-[p-(dimethylamino)phenyl]-17\(\beta\)-hydroxy-17-(1-propynyl)estra-4,9-dien-3-one), a glucocorticoid receptor antagonist, decreased adipose mRNA levels of 11\(\beta\)-HSD1 as well as adipose triglyceride lipase. RU486 also improved plasma NEFA, glycerol, and glucose levels in DIO/CP mice. These results demonstrate that lipolysis in adipose tissues caused by GC activation via 11\(\beta\)-HSD1 serves as a trigger for diabetes with ectopic fat accumulation. Our findings also indicate the possibility of a vicious circle of GC signals via 11\(\beta\)-HSD1 up-regulation in adipose tissues, contributing to deterioration of glucose metabolism to result in diabetes. Our DIO/CP mouse could be a suitable model of type 2 diabetes to evaluate adipose dysfunction via 11\(\beta\)-HSD1.

Introduction

Glucocorticoids (GCs) are important regulators of glucose and lipid homeostasis. As more than 90% of circulating cortisol, the active GC in humans, is predominantly bound to corticosteroid-binding globulin in plasma, less than 10% of cortisol can enter the cells across the plasma membrane. On the other hand, because cortisone, the inactive form of cortisol, is rarely protein bound, it is continuously present at higher free plasma concentrations than cortisol and can easily cross the cell membrane of target organs (Andrews and Walker, 1999; Stulnig and Waldhäusl, 2004). The main regulators of intracellular GC activation are 11\(\beta\)-hydroxysteroid dehydrogenase (11\(\beta\)-HSD) enzymes. Two isoforms of 11\(\beta\)-HSD (11\(\beta\)-HSD1 and 11\(\beta\)-HSD2) have been cloned and characterized (Lakshmi and Monder, 1988; Agarwal et al., 1989; Brown et al., 1993; Albiston et al., 1994; Brown et al., 1996). 11\(\beta\)-HSD1 is a NADP(H)-dependent enzyme that acts primarily as a reductase, converting the inactive 11-keto metabolites, cortisol (in humans) or 11-dehydrocorticosterone (11-DHC, in rodents) into the active GCs, cortisol or corticosterone, respectively. It is highly expressed in GC target tissues such as the liver, adipose tissues, and the brain. In contrast, 11\(\beta\)-HSD2 is an NAD(H)-dependent enzyme that catalyzes the reverse reaction, oxidizing active GCs to their inactive 11-keto forms, and its expression is limited to tissues that express mineralocorticoid receptors, such as the kidney, colon, and the placenta.

Excess GCs, epitomized by Cushing’s syndrome in humans, lead to central obesity, insulin resistance, and dyslipidemia associated with increased cardiovascular risk (Newell-Price et al., 2006). It is noteworthy that the Cushing’s patient with a partial defect in 11\(\beta\)-HSD1 activity displays hypercortisolemia.
but is protected against these phenotypes, which suggests that increased GC action inside the cells may contribute to progression of the metabolic syndrome (Tomlinson et al., 2002).

Mice with overexpression of 11β-HSD1 in adipose tissues show elevated adipose corticosterone and present a phenotype resembling the metabolic syndrome, including central obesity, hypertension, dyslipidemia, and insulin resistance (Masuzaki et al., 2001). On the other hand, homozygous 11β-HSD1 knockout mice are resistant to hyperglycemia by high-fat diet feeding (Kotelevtsev et al., 1997) and show an improved lipid profile (Morton et al., 2001). Furthermore, overexpression of 11β-HSD2 in adipose tissues leads to improvement of the metabolic disorders in diet-induced obesity (DIO) mice (Kershaw et al., 2005). In obese human subjects, hepatic 11β-HSD1 activity is reported to be reduced (Rask et al., 2001, 2002), whereas adipose 11β-HSD1 activity is positively correlated with body-mass index (Rask et al., 2001, 2002; Wake et al., 2003; DeSchoonmeester et al., 2013). Previous findings support the hypothesis that GC activation by 11β-HSD1 in adipose tissues contributes to the development of metabolic syndrome, including obesity-related type 2 diabetes. However, the details of how GC activation in adipose tissues contributes to systemic metabolic disorders remain unclear. Elucidation of this mechanism should provide important clues for the treatment of obesity-related type 2 diabetes.

