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11β-Hydroxysteroid dehydrogenase type 1 shRNA ameliorates glucocorticoid-induced insulin resistance and lipolysis in mouse abdominal adipose tissue

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[1]*Division of Endocrinology, Metabolism, and Molecular Medicine, Charles R. Drew University of Medicine and Sciences, University of California Los Angeles (UCLA) School of Medicine, Los Angeles, California;* [2]*Department of Pediatrics, First Hospital, Jilin University, ChangChun, China;* [3]*Department of Endocrinology and Metabolism, Shanghai Jiaotong University Affiliated Sixth People's Hospital, Shanghai Diabetes Institute, Shanghai, China;* [4]*Department of Pharmaceutical Sciences, Western University of Health Sciences, Pomona, California; and* [5]*Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, UCLA, Los Angeles, California*

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Wang Y, Yan C, Liu L, Wang W, Du H, Fan W, Lutfy K, Jiang M, Friedman TC, Liu Y. 11β-Hydroxysteroid dehydrogenase type 1 shRNA ameliorates glucocorticoid-induced insulin resistance and lipolysis in mouse abdominal adipose tissue. *Am J Physiol Endocrinol Metab* 308: E84–E95, 2015. First published November 11, 2014; doi:10.1152/ajpendo.00205.2014.—Long-term glucocorticoid exposure increases the risk for developing type 2 diabetes. Prereceptor activation of glucocorticoid availability in target tissue by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) coupled with hexose-6-phosphate dehydrogenase (H6PDH) is an important mediator of the metabolic syndrome. We explored whether the tissue-specific modulation of 11β-HSD1 and H6PDH in adipose tissue mediates glucocorticoid-induced insulin resistance and lipolysis and analyzed the effects of 11β-HSD1 inhibition on the key lipid metabolism genes and insulin-signaling cascade. We observed that corticosterone (CORT) treatment increased expression of 11β-HSD1 and H6PDH and induced lipase HSL and ATGL with suppression of p-Thr^{172} AMPK in adipose tissue of C57BL/6J mice. In contrast, CORT induced adipose insulin resistance, as reflected by a marked decrease in IR and IRS-1 gene expression with a reduction in p-Thr^{308} Akt/PKB. Furthermore, 11β-HSD1 shRNA attenuated CORT-induced 11β-HSD1 and lipase expression and improved insulin sensitivity with a concomitant stimulation of pThr^{308} Akt/PKB and p-Thr^{172} AMPK within adipose tissue. Addition of CORT to 3T3-L1 adipocytes enhanced 11β-HSD1 and H6PDH and impaired p-Thr^{308} Akt/PKB, leading to lipolysis. Knockdown of 11β-HSD1 by shRNA attenuated CORT-induced lipolysis and reversed CORT-mediated inhibition of pThr^{172} AMPK, which was accompanied by a parallel improvement of insulin signaling response in these cells. These findings suggest that elevated adipose 11β-HSD1 expression may contribute to glucocorticoid-induced insulin resistance and adipolysis.

adipose tissue; insulin signaling; lipolysis; hexose-6-phosphate dehydrogenase; glucocorticoid; 11β-hydroxysteroid dehydrogenase type 1; short-hairpin RNA

INSULIN RESISTANCE IS A MAJOR RISK FACTOR for the development of type 2 diabetes (T2DM). Glucocorticoids (GCs) lead to insulin resistance (38), and excess production of GCs such as cortisol and corticosterone can induce central obesity (Cushing's syndrome), insulin resistance, and T2DM (1, 33). GCs are also potent pharmacological agents, and prolonged use of GCs often leads to adiposity and T2DM (15, 17, 22, 35, 36). More than 2.5 million people in the US are currently exposed to long-term GCs and potentially to their diabetogenic effects. These studies highlight the importance of GCs in the pathogenesis of T2DM and emphasize the urgent need for understanding the underlying mechanisms of GC-induced insulin resistance to develop new treatments.

GCs exert pleiotropic effects on multiple organ systems. In adipose tissue, GCs not only promote differentiation of preadipocytes and adipose distribution (18, 27) but also induce lipolysis by stimulation of both hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) to release free fatty acids into the circulation that is linked to hyperlipidemia and systemic insulin resistance (40, 46). However, tissue-specific GC action not only depends on circulating GCs levels but can also be regulated by an intracellular endoplasmic reticulum (ER) lumen resident enzyme, 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), that converts inactive cortisone (11-dehydrocorticosterone in rodents) to active cortisol (corticosterone) and therefore amplifies intracellular GC action, particularly in adipose tissue (6, 21, 24, 41). 11β-HSD1 is regulated by its cofactor NADPH, which is provided by the enzyme hexose-6-phosphate dehydrogenase (H6PDH) (32, 34). In the ER lumen, H6PDH can metabolize glucose 6-phosphate and NADP to generate NADPH, thus ensuring 11β-HSD1 activity. Enhanced 11β-HSD1 and H6PDH expression results in production of excess adipose GCs and produces features of the metabolic syndrome in transgenic mice (28, 48). Pharmacological inhibition of 11β-HSD1 modulates lipid metabolism (3), improves GC-induced hyperglycemia in animals (4), and decreases glycerol concentrations (44). 11β-HSD1 inhibition also regulates GC-induced insulin resistance and lipid metabolic profile in skeletal muscle (30). Thus prereceptor modulation of tissue-specific GC action by 11β-HSD1 may represent an emerging etiology for GC-induced diabetes.

GCs also have a direct inhibitory effect on insulin sensitivity at one or more sites along the signaling pathway by decreasing the expression of insulin-signaling proteins in rodent liver and skeletal muscle (12, 39), such as insulin receptor (IR), insulin receptor substrate (IRS)-1–4, IRS-1-associated phosphatidylinositol 3-kinase (PI3K), and protein kinase B (PKB)/Akt, the

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key elements of the insulin-signaling cascade. Moreover, GCs impair insulin signaling and glucose transporter (GLUT4) expression in cultured adipocytes (5, 16). These studies substantiate the importance of GCs in the regulation of tissue-specific insulin sensitivity. However, the contributions of tissue-specific alterations of 11β-HSD1 coupled with H6PDH amplifying intracellular GC signaling to the molecular mechanisms underlying GC-induced insulin resistance, and whether 11β-HSD1 inhibitors modulate the mechanistic link between insulin signaling and adipolytic pathway, remain unclear.

The aim of the present study was to determine whether GC-induced changes in adipose insulin signaling and lipid metabolic profile are associated with the metabolic phenotype of 11β-HSD1 and H6PDH. The study also aimed to investigate the functional impact of the selective 11β-HSD1 inhibitor in key adipose insulin-signaling cascade and lipid metabolism genes using shRNA-silencing technology in vivo in mouse adipose tissue as well as in 3T3-L1 adipocytes.

MATERIALS AND METHODS

Preparation of recombinant adenoviruses expressing short-hairpin RNAs. For generation of shRNA constructs against mouse 11β-HSD1, a small DNA insert (76-bp 5′-*Mlu* ACGCGTCGTTGAGTTCATCACAGCGACTGTtgatatccgCAGTCGCTGTGATGAACTCAAttttttccaactcgag *Xho*I-3′) encoding a short-hairpin RNA (shRNA) targeting mouse 11β-HSD1 (Gene bank: S75207) and scrambled shRNA was obtained from GenScript (SA Biosciences, Frederick, MD) and cloned into adenoviral shuttle vector pRNATin-H1.2-green fluorescent protein (GFP) (SD1229) at the multiple cloning sites by GenScript. The β-galactosidase gene was inserted in the multiple cloning sites of the same vector to produce pShuttle-CMV-LacZ (LacZ) as a control for the production of recombinant adenovirus. The adenoviral vectors were transfected into 293 cells to generate recombinant adenovirus expressing 11β-HSD1 (Ad-HSD1) or LacZ (Ad-LacZ) using the MBS Mammalian Transfection Kit (Stratagene). Viruses harvested 6 days after transfection were purified and dissolved in PBS according to the manufacturer's protocol.