The cortisone implantation model in obese KK mice, the polygenic diabetic mouse model, has been reported to address the question of the mechanisms from GC activation to systemic metabolic disorders (Bhat et al., 2008). Long-term treatment with cortisone was reported to cause severe hyperglycemia in KK mice, whereas lean C57BL/6 mice implanted with a cortisone pellet exhibited only hyperinsulinemia. This article suggests that changes in hepatic glucose metabolism caused by GC activation via 11β-HSD1 might be involved in the diabetic phenotype. Based on the differences in phenotypes between lean and obese mice, we hypothesized that visceral adipose tissues might be an important driving force to accelerate the diabetic condition induced by GCs. The present study was conducted to test this hypothesis. To exclude polygenic factors in KK mice, we conducted cortisone pellet implantation into DIO mice characterized by obesity and insulin resistance. To discriminate exogenous GCs from endogenous GCs, we used cortisone, an inactive GC in humans, as a substrate of 11β-HSD1, as done in a previous study (Bhat et al., 2008). Although both cortisone pellet–implanted lean and DIO mice showed the same levels of plasma cortisone and cortisol, metabolic disorders such as hyperlipidemia, hyper-glycemia, and marked insulin resistance were seen only in cortisone pellet–implanted DIO mice, as reported in previous literature for KK mice (Bhat et al., 2008). In the present article, we further report and discuss the central role of adipose tissues in the development of metabolic syndrome by GC activation.

Materials and Methods

**Animal Experiments.** Male C57BL/6J mice were purchased at 6 weeks of age from Clea Japan (Tokyo, Japan). All mice were housed under standard conditions on a 12/12-hour light/dark cycle (lights on at 8:00 AM), and water was given ad libitum. Mice were fed either a high-fat diet (Test Diet 58Y1, 5.16 kcal/g, 60% of calories from fat; Purina Mills, Gray Summit, MO) or normal chow (CE-2; Clea Japan) from 7 weeks of age. Animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee of Shionogi. Approximately 16 weeks after the start of high-fat diet feeding, mice were housed individually in cages and then subcutaneously implanted with a cortisone pellet (35 mg; 21-day release; Innovative Research of America, Sarasota, FL) or sham operated. The day the cortisone pellet was implanted was defined as day 0, and blood samples were collected at the indicated points and conditions for each experiment to measure metabolic parameters.

**RU486** (10 and 30 mg/kg, mifepristone, 11β-3-(dimethylamino)phenyl-17β-hydroxy-17-(1-propynyl)estradiol-4,9-dien-3-one; Sigma-Aldrich, St. Louis, MO) or vehicle (0.5% w/v hydroxypropyl methyl cellulose aqueous solution; Shin-Etsu Chemical, Tokyo, Japan) was orally administered twice daily to cortisone pellet–implanted DIO (DIO/CP) mice for 5 days, from day 0 to day 4. On day 5, blood samples and mesenteric adipose tissues were collected for determination of metabolic parameters [nonfasting glucose, nonfasting nonesterified fatty acids (NEFAs), and nonfasting glycerol], as well as mRNA expression levels of 11β-HSD1 and adipose triglyceride lipase (ATGL).