Animal treatment. Five-week-old male C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME) and housed in a room maintained on a 12:12-h light-dark cycle with free access to water and standard laboratory chow. Animal experiments were approved by the Shanghai Jiaotong Uinversity of Medicine and the Charles R. Drew University Institutional Animal Care and Use Committee. Mice were given exogenous corticosterone (CORT; 100 μg/ml; Sigma- Aldrich, St. Louis, MO) or vehicle for 3 wk before the initiation of 11β-HSD1 shRNA or LacZ treatment (20). CORT was dissolved in 100% ethanol and then diluted in tap water to a 1% final ethanol concentration. Control mice received 1% ethanol alone as vehicle in tap water. Mice were divided into four groups: *1*) vehicle-treated mice that received targeting shRNA, *2*) vehicle-treated mice that received nontargeting shRNA, *3*) CORT-treated mice that received nontargeting shRNA, and *4*) CORT-treated mice that received targeting 11β-HSD1 shRNA. Ad-11β-HSD1 shRNA (3×10^7 plaque-forming units virus/g) or an equal volume of nontargeting shRNA (Ad-LacZ) was injected intraperitoneallly (ip) once/wk for 3 wk. Body weights and food intake were recorded weekly. At the end of the 3rd wk after Ad-LacZ or Ad-11β-HSD1shRNA administration, epididymal fat and abdominal subcutaneous fat pads were removed, weighed, and frozen in liquid nitrogen. Blood samples were collected between 0900 and 1000 and then stored at −80°C until measurement of glucose, insulin, CORT, free fatty acid, and triglycerides using commercially available kits, as described previously (48).

Intraperitoneal insulin tolerance and glucose tolerance tests. Twenty days following 11β-HSD1 shRNA treatment, intraperitoneal insulin tolerance test (ITT) was performed on mice after an 8-h fast, and blood samples were drawn at different times following insulin injection (0.75 U/kg ip) (29). For the glucose tolerance test (GTT), mice were fasted on 21 days for 12 h, and blood samples were taken from a tail vein after the 1 g/kg glucose injection.

Measurement of glucose uptake in adipose tissue. Adipose glucose uptake was determined by measuring the incorporation of 2-[^{3}H]deoxyglucose (2-[^{3}H]DG), as described earlier (42). Briefly, fresh adipose tissue was cut into ~5-mg pieces and incubated at 37°C for 30 min in DMEM (25 mmol/l glucose) solution supplemented with 0.5% BSA. After three washes with Krebs-Ringer bicarbonate-HEPES solution containing 1% BSA (pH 7.4), tissues were incubated in 0.5 ml of Krebs-Ringer bicarbonate-HEPES containing 0.1% BSA with either 0 or 100 nM insulin for 5 min at 37°C. The uptake was initiated by adding 0.1 ml of the same buffer containing 10 mmol/l glucose and 2.0 μCi 2-[^{3}H]DG (NEN Life Science Products, Boston, MA). At the end of the 30-min incubation the uptake was terminated, and the fat tissues were digested in 0.5 N NaOH, and radioactivity was measured in the samples using a liquid scintillation counter.

Microsomal enzymatic activity assays. The adipose microsomal pellet was obtained by centrifugation of the supernatant for 1 h at 100,000 *g*. 11β-HSD1 reductase activity was measured by addition of 1 mM NADPH and 250 nmol/l 11-DHC, with [^{3}H]11-DHC as tracer to microsomes in KRB solution at 37°C for 1–2 h, as in our previous study (49). Adipose corticosterone concentrations were measured by methanol extraction using a corticosterone ELISA kit (Abcam), as in our previous report (48).

shRNA for 11β-HSD1 in 3T3-L1 cells. 3T3-L1 cells were transfected with 11β-HSD1 shRNA (SA Biosciences) or scrambled control shRNA using lipofectamine 2000 (Invitrogen). Positive transfected cells were selected with medium containing G418 (600 μg/ml). Stable cell lines were established after selection with G418 for further experiments. Stable transfected 3T3-L1 cells were induced to differentiate with 10 μg/ml insulin, 0.5 mM 3-isobutyl-1-methylxanthine, and 0.5 μM dexamethasone in DMEM supplemented with 10% FBS for 2 days. The cells were then maintained in 10% FBS-DMEM containing 10 μg/ml insulin to fully differentiate into adipocytes. On *day 8* after differentiation, the medium was changed to charcoal-stripped FBS-DMEM for overnight, and then 3T3-L1 adipocytes were incubated in the absence or presence of corticosterone and harvested at 48 h after treatment (25).

Oil Red O staining. At the end of the treatment, adipocytes were fixed in freshly prepared 4% paraformaldehyde and stained with Oil Red O (Sigma, St. Louis, MO). Cells were mounted with Vestashield mounting solution with 4′,6′-diamidino-2-phenylindole (Vector Laboratories) and visualized under a fluorescence microscope.

RNA analysis by real-time RT-PCR. Adipose RNA was extracted using RNAzol B kit (Invitrogen). Real-time primers for mouse 11β-HSD1 (forward: 5′-CCTTGGCCTCATAGACACAGAAAC-3′; reverse: 5′-GGAGTCAAAGGCGATTTGTCA-3′), H6PDH (forward: 5′-TGGCTACGGGTTGTTTTTGAA-3′; reverse: 5′-TATACACGGTACATCTCCTCTTCCT-3′), HSL (forward: 5′-GGGCAAAGAAGGATCGAAGAA-3′; reverse: 5′-GCGTTAAATCCATGCTGTGTGA-3′), ATGL (forward: 5′-TCGTGGATGTTGGTGGAG CT-3′; reverse: 5′-TGTGGCCTCATTCCTCCTA-3′), IR (forward: 5′-AGTGAGCTATCGCCGATATGGT-3′; reverse: 5′-CCGGACTCGAACACTGTAGTTTC-3′), IRS-1 (forward: 5′-GGGCTTGAAGCGGCTAAGT-3′; reverse: 5′-CTGCACGGATGACCTTAGCA-3′), glucocorticoid receptor (GR; forward: 5′-TGCTATGCTTTGCTCCT GATCTG-3′; reverse: 5′-TGTCAGTTGATAAAACCGCTGC-3′), phospho*enol*pyruvate carboxykinase (PEPCK; forward: 5′TGAACTGACAGACTCGCCCTAT-3′; reverse: 5′-GCACTTGATGAACTCCCCATCT-3′), CCAAT/enhancer-binding protein-α (C/EBPα; forward: 5′TGGACAAGAACAGCAACGAGTAC-3′; reverse: 5′-CGGTCATTGTCACTGGTCAACT-3′), and peroxisome proliferator-activated receptor-γ (PPARγ; forward: 5′-CAA GAATACCAAAGTGCGATCAA-3′; reverse: 5′-GAGCTGG-

Table 1. *Body weight, fat mass, glucose, insulin, corticosterone, FFA, and triglyceride*

Metabolic Parameter	Vehicle-Treated Mice		CORT-Treated Mice	
	LacZ	11β-HSD1 shRNA	LacZ	11β-HSD1 shRNA
Body weight, g	27.3 ± 0.3	26.7 ± 0.4	29.6 ± 0.6*	27.5 ± 0.5†
Food intake, g/wk	32 ± 2.1	30 ± 3.5	44 ± 4.2*	36 ± 3.8†
Epi fat pad weight, g	0.81 ± 0.04	0.78 ± 0.05	1.25 ± 0.06‡	0.83 ± 0.05
Sub Fat pad weight, g	0.68 ± 0.07	0.63 ± 0.06	0.74 ± 0.08	0.69 ± 0.07
Blood glucose, mg/dl	138 ± 12	117 ± 9*	225 ± 18*	127 ± 10#
Insulin, ng/ml	5.9 ± 0.4	4.8 ± 0.2	16.7 ± 1.5*	12.8 ± 0.9#
CORT, ng/ml	39 ± 4.3	51 ± 4.5*	236 ± 37‡	127 ± 28†
FFA, ng/ml	4.7 ± 0.6	3.8 ± 0.3**	9.8 ± 0.4‡	5.1 ± 0.8#
Triglyceride, ng/ml	10.7 ± 2.3	7.7 ± 1.3**	23.5 ± 3.7*	12.6 ± 1.2#

Data are means ± SE of 7–8 mice/group. FFA, free fatty acid; CORT, corticosterone; shRNA, short-hairpin RNA; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; Epi, epididymal; Sub, subcutaneous. *$P < 0.01$ vs. LacZ-treated vehicle mice; **$P < 0.05$ vs. LacZ-treated vehicle mice; ‡$P < 0.001$ vs. LacZ-treated vehicle mice; †$P < 0.05$ vs. LacZ-treated CORT-exposed mice; #$P < 0.01$ vs. LacZ-treated CORT-exposed mice.

GTCTTTTCAGAATAATA AG-3′) were designed with Primer Express software 2.0. Real-time RT-PCR was performed using SYBR green kit detection of products in the ABI Prism 7700 system. Results were expressed as a relative value after normalization to 18S rRNA.