**Measurement of Metabolic Parameters in Plasma.** Blood samples were collected from the tail vein with heparinized hematocrit capillary tubes (Terumo, Tokyo, Japan) and centrifuged (15,000 upside down) at 4 hours before the experiments, and plasma samples were transferred to collection tubes on ice. Plasma glucose, triglycerides, and NEFAs were measured with a blood chemistry automatic analyzer (Clinical Analyzer 7150; Hitachi High-Technologies, Tokyo, Japan). Plasma insulin and glycerol levels were determined using the Insulin ELISA Kit (Shibayagi, Gunma, Japan) and the free glycerol determination kit (Sigma-Aldrich), respectively. GCs in plasma were extracted and analyzed by high-performance liquid chromatography (Waters 2690 System; Waters, Milford, MA) with dexamethasone (Sigma-Aldrich) used as an internal standard.

**Measurement of Triglycerides in Liver.** The animals were sacrificed by inhalation of CO2 gas and livers were dissected. The samples were thoroughly homogenized in 4–10 volumes of isopropanol and cleared of debris by centrifugation (×10,000g). The extract was measured with a biochemistry automatic analyzer (Clinical Analyzer 7150; Hitachi High-Technologies).

**Hyperinsulinemic-Euglycemic Clamp Studies.** Hyperinsulinemic-euglycemic clamp studies were performed in DIO and DIO/CP mice at 5 days after sham operation or cortisone pellet implantation. The mice used in the clamp studies were distinct from those in metabolic parameters studies. After an overnight fast, the mice were anesthetized with isoflurane at 4 hours before the experiments, and the right jugular vein was catheterized with MRE-025 tubes (inside diameter 0.0305 and 0.0635 mm, respectively; Braintree Scientific, Braintree, MA) filled with saline. Insulin (Humalin R; Eli Lilly, Indianapolis, IN) was infused continuously at 100 mU/kg/min, and blood glucose concentration was monitored.

### TABLE 1

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Cortisone</th>
<th>Cortisol</th>
<th>11-DHC</th>
<th>Corticosterone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean/CP</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>Lean</td>
<td>202 ± 12.9</td>
<td>314 ± 29.2</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td>DIO</td>
<td>N.D.</td>
<td>N.D.</td>
<td>55 ± 13.4</td>
<td>N.D.</td>
</tr>
<tr>
<td>DIO/CP</td>
<td>135 ± 8.4</td>
<td>284 ± 20.7</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D., Not detected.
every 10 minutes and maintained at 115–135 mg/dl by infusion of 20% glucose. These hyperinsulinemic-euglycemic clamp studies were performed within around 3 hours to maintain the desired glucose level. The glucose infusion rate was calculated during the last 20 minutes of the clamp.

**RNA Extraction and Real-Time Polymerase Chain Reaction.** Tissue samples from the liver, gastrocnemius muscle, and mesenteric fat were snap-frozen in liquid nitrogen and homogenized in QIAzol (Qiagen, Valencia, CA). Total RNA was extracted using the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. Equal amounts of total RNA from each sample were reverse-transcribed using the High Capacity RNA-to-cDNA Kit (Life Technologies, Carlsbad, CA). The primer sets for 11β-HSD1, ATGL, and ribosomal protein S18 were purchased from Takara (Shiga, Japan). 11β-HSD1 and ATGL mRNA levels were analyzed using the 7500 Real Time Polymerase Chain Reaction System (Life Technologies) and were normalized to those of ribosomal protein S18 and expressed as the fold increase based on the expression levels in sham-operated DIO mice.

**Statistical Analysis.** Data are represented as mean ± S.E.M. with statistical significance defined as \( P < 0.05 \). Statistical analysis was performed by \( t \) test, two-way analysis of variance, or