Immunoblotting analysis. Adipose tissue proteins were separated on 4–12% acrylamide SDS-PAGE gels (Bio-Rad, Hercules, CA) for analysis of 11β-HSD1, HSL, ATGL, IR, IRS-1, GLUT1, GLUT4, PI3K, phospho-Thr^{308} kinase PKB, phospho-Thr^{172}

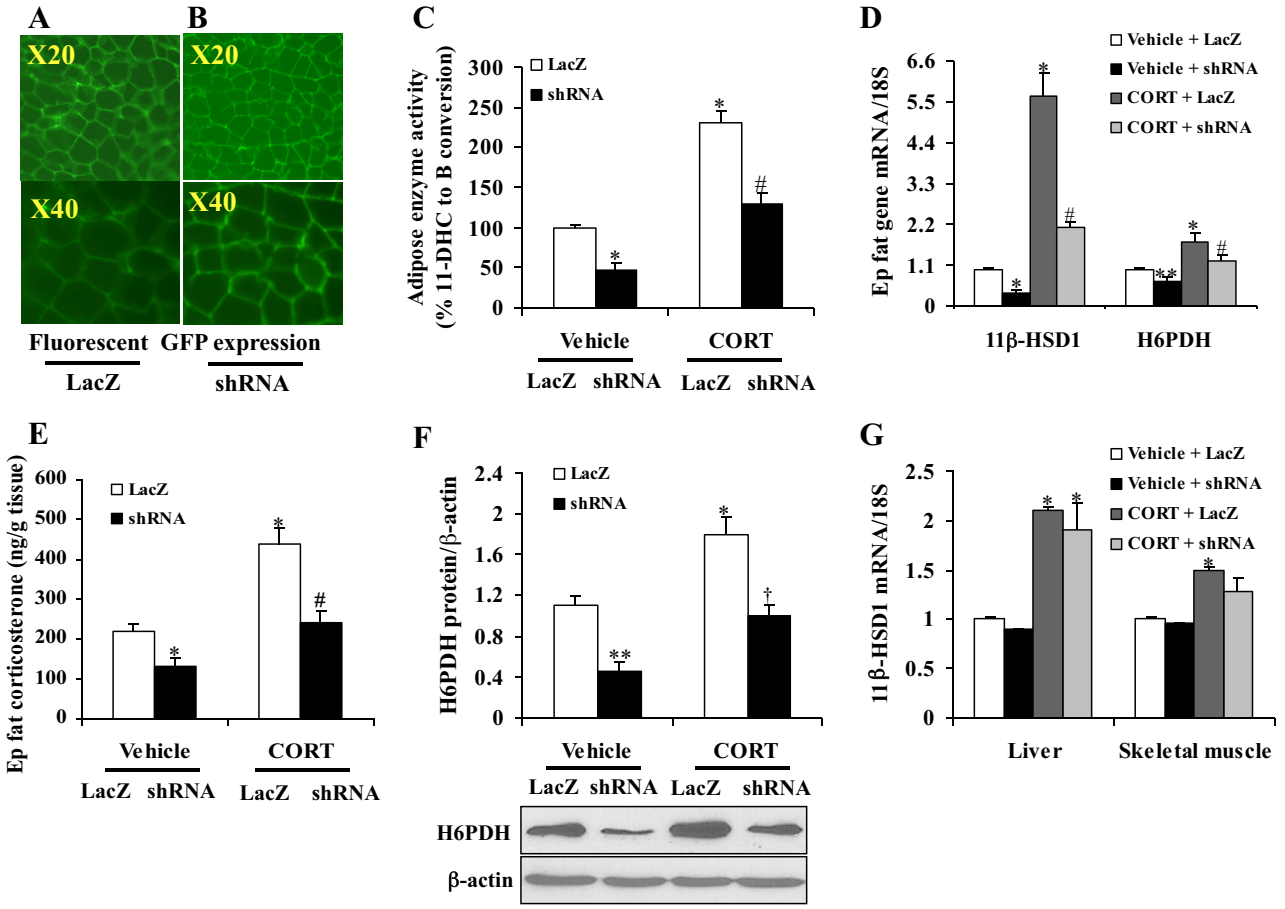

Fig. 1. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) and hexose-6-phosphate dehydrogenase (H6PDH) expression in the epididymal fat (Ep fat) of vehicle- and corticosterone (CORT)-exposed C57BL/6J mice after 11β-HSD1 short-hairpin RNA (shRNA) or control LacZ treatment. *A* and *B*: representative fluorescence microscope images of Ep fat sections with green fluorescent protein expression from LacZ- and shRNA-treated mice at 3 wk postinjection. Original magnification, ×20 and ×40. *C*: Ep fat 11β-HSD1 reductase activity was measured in adipose microsomes, using 11-dehydrocorticosterone (11-DHC) as substrate in the presence of NADPH. Enzyme activity was expressed as %11-DHC converted to corticosterone (*B*). *D*: relative expression of adipose 11β-HSD1 and H6PDH mRNA levels were measured by real-time PCR and normalized to 18S ($n = 8$). *E*: Ep fat CORT concentrations. *F*: expression and quantification of H6PDH protein levels were done relative to the amount of β-actin ($n = 5$). *G*: hepatic and skeletal muscle 11β-HSD1 mRNA levels in vehicle- and CORT-exposed C57BL/6J mice after 11β-HAD1 shRNA or LacZ treatment ($n = 5$). Data are means ± SE. *$P < 0.01$ vs. LacZ-treated control mice; #$P < 0.01$ vs. LacZ-treated CORT-exposed mice; †$P < 0.05$ vs. LacZ-treated CORT-exposed mice; **$P < 0.05$ vs. LacZ-treated control mice.

AMPK, total AMPK, and β-actin (Cell Signaling Technology, Danvers, MA).

Statistical analyses. All values are expressed as means ± SE. Data were analyzed using two-way analysis of variance (ANOVA). The post-hoc Tukey test was performed to reveal significant different between groups. $P < 0.05$ was considered to be significant.

RESULTS

Chronic CORT treatment induces the phenotype of diabetes and enhances adipose 11β-HSD1 and H6PDH expression. As shown in Table 1, mice exposed to exogenous CORT for 7 wk gained more weight and had increased weight gain and elevated plasma levels of CORT, blood glucose, insulin, free fatty acid, and triglycerides compared with vehicle-treated controls. Epididymal fat pad weight increased markedly, but subcutaneous fat pad weight was not affected in CORT-treated mice compared with vehicle-treated controls. 11β-HSD1 HSD1 activity in epididymal fat from CORT-treated mice was increased 2.3-fold over that of vehicle-treated mice ($P < 0.01$; Fig. 1*C*). 11β-HSD1 mRNA and CORT levels in epididymal fat in CORT-treated mice were increased 5.5- and 2.0-fold, respectively, compared with that of vehicle-treated mice ($P < 0.01$; Fig. 1, *D* and *E*). Consistent with these observations, H6PDH mRNA and protein expression in epididymal fat of CORT-treated mice were increased 1.7- and 1.6-fold, respectively (Fig. 1, *D* and *F*). Parallel to the increase in 11β-HSD1, adipose HSL and ATGL mRNA levels were increased 3.0- and 3.5-fold, respectively, in CORT-treated mice over their respective vehicle-treated mice (Fig. 2*A*). Western blot analysis revealed that HSL and ATGL protein levels were elevated, whereas pThr^{172} AMPK activity was reduced in epididymal fat from CORT-treated mice ($P < 0.01$ vs. respective vehicle-treated mice; Fig. 2, *B–D*). Similarly, subcutaneous fat 11β-HSD1 and H6PDH mRNA levels were markedly increased ($P < 0.01$; Fig. 3*A*) with simultaneous induction of HSL, ATGL, and PEPCK expression (Fig. 3*B*). However, pThr^{172} AMPK protein levels were decreased without changes in total AMPK protein level in CORT-treated mice vs. controls (Fig. 3*C*).

Effects of Ad-11β-HSD1 shRNA treatment on CORT-induced metabolic phenotype. Histological fluorescence assay showed that the Ad-shRNA or LacZ vector containing GFP construct expression was present in epididymal fat from mice treated with LacZ or 11β-HSD1 shRNA (Fig. 1, *A* and *B*), indicating efficient delivery of shRNA or LacZ virus into the adipose tissues. 11β-HSD1 shRNA treatment for 3 wk lowered weight gain, food intake, blood glucose and plasma insulin, CORT, and lipid levels in CORT-treated mice compared with

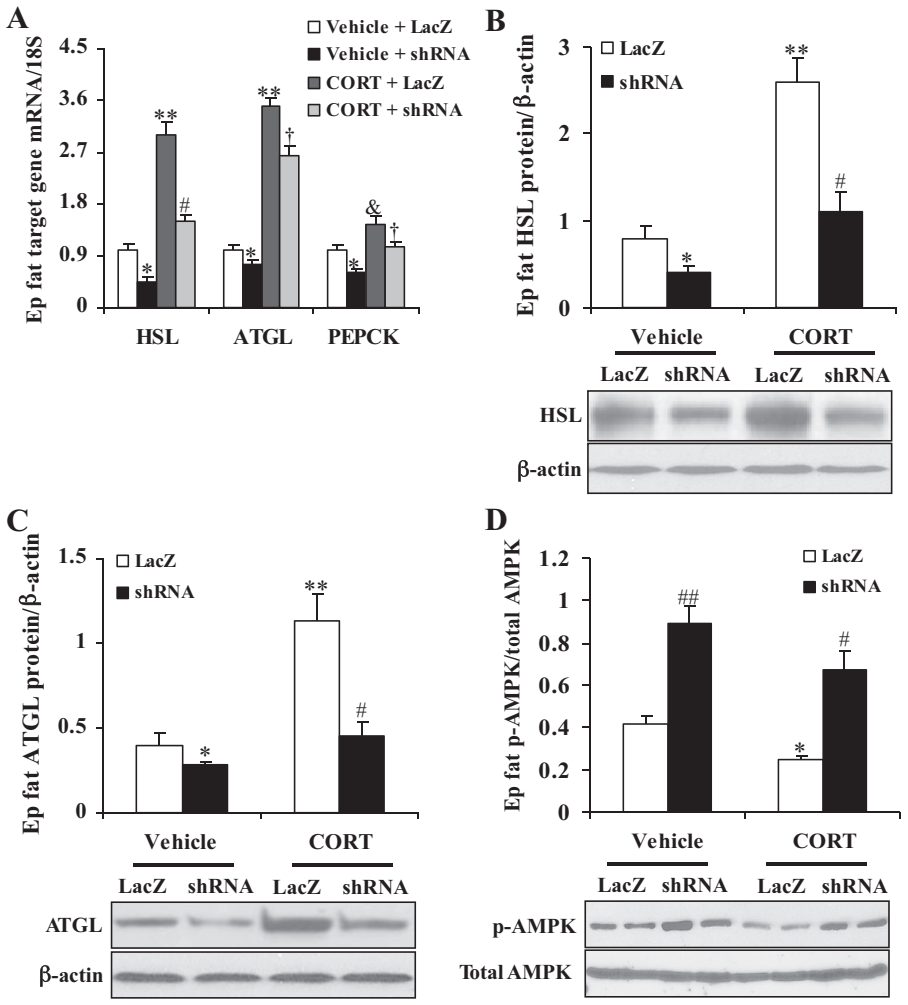

Fig. 2. Alterations of target gene expression and the Thr^{172} phosphorylation of AMP-activated protein kinase (AMPK) in Ep fat from vehicle- and CORT-exposed mice treated with 11β-HSD1 siRNA or LacZ. *A*: quantitative real-time RT-PCR analysis demonstrating the relative alterations of HSL and ATGL and phospho*enol*pyruvate carboxykinase (PEPCK) mRNA expression ($n = 8$). *B* and *C*: Western blot analysis of expression of hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) ($n = 5$). HSL and ATGL protein was standardized to the amount of β-actin. *D*: Thr^{172} phosphorylation of AMPK was normalized to total AMPK. Data are means ± SE. *$P < 0.01$ vs. LacZ-treated control mice; **$P < 0.001$ vs. LacZ-treated control mice; †$P < 0.05$ vs. LacZ-treated CORT-exposed mice; #$P < 0.01$ vs. LacZ-treated CORT-exposed mice; ##$P < 0.05$ vs. LacZ-treated control mice; &$P < 0.05$ vs. LacZ-treated control mice.

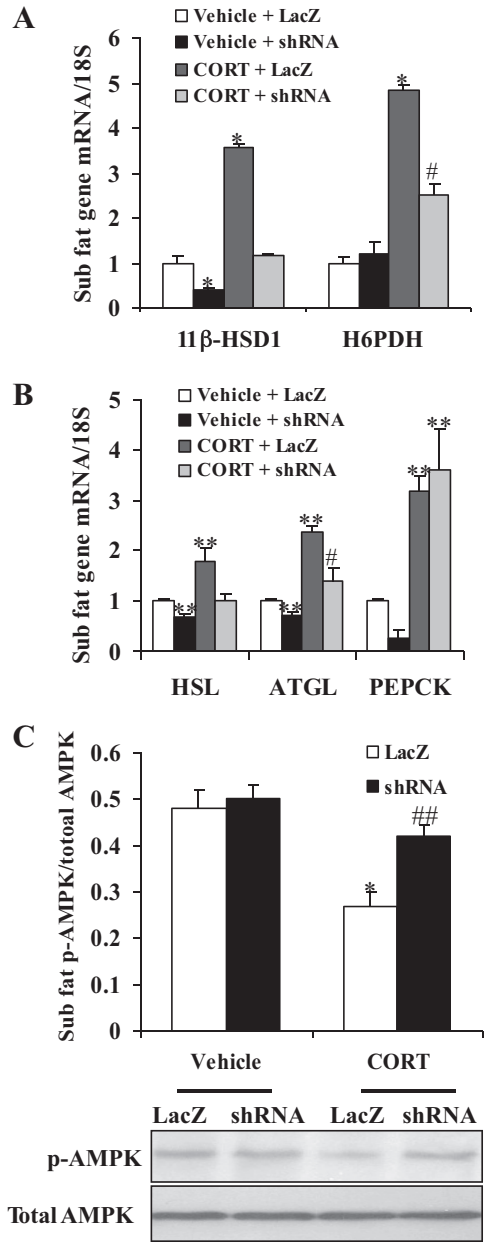

Fig. 3. Adipose 11β-HSD1 and H6PDH (*A*), HSL, ATGL, and PEPCK mRNA expression (*B*), and Thr^{172} AMPK phosphorylation (*C*) in subcutaneous fat from vehicle- and CORT- exposed mice treated with 11β-HSD1 shRNA or LacZ. Relative expression of mRNA levels was measured by RT-PCR and normalized to 18S rRNA expression ($n = 8$). HSL and ATGL protein were standardized to the amount of β-actin ($n = 6$). Thr^{172} phosphorylation of AMPK was normalized to total AMPK. Data are means ± SE. *$P < 0.01$ vs. LacZ-treated control mice; **$P < 0.05$ vs. LacZ-treated control mice; #$P < 0.05$ vs. LacZ-treated CORT-exposed mice; ##$P < 0.01$ vs. LacZ-treated CORT-exposed mice.

LacZ control. However, plasma CORT levels were increased and lipid levels decreased, with no effect on weight and insulin levels in vehicle-treated mice after 11β-HSD1 shRNA (Table 1). In contrast, 11β-HSD1 shRNA reduced epididymal fat 11β-HSD1 activity by 55% in vehicle-treated mice and by 44% in CORT-treated mice (Fig. 1*C*); this reduction in enzyme activity occurred in parallel with decreased epididymal fat 11β-HSD1 mRNA level and corticosterone concentrations in both vehicle- and CORT-treated mice after 11β-HSD1 shRNA (Fig. 1, *D* and *E*). In addition, epididymal fat H6PDH mRNA and protein expression were decreased after 11β-HSD1 shRNA in both vehicle- and CORT-treated mice (Fig. 1, *D* and *F*). However, 11β-HSD1 shRNA did not affect hepatic and skeletal muscle 11β-HSD1 mRNA levels in vehicle- or CORT-treated mice, although 11β-HSD1 mRNA expression was increased in both liver and muscle in CORT-treated mice (Fig. 1*G*). In parallel with the decrease in 11β-HSD1 and H6PDH, epididymal fat HSL and ATGL at both mRNA and protein levels were decreased in both vehicle- and CORT-treated mice after 11β-HSD1 shRNA (Fig. 2, *A–C*). Moreover, 11β-HSD1 shRNA reversed CORT-mediated inhibition of epididymal fat p-Thr^{172} AMPK but did not affect total AMPK level (Fig. 2*D*). Similarly, 11β-HSD1 shRNA decreased CORT-induced epididymal fat PEPCK mRNA level (Fig. 2*A*). In addition, 11β-HSD1 shRNA attenuated CORT-induced subcutaneous fat 11β-HSD1 and H6PDH expression and reduced lipase mRNA levels (Fig. 3, *A* and *B*) in both vehicle- and CORT-treated mice but reversed the suppression of p-Thr^{172} AMPK in CORT-treated mice and maintained p-Thr^{172} AMPK in vehicle-treated mice (Fig. 3*C*). However, 11β-HSD1 shRNA failed to alter CORT-induced subcutaneous fat PEPCK mRNA level but decreased the subcutaneous fat PEPCK expression in vehicle-treated mice (Fig. 3*B*).