---

**Fig. 1.** Effects of cortisone treatment on metabolic parameters in lean and DIO mice. (A–C) Plasma levels of glucose (A), triglycerides (B), and NEFAs (C) in lean and DIO mice 5 days after treatment with sham operation or cortisone pellet implantation (lean, \( n = 19 \); lean/CP, \( n = 4 \); DIO, \( n = 12 \); DIO/CP, \( n = 25 \)). (D) Glucose infusion rate during hyperinsulinemic-euglycemic clamp studies performed in DIO mice 5 days after treatment with sham operation or cortisone pellet implantation (DIO, \( n = 5 \); DIO/CP, \( n = 6 \)). (E and F) Plasma levels of glucose (E) and insulin (F) in overnight-fasted DIO mice 7 days after treatment with sham operation or cortisone pellet implantation (DIO, \( n = 21 \); DIO/CP, \( n = 20 \)). Values are mean ± S.E.M. ***\( P < 0.001 \) versus sham-operated DIO mice. N.S., Not significant.
Dunn’s multiple comparison test. All analyses were performed by using the SAS Version 9.2 for Windows (SAS Institute, Cary, NC).

Results

Plasma Levels of GCs in Lean/CP and DIO/CP Mice. As shown in Table 1, 11-DHC, endogenous inactive GC in rodents, was not detected in lean or DIO mice. On the other hand, corticosterone, endogenous active GC in rodents, was detected only in DIO mice. To adjust the inactive GC storage pool in plasma to that in humans, we implanted a cortisone pellet into lean and DIO mice. In cortisone pellet–implanted lean (lean/CP) and DIO (DIO/CP) mice, detectable levels of cortisone and cortisol, the inactive and active GCs in humans, respectively, appeared in the plasma.

Metabolic Parameters in Lean/CP and DIO/CP Mice. To investigate metabolic plasma parameters in lean/CP and DIO/CP mice, nonfasting plasma glucose, triglyceride, and NEFAs were measured 5 days after cortisone pellet implantation. The implantation in DIO mice strongly increased nonfasting plasma glucose from 183 ± 18.5 mg/dl in sham-operated DIO mice to 351 ± 15.7 mg/dl (Fig. 1A). On the other hand, cortisone pellet implantation in lean mice did not significantly induce hyperglycemia. Furthermore, in DIO/CP mice, nonfasting plasma triglyceride and NEFA levels were increased compared with sham-operated DIO mice (Fig. 1, B and C). In the fasting state, DIO/CP mice showed higher plasma glucose and insulin levels relative to sham-operated DIO mice (Fig. 1, E and F). A hyperinsulinemic-euglycemic glucose clamp study was performed to investigate the insulin resistance of DIO/CP mice. In DIO/CP mice, the glucose infusion rate was significantly reduced (Fig. 1D, 44.2 ± 4.4 mg/kg/min in DIO mice versus 14.0 ± 2.1 mg/kg/min in DIO/CP mice).

Effects of Cortisone Treatment on Adiposity and Hepatic Triglyceride Contents in DIO Mice. To evaluate the distribution of adiposity in DIO/CP mice, we measured the weights of the liver and adipose tissues. In DIO/CP mice, the liver weight was greater than that of sham-operated DIO mice when measured 11 days after cortisone pellet implantation (Table 2). In agreement with the increase in hepatic weight, DIO/CP mice exhibited elevated triglyceride contents in the liver (Table 2). On the other hand, the weights of mesenteric and epididymal adipose tissues were decreased in DIO/CP mice.

TABLE 2

Effects of cortisone treatment on adiposity and hepatic triglyceride contents in DIO mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DIO</th>
<th>DIO/CP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>43.4 ± 0.51</td>
<td>39.0 ± 0.59*</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>1.67 ± 0.07</td>
<td>2.57 ± 0.13**</td>
</tr>
<tr>
<td>Mesenteric fat (g)</td>
<td>1.15 ± 0.04</td>
<td>0.62 ± 0.03**</td>
</tr>
<tr>
<td>Epididymal fat (g)</td>
<td>1.75 ± 0.06</td>
<td>1.28 ± 0.05**</td>
</tr>
<tr>
<td>Liver triglycerides (mg/g tissue)</td>
<td>129.1 ± 14.6</td>
<td>172.3 ± 9.7*</td>
</tr>
</tbody>
</table>

*P < 0.05 versus sham-operated DIO mice.