11β-HSD1 shRNA attenuates CORT-induced insulin resistance with modulation of the adipocyte insulin signaling cascade. We next performed an ITT and measured adipose insulin signaling action. The results of the ITT experiments revealed that CORT treatment impaired the glucose-lowering effect of insulin (Fig. 4, *A* and *C*) and increased the slopes of the ITT curve at 0–10 min and 10–20 min but did not significantly affect its slope at 0–30 min (Fig. 4*B*). In contrast, 11β-HSD1 shRNA treatment enhanced glucose clearance in response to exogenous insulin during the ITT (Fig. 4, *A–C*). The results of the GTT experiments showed that CORT-treated mice had higher glucose levels (Fig. 4, *D* and *E*) and elevated fasted insulin concentrations (Fig. 4*F*) compared with LacZ-treated mice. In contrast, 11β-HSD1 shRNA treatment improved glucose tolerance and decreased insulin levels in CORT-treated mice (Fig. 4, *D* and *F*). Similarly, the decreased epididymal fat 2-[^{3}H]DG uptake in CORT-treated mice was reversed by 11β-HSD1 shRNA (Fig. 5*A*). Western blot analysis revealed that 11β-HSD1 shRNA prevented CORT-induced decreases in both GLUT1 and GLUT4 protein expression in epididymal fat (Fig. 5, *B* and *C*), supporting the improvement of adipose glucose uptake in these mice.

Consistent with these observations, the expression of both IR and IRS-1 mRNA (Fig. 6*A*) and their total protein levels (Fig. 6, *B* and *C*) was reduced in epididymal fat from CORT-treated mice, which were restored by 11β-HSD1 shRNA treatment to levels similar to those found in their respective LacZ-treated control groups. However, 11β-HSD1 shRNA increased IRS-1 mRNA and protein expression, with no effect on IR mRNA in epididymal fat from vehicle-treated mice. Likewise, the total IR and IRS-1 that was associated with Thr^{308} phosphorylation of Akt/PKB was decreased in epididymal fat of CORT-treated mice ($P < 0.01$), which were restored by 11β-HSD1 shRNA to levels similar to those found in their respective LacZ-treated controls (Fig. 6, *B* and *D*). However, 11β-HSD1 shRNA did not affect the PI3K protein level but in-

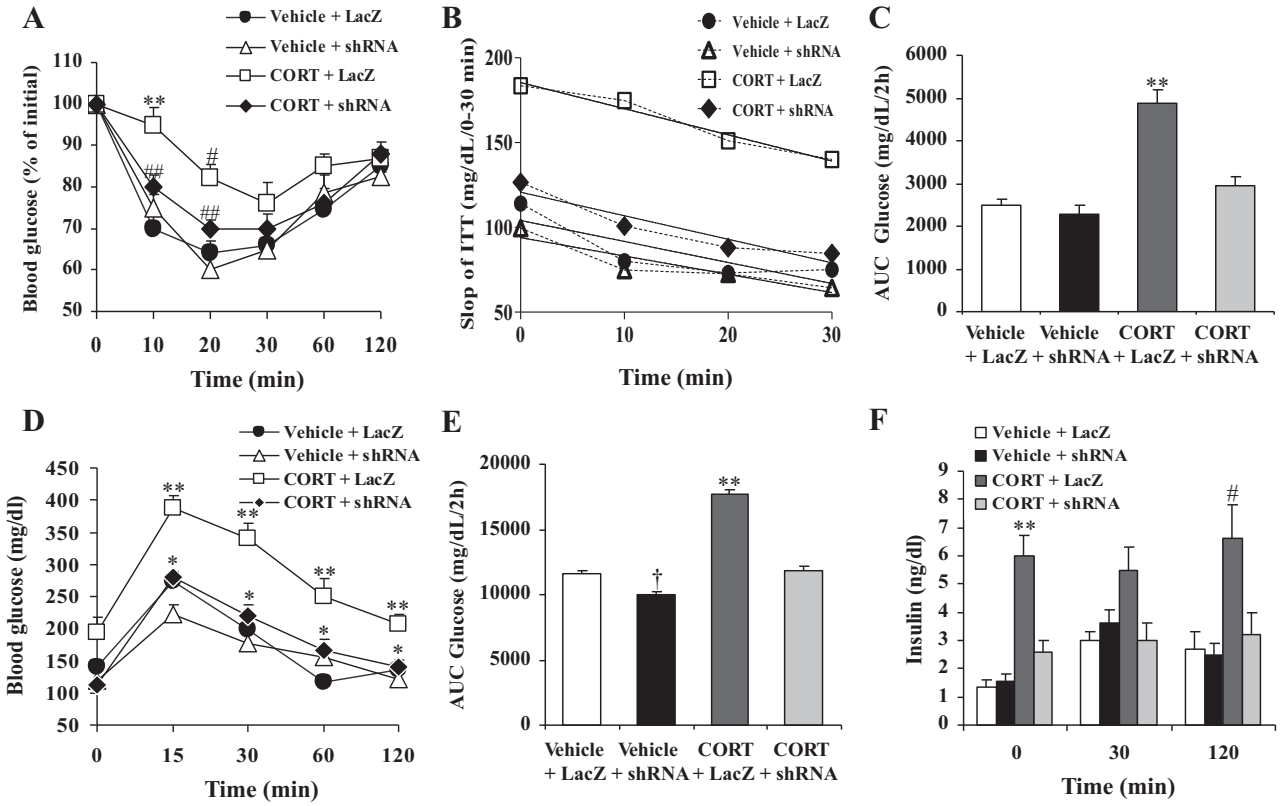

Fig. 4. CORT treatment impairs glucose homeostasis. *A*: insulin tolerance test (ITT); vehicle-exposed mice treated with LacZ (vehicle + LacZ; ●), vehicle-exposed mice treated with 11β-HSD1 shRNA (vehicle + shRNA; △), CORT-exposed mice treated with LacZ (CORT + LacZ; □), and CORT-exposed mice treated with 11β-HSD1 shRNA (CORT + shRNA; ◆). *B*: slopes of the ITT curve at 0–30 min. *C*: glucose area under the curve (AUC) during ITT. *D*: glucose tolerance test (GTT; ip). *E*: glucose AUC during GTT. *F*: insulin concentrations at 0, 30, and 120 min during the GTT. Data are means ± SE of 6 mice/group. $^{**}P < 0.01$ vs. LacZ-treated vehicle/control mice; $^{*}P < 0.01$ vs. LacZ-treated CORT-exposed mice; $^{\#\#}P < 0.05$ vs. LacZ-treated CORT-exposed mice; $^{\#}P < 0.05$ vs. LacZ-treated controls; $^{\dagger}P < 0.05$ vs. LacZ-treated vehicle/control mice.

creased p-Thr^{308} Akt/PKB activity in epididymal fat of vehicle-treated mice compared with their respective LacZ-treated mice ($P < 0.05$). In addition, CORT-impaired IR and IRS-1 mRNA and protein expression (Fig. 7, *A–C*) in subcutaneous fat of these mice were attenuated with the restoration of p-Thr^{308} Akt/PKB and maintained PI3K protein level (Fig. 7*D*) after 11β-HSD1 shRNA treatment compared with their respective LacZ-treated mice.

The effect of adipose 11β-HSD1 knockdown in the key lipid metabolism genes and insulin-signaling cascade in 3T3-L1 adipocytes. To further explore whether the functional consequence of 11β-HSD1 suppression could be observed in an in vitro setting, 11β-HSD1 shRNA-stable adipocytes cells were generated and showed an ~80% decrease in 11β-HSD1 mRNA and protein levels ($P < 0.001$; Fig. 8, *A* and *B*) with reduction of H6PDH mRNA level compared with scrambled LacZ cells (Fig. 8*A*). Similarly, 11β-HSD1 silencing reduced the mRNA levels of HSL and ATGL in 11β-HSD1 shRNA-cells ($P < 0.01$; Fig. 8*C*). A concentration of CORT (10^{-6} mol/l) similar to what occurs in mice following doses of exogenous CORT increased 11β-HSD1 expression in LacZ-cells, an effect that was abolished in 11β-HSD1 shRNA cells (Fig. 8, *A* and *B*). Consequently, 11β-HSD1 shRNA attenuated CORT-induced H6PDH mRNA levels and reversed CORT-enhanced HSL and ATGL mRNA expression in these intact cells (Fig. 8, *A* and *C*). Western blot analysis showed that 11β-HSD1 silencing enhanced pThr^{172} AMPK and blocked CORT-induced reduction of pThr^{172} AMPK in these cells compared with that in LacZ-cells (Fig. 8*D*). In addition, 11β-HSD1 shRNA-cells decreased lipid droplet accumulation (Fig. 8*E*) and attenuated CORT-induced C/EBPα and PPARγ mRNA expression with reduction of GR mRNA levels (Fig. 8*F*).