Acute Effects of Cortisone Treatment on Plasma Glucose and Parameters of Lipolysis in Lean and DIO Mice. Nonfasting plasma glucose levels were not affected by cortisone pellet implantation in either lean or DIO mice, when measured 1 day after the implantation (Fig. 2A). Nonfasting plasma glycerol and NEFA levels were markedly increased in DIO/CP mice compared with sham-operated DIO mice on day 1 (Fig. 2, C and E). However, nonfasting plasma glucose, glycerol, and NEFA levels were not changed by cortisone pellet implantation in lean mice at all (Fig. 2).

Expression Change of 11β-HSD1 mRNA in Adipose Tissues. Five days after cortisone pellet implantation, 11β-HSD1 mRNA expression in mesenteric adipose tissues of DIO/CP mice was 2.9-fold higher than that of DIO mice (Fig. 3). On the other hand, 11β-HSD1 mRNA expressions in liver and gastrocnemius muscle were not changed by cortisone pellet implantation in DIO mice.

Effects of RU486 on 11β-HSD1 and ATGL mRNA Expression in Adipose Tissues and Metabolic Parameters. On day 5, DIO/CP mice showed statistically significant increases in the levels of adipose 11β-HSD1 and ATGL mRNA, and metabolic parameters (glucose, glycerol, and NEFAs) compared with sham-operated DIO mice (Fig. 4). Administration of RU486, a glucocorticoid receptor (GR) antagonist, for 5 days dose-dependently decreased adipose 11β-HSD1 and ATGL mRNA expression in mesenteric adipose tissues in DIO/CP mice (Fig. 4, A and B). Furthermore, treatment with RU486 dose-dependently ameliorated nonfasting plasma glucose, glycerol, and NEFA levels compared with those in vehicle-treated DIO/CP mice (Fig. 4, C–E).

Discussion

To elucidate the role of 11β-HSD1 in the development of diabetes based on obesity, we established novel diabetic mice by implantation of a cortisone pellet in DIO mice. We report here that cortisone pellet–implanted DIO mice (DIO/CP mice) display hyperglycemia, insulin resistance, hyperlipidemia, and ectopic fat accumulation. Furthermore, this report proposes that increased lipolysis with the upregulation of adipose GC signals through 11β-HSD1 plays an important role in systemic hyperglycemia and insulin resistance.

Inactive GCs such as cortisone or 11-DHC, in humans or rodents, respectively, are substrates for the 11β-HSD1 enzyme. However, the plasma GC levels differ greatly between humans and rodents. Humans have a store of cortisone circulating at a concentration of about 50–100 nM in plasma (Walker et al., 1992). On the other hand, the plasma 11-DHC level in rodents is much lower. Therefore, we thought it would be difficult to fully elucidate the pathophysiologic role of 11β-HSD1 in rodent models without manipulating the inactive GC levels. To resolve this issue, we referred to two papers reporting surgical implantation of a cortisone pellet into diabetic KK mice (Bhat et al., 2008; Morgan et al., 2009). They reported that long-term treatment with cortisone caused severe hyperglycemia in these mice; when they administered cortisone, cortisol was produced by 11β-HSD1 in these mice as occurs in humans. On the other hand, lean C57BL/6 mice implanted with...
a cortisone pellet exhibited only hyperinsulinemia (Bhat et al., 2008). Based on this report, we hypothesized that visceral adipose tissues might be essential for the progress of type 2 diabetes induced by GC activation. To test our hypothesis, we used DIO mice instead of KK mice, because DIO mice have abundant visceral fat and are recognized as a general obesity model. Furthermore, since KK mice are hyperglycemic due to polygenic factors, DIO mice are considered to be a more suitable model for investigating the mechanisms of diabetes based on obesity. In accordance with previous reports, we implanted a cortisone pellet into lean and DIO mice to adjust inactive GC pool sizes in plasma to simulate those in humans. As shown in Results, DIO/CP mice displayed hyperglycemia, hyperinsulinemia, and hyperlipidemia. On the other hand, although cortisone pellet–implanted lean mice (lean/CP mice) showed the same levels of plasma cortisol and cortisone as DIO/CP mice, the lean/CP mice did not become hyperglycemic. The differences in phenotypes between lean/CP and DIO/CP mice indicate that obesity plays an important role in causing diabetes associated with 11β-HSD1.