We next examined the effect of 11β-HSD1 suppression on insulin signaling in these intact cells. As shown in Fig. 9, CORT treatment decreased both IR and IRS-1 mRNA and total protein levels in LacZ cells (Fig. 9, *A–C*), respectively. Consistent with our in vivo data, 11β-HSD1 silencing reversed CORT-induced decrease in the IR and IRS-1 gene expression at both mRNA and protein levels in 11β-HSD1 shRNA-cells. Additionally, p-Thr^{308} Akt/PKB content and the protein expression of GLUT1 and GLUT4 were reduced in LacZ cells after CORT compared with that of controls ($P < 0.05$; Fig. 9, *C* and *D*). These effects were completely reversed in 11β-HSD1 shRNA cells.

DISCUSSION

GC excess increases the risk of developing T2DM and changes adipocyte function, which is directly relevant to obesity and insulin resistance. In the present study, we found that chronic GC-exposed mice exhibited hyperglycemia and re-

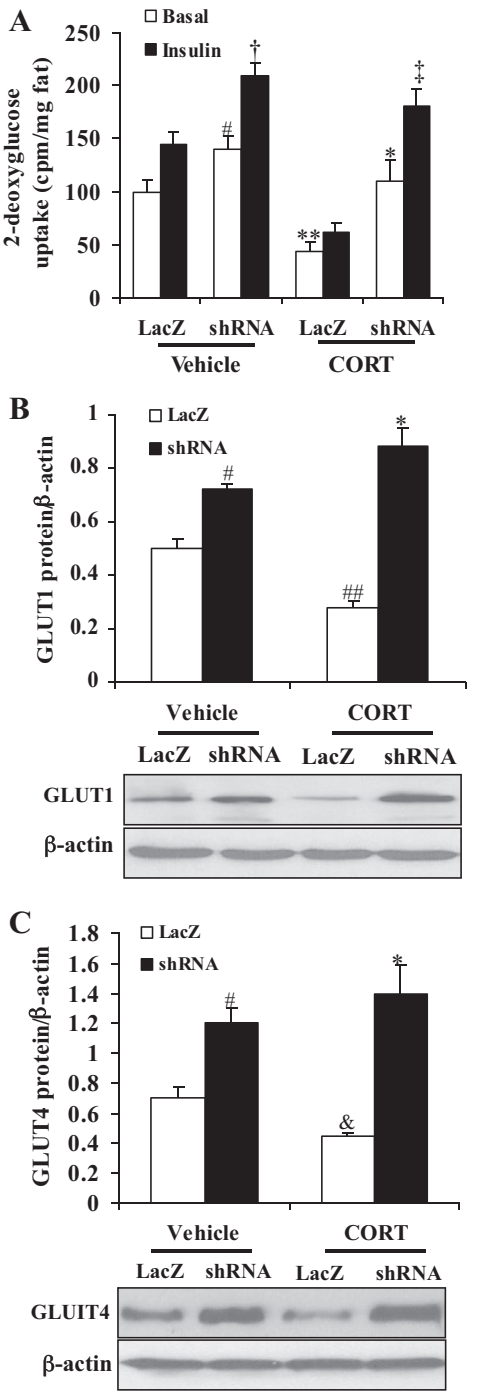

Fig. 5. Adipose glucose uptake and glucose transporter (GLUT) expression in vehicle- and CORT-exposed mice after 11β-HSD1 shRNA or LacZ treatment. *A*: 2-[^{3}H]deoxyglucose uptake in Ep fat from vehicle- and CORT-treated mice after 11β-HSD1 shRNA or LacZ. *B* and *C*: expression of adipose GLUT1 and GLUT4 total protein was done relative to the amount of β-actin. Data are means ± SE of 6 mice/group. #$P < 0.05$ vs. LacZ-treated controls; †$P < 0.05$ vs. basal and insulin-stimulated vehicle-exposed mice; **$P < 0.01$ vs. basal and insulin-stimulated LacZ-treated vehicle/control mice; *$P < 0.01$ vs. LacZ-treated CORT-exposed mice; ‡$P < 0.05$ vs. basal and insulin-stimulated CORT-exposed mice; ##$P <$ vs. LacZ-treated vehicle/control mice; &$P < 0.05$ vs. LacZ-treated vehicle/control mice.

duced insulin tolerance, which was associated with the induction of 11β-HSD1 and H6PDH in abdominal adipose tissue. We observed that CORT upregulated adipose 11β-HSD1 and H6PDH expression with corresponding body weight gain, hyperglycemia, hyperlipidemia, hyperinsulinemia, and reduced insulin tolerance. These findings imply that increased 11β-HSD1 and H6PDH expression in the abdominal fat may contribute to the development of GC-induced metabolic disorders. Moreover, the induction of adipose 11β-HSD1 was accompanied by the elevation of both lipase HSL and ATGL expression in response to elevated circulating levels of CORT (approaching stress-induced levels). Our current data are consistent with an earlier report that GCs increased 11β-HSD1 in human adipose stromal cells (6) and stimulated lipase activity in isolated rat adipocytes (8, 40). Induction of 11β-HSD1 enhanced intracellular GC action to induce adipose lipolysis, which contributes to free fatty acid release into the circulation, which is linked to the reduction of insulin and glucose tolerance. Additionally, H6PDH mRNA was elevated in abdominal adipose tissue of CORT-treated mice, suggesting that increased adipose H6PDH expression may contribute to 11β-HSD1-mediated promotion of intracellular GC's ability to drive fat lipolysis and alter the lipid profile in response to exogenous GCs. To our knowledge, the alterations of 11β-HSD1 coupled with H6PDH and their contributions to modulation of lipolysis have not been explored in humans or in in animals after GC challenge. In accord with this concept, adipose 11β-HSD1 was induced through the availability of local NADPH and caused lipolysis in adipose tissue in H6PDH transgenic mice (48). These data support the possibility that prereceptor induction of adipose 11β-HSD1 and H6PDH may contribute to GC excess-induced metabolic syndrome. In addition, elevated 11β-HSD1 mRNA levels in liver and muscle tissues were observed in mice treated with CORT, consistent with studies in mice as well as in humans where elevated GC levels induce hepatic and muscle 11β-HSD1 expression, which is linked to the hypercortisolemia-induced hyperglycemia and insulin resistance (24, 47).

11β-HSD1 inhibitors improved adipose lipid metabolism and reduced lipemia in humans as well as in rodents (3, 44). In accord with this concept, we demonstrated that administration of 11β-HSD1 shRNA effectively entered into adipose tissue, leading to knockdown of adipose 11β-HSD1, and attenuated GC-induced hyperglycemia and hyperlipidemia. Moreover, 11β-HSD1 shRNA also inhibited adipose H6PDH and resulted in suppression of local HSL and ATGL, leading to improvement of lipid profiles and metabolic phenotype. In addition, 11β-HSD1 shRNA adipocytes are resistant to GC-induced lipolysis. These data indicate that synthetic 11β-HSD1 shRNA can effectively inhibit adipose 11β-HSD1 activity and H6PDH expression and thus decrease exogenous GC availability to enhance adipolysis. This is supported by recent reports that inhibition of 11β-HSD1 modulates adipose tissue lipid metabolism in rats and limits prednisone exposure to human subcutenous adipose tissue and decreases glycerol levels (3, 44). Our data suggest that the some beneficial effects of 11β-HSD1 shRNA on the phenotype of GC-induced metabolic syndrome may be due partly to knockdown of 11β-HSD1 and H6PDH amplifying adipose GC action.