As also shown in Results, increased plasma NEFA and glycerol levels were observed in DIO/CP mice. We also demonstrated that DIO/CP mice exhibited increased hepatic triglyceride content and weight, indicating serious hepatic steatosis. Furthermore, we found a decrease in the volume of mesenteric fat in DIO/CP mice compared with DIO mice, but the adipose volume of DIO/CP mice exceeded that of lean
mice. These results suggest that elevated lipolysis in adipose tissues induces severe ectopic fat accumulation in DIO/CP mice. Several previous reports have shown that increased GC signals potently induce adipose lipolysis in humans (Divertie et al., 1991; Gravholt et al., 2002). Therefore, the induction of lipolysis is considered to play a crucial role in all metabolic disorders evoked by GC activation via 11β-HSD1.

ATGL cleaves triacylglycerol, releasing NEFAs into the bloodstream, and plays an important role in basal lipolytic rates (Jenkins et al., 2004; Zimmermann et al., 2004; Haemmerle et al., 2006). In addition, it has been reported that adipose ATGL expression and lipolysis are regulated by GC signals (Villena et al., 2004; Berthiaume et al., 2007; Xu et al., 2009; Campbell et al., 2011; Serr et al., 2011; Sano et al., 2012; Wang et al., 2012). As shown in these previous similar studies, we confirmed that cortisone treatment increased the ATGL mRNA level and glycerol release in 3T3-L1 adipocytes (data not shown). In this study, we showed that the adipose ATGL mRNA levels were higher in DIO/CP mice than in DIO mice. These results suggest that up-regulation of adipose ATGL expression contributes to the enhanced lipolysis observed in DIO/CP mice.

As a mechanism of hyperglycemia induced by lipolysis in adipose tissues, glycerol has been reported to be a substrate for hepatic gluconeogenesis (Postic et al., 2004). As shown in the previous study (Jelen et al., 2011), we also confirmed that glycerol in the range of the plasma level dose-dependently increased the glucose output from rat primary hepatocytes (data not shown). The rates of glycerol appearance and gluconeogenesis from glycerol are increased in patients with type 2 diabetes (Nurjhan et al., 1992; Puhakainen et al., 1992). Acipimox, a niacin derivative used to reduce plasma lipids by inhibiting lipolysis, lowers plasma glycerol and glucose in streptozotocin-induced diabetic rats (Claus et al., 2005). In a clinical study, acipimox decreased plasma free fatty acid levels and ameliorated fasting plasma glucose and insulin resistance in patients with type 2 diabetes (Bajaj et al., 2005). Based on the finding that visceral adipose dysfunction might closely influence hepatic glucose output, we focused on the relationship between hyperglycemia and lipolysis in DIO/CP mice. One day after cortisone pellet implantation, plasma glycerol and NEFA levels markedly increased in DIO/CP mice before the onset of hyperglycemia, indicating that induction of lipolysis in adipose tissue occurs upstream of hyperglycemia caused by GC activation. On the other hand, in lean/CP mice, plasma glycerol and NEFA levels were not changed on day 1 or day 4, and hyperglycemia was not induced at all. The phenotype of lean/CP mice suggests that glycerol supply from adipose tissues is insufficient for the elevation of hepatic gluconeogenesis because of the small mass of visceral adipose tissues in lean mice. From the difference of phenotypes between lean/CP mice and DIO/CP mice, we speculate that glycerol release produced by adipose lipolysis plays a crucial role in the hyperglycemia caused by GC activation. Furthermore, plasma glycerol may be a good marker of the activation of 11β-HSD1 in adipose tissues.

Our hyperinsulinemic-euglycemic clamp data showed that DIO/CP mice exhibited severe systemic insulin resistance. Insulin has been reported to be a functional regulator of lipolysis (Giorgino et al., 2005). GCs have been shown to directly decrease insulin sensitivity in adipocytes. In adipose tissues, GCs decrease the expression of insulin receptor substrate proteins 1 and 2 (Caperuto et al., 2006) and the activity of phosphoinositide 3-kinase (Corporeau et al., 2006). Therefore, insulin resistance in adipose tissues could also contribute to the elevated lipolysis seen in DIO/CP mice.

Previous reports showed that active GCs increase 11β-HSD1 mRNA levels in in vitro experiments (Whorwood et al., 2001; Sun et al., 2002; Yang et al., 2007). Furthermore, 11β-HSD1 mRNA expression is up-regulated by chronic administration of active GCs in adipose tissues in rodent models (Michailidou et al., 2007; Balachandran et al., 2008; Sai et al., 2008). In obese patients with type 2 diabetes, basal lipolysis and 11β-HSD1 mRNA levels are increased in mesenteric adipose tissue compared with those of obese patients (Yang et al., 2008). Thus, a vicious circle of GC signals with increased 11β-HSD1 expression is thought to exist in adipose tissues in obesity-related diabetic conditions. We have demonstrated here the increased expression levels of 11β-HSD1 in mesenteric adipose tissue of DIO/CP mice, suggesting that a vicious circle of GC signals exists in adipose tissues of DIO/CP mice. Antidiabetic agents of the thiazolidinedione class, ligands for peroxisome proliferator-activated receptor γ, have been reported to reduce adipose 11β-HSD1 mRNA expression and activity in vitro and in vivo models (Berger et al., 2001; Nakano et al., 2007). Pioglitazone, a representative drug of the thiazolidinedione class, decreases whole-body lipolysis in patients with type 2 diabetes (Gastaldelli et al., 2009). These reports support the possibility that normalization of the GC condition in adipose tissue can lead to improvement in diabetic conditions.

To further investigate the contribution of GRs on pathologic conditions in DIO/CP mice, we evaluated the efficacy of RU486, a GR antagonist. Pharmacological blockade of GR has been shown to ameliorate hyperglycemia and insulin resistance in animal models (Kusunoki et al., 1995; Gettys et al., 1997; Liu et al., 2005). As expected, up-regulated adipose 11β-HSD1 and ATGL mRNA expression in DIO/CP mice were dose-dependently decreased by RU486 treatment. Furthermore, RU486 dose-dependently decreased plasma NEFA, glycerol, and glucose levels in DIO/CP mice. These
results show that there is a close relationship between hyperglycemia and enhancement of lipolysis caused by GC signal activation.

In conclusion, we have established a novel obese diabetic mouse model caused by GC activation through 11β-HSD1. We showed that lipolysis in visceral adipose tissue was important for inducing diabetes with the GC signal in the mice. Our findings also raise the possibility that the vicious circle of adipose GC signal activation with increased 11β-HSD1 expression levels contributes to the dramatic development of diabetes. Thus, we propose that blockade of the adipose GC signal can be an attractive therapeutic approach for obese patients with type 2 diabetes. This diabetic mouse model may be suitable for understanding how GCs induce diabetes in obesity and for characterizing 11β-HSD1 inhibitors.


**Address correspondence to:** Dr. Kenji Asakura, Shionogi Pharmaceutical Research Center 3-1-1, Futaba-cho, Toyonaka-shi, Osaka 561-0825, Japan. E-mail: kenji.asakura@shionogi.co.jp