Multiple mechanisms may be involved in the regulation of 11β-HSD1 shRNA-mediated suppression of GC-induced adi-

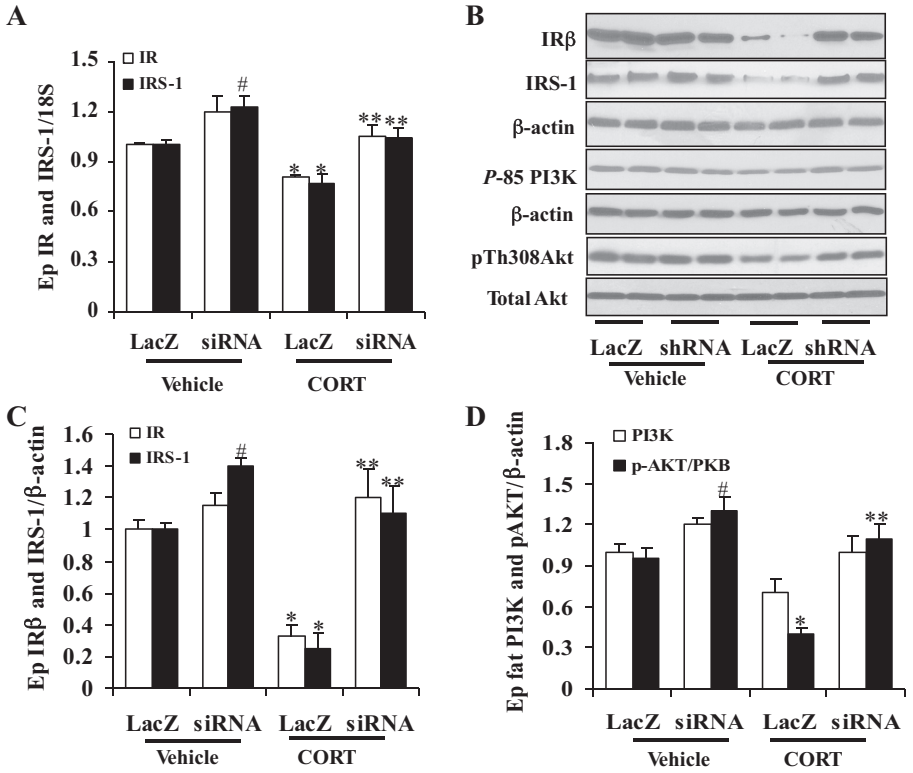

Fig. 6. Comparison of insulin receptor (IR), insulin receptor substrate-1 (IRS-1), p85 phosphatidylinositol 3-kinase (PI3K), and Thr^{308} Akt/PKB phosphorylation in Ep fat of vehicle- and CORT-exposed mice after 11β-HSD1 shRNA or LacZ treatment. *A*: relative expression of IR and IRS-1 mRNA levels were measured by RT-PCR and normalized to 18S ($n = 8$). *B–D*: relative epididymal fat IRβ, IRS-1, and PI3K protein was standardized to the amount of β-actin ($n = 6$). p-Thr^{308} Akt/PKB protein was normalized to the amount of total Akt. Data are means ± SE. #$P < 0.05$ vs. LacZ-treated control mice; *$P < 0.01$ vs. LacZ-treated control mice; **$P < 0.01$ vs. LacZ-treated CORT-exposed mice.

polysis. In fact, AMPK has been emerging as a negative regulator of HSL, and therefore, activation is limited (14, 26). We found that 11β-HSD1 shRNA blocked the GC-mediated inhibition of p-Thr^{172} AMPK with concomitant reduction of lipase expression in adipose tissue. Conversely, the expression levels of p-Thr^{172} AMPK were decreased in response to the activation of 11β-HSD1 and H6PDH with corresponding induction of HSL and ATGL expression in adipose tissue in

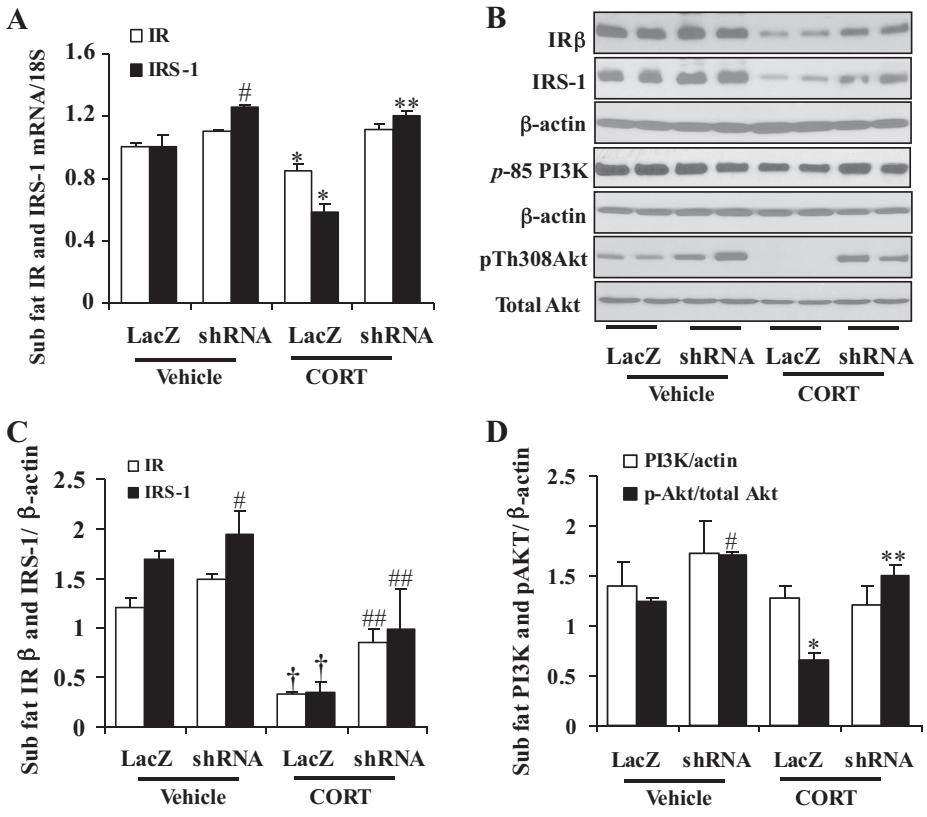

Fig. 7. Adipose IR, IRS-1, p85 PI3K expression, and Thr^{308} Akt/PKB phosphorylation in subcutaneous fat (Sub fat) of vehicle- and CORT-exposed mice after LacZ or 11β-HSD1 shRNA treatment. *A*: relative expression of mRNA levels were measured by RT-PCR and normalized to 18S expression. *B–D*: relative Sub fat IRβ, IRS-1, PI3K, and p-Thr^{308} Akt/PKB protein expression were standardized to the amount of β-actin. p-Thr^{308} Akt/PKB protein was normalized to the amount of total Akt. Data are means ± SE of 6 mice/group. #$P < 0.05$ vs. LacZ-treated control mice; *$P < 0.01$ vs. LacZ-treated control mice; **$P < 0.01$ vs. LacZ-treated CORT-exposed mice; †$P < 0.001$ vs. LacZ-treated vehicle-exposed mice; ##$P < 0.05$ vs. LacZ-treated CORT-exposed mice.

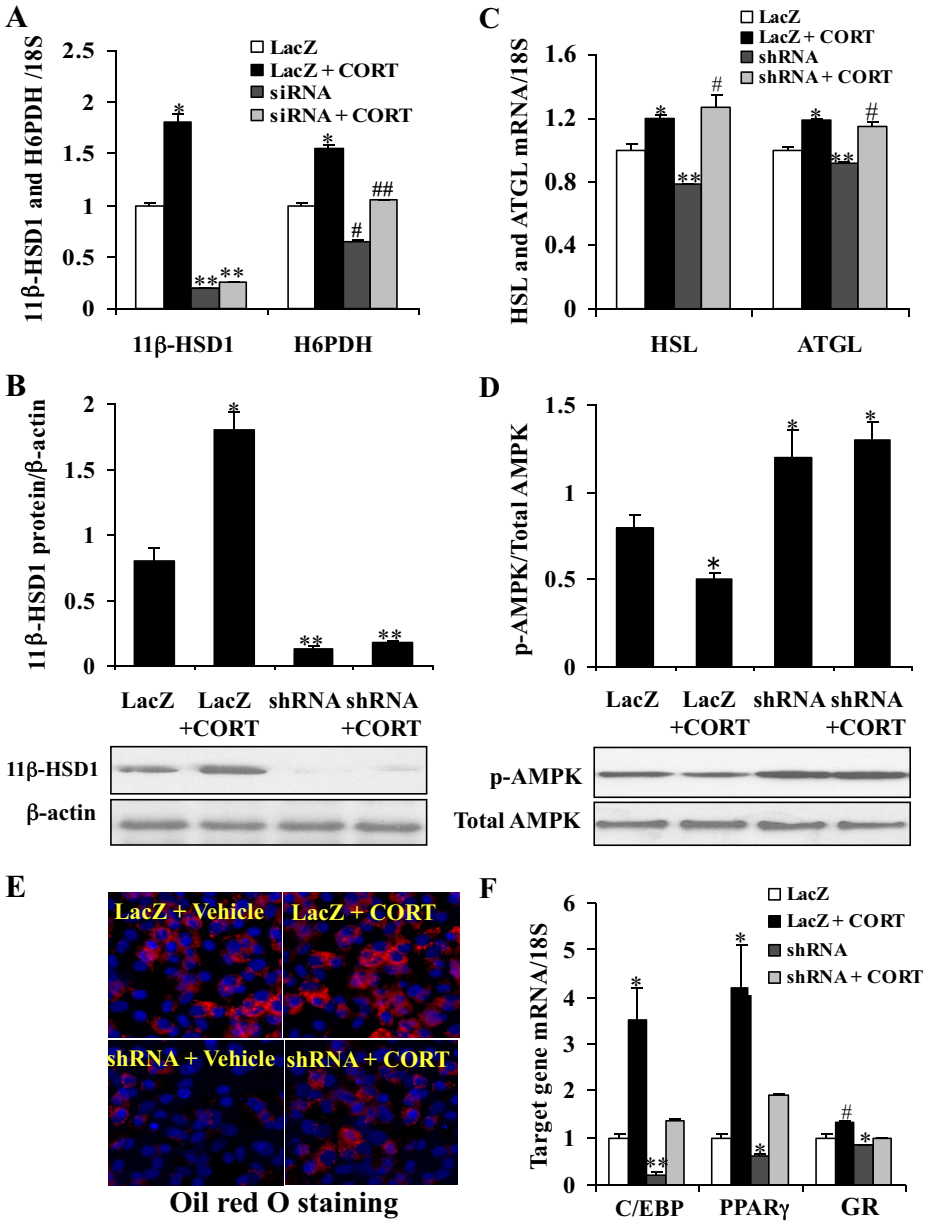

Fig. 8. Effects of CORT and 11β-HSD1 shRNA on 11β-HSD1, H6PDH, and p-Thr^{172} AMPK as well as lipid content and gene expression in 11β-HSD1 shRNA-stable 3T3-L1 adipocyte cells or LacZ control cells. Cells were incubated with CORT (10^{-6} mol/l) for 48 h. Relative expression of 11β-HSD1 and H6PDH (*A*), HSL and ATGL (*C*), and CCAAT/enhancer-binding protein-α (C/EBP), peroxisome-proliferator-activated receptor-γ (PPARγ), and glucocorticoid receptor (GR) (*F*) mRNA levels were normalized to 18S rRNA. *B* and *D*: 11β-HSD1 protein and Thr^{172} AMPK phosphorylation in LacZ cells or 11β-HSD1 shRNA-stable cells. *E*: lipid droplet accumulation was monitored by fluorescent microscopy using Oil Red O staining with mounting medium DAPI. Original magnification, ×20. Values are means ± SE from 3 separate culture preparations. *$P < 0.01$ vs. LacZ control cells; **$P < 0.001$ vs. LacZ control cells; #$P < 0.05$ vs. LacZ control cells.

GC-treated mice. Our present results support the concept that the suppression of AMPK activity in adipose tissue may be a potential mechanism for hypercortisolism-mediated activation of lipase HSL and ATGL with induction of 11β-HSD1 expression. This is in accord with previous reports that enhanced HSL activity is closely associated with a decrease in p-Thr^{172} AMPK in adipocytes (23), whereas AMPK activation is suppressed by GCs (9). These data support the possibility that the specific 11β-HSD1 inhibitor shRNA improvement of GC-mediated activation of lipase expression may be mediated in part by stimulation of adipose p-Thr^{172} AMPK. In addition, 11β-HSD1 shRNA also impaired CORT-induced C/EBPα and PPARγ mRNA levels, which are well known to be the key lipogenic transcriptional regulators for terminal adipogenesis, suggesting that 11β-HSD1 shRNA-mediated inhibition of C/EBPα and PPARγ could decrease lipogenesis, which could contribute to the attenuation of GC-induced fat breakdown. Moreover, C/EBP is required for the transcriptional activator of 11β-HSD1 in adipocytes (13). Reduction of C/EBP may thus provide an additional mechanism to adipose 11β-HSD1 knockdown, which is linked to the improvement of GC-induced adipolysis. Additionally, CORT induced the expression of adipose PEPCK mRNA, a key regulatory enzyme of fatty acid reesterification for adipose fatty acid release that is activated by GCs (49). In contrast, 11β-HSD1 shRNA blocked GC-mediated induction of epididymal fat PEPCK, although it maintained subcutaneous fat PEPCK expression in GC-treated mice. These data suggest that the activation of adipose PEPCK may also be involved in the metabolic phenotype of GC-induced adipolysis.

In addition, the current study also demonstrates that prereceptor modulation of 11β-HSD1 may also contribute to GC-induced changes in insulin sensitivity in adipose tissue. We found that chronic CORT-exposed mice developed insulin

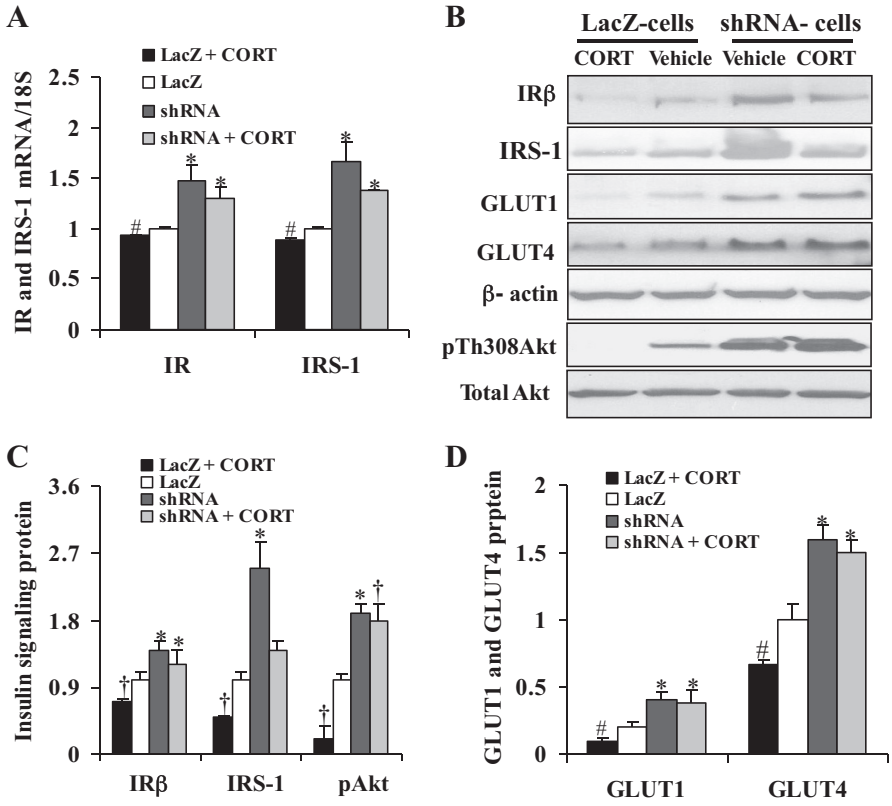

Fig. 9. Effects of CORT on insulin-signaling cascade in 11β-HSD1 shRNA-stable 3T3-L1 adipocytes cells or LacZ controls. Cells were generated and incubated with CORT (10^{-6} mol/l) for 48 h. CORT decreased IR and IRS-1 mRNA (*A*) and protein expression (*B* and *C*), leading to the inhibition of GLUT1 and GLUT4 (*D*) with suppression of Thr^{308} Akt/PKB phosphorylation (*B* and *C*) in LacZ-3T3-L1 adipocytes cells; effects were reversed in 11β-HSD1 shRNA adipocytes cells. *A*: relative expression of mRNA levels was measured by RT-PCR and normalized to 18S rRNA. *C* and *D*: relative expression of IRβ, IRS-1, GLUT1, and GLUT4 protein was standardized to the amount of β-actin. Values are means ± SE from 3 separate culture preparations. #$P < 0.05$ vs. LacZ control cells; *$P < 0.01$ vs. LacZ control cells; †$P < 0.05$ vs. LacZ control cells.

resistance in abdominal adipose tissue, as evidenced by the reduction of adipose IR and IRS-1 protein expression with suppression of p-Thr^{308} Akt/PKB accompanied by elevated adipose 11β-HSD1. These findings in vivo in adipose tissue support the finding that GCs impair the insulin-signaling cascade in vitro in cultured adipose cells with inhibition of IR, IRS-1, PI3K, and PKB activity (5, 16, 45). In contrast, decreased adipose 11β-HSD1 after 11β-HSD1 shRNA treatment attenuated GC-induced decrease in the IR and IRS-1 gene expression with the induction of p-Thr^{308} Akt/PKB and the maintaining of PI3K levels, both of which act to improve local insulin sensitivity impaired by CORT. In agreement with these findings, silencing 11β-HSD1 effectively attenuated GC-induced reduction of p-Thr^{308} Akt/PKB-mediated IR and IRS-1 signaling action in 11β-HSD1 shRNA-adipocytes cells, indicating that 11β-HSD1 is able to regulate GC-induced insulin resistance. These data support our hypothesis that the prereceptor modulation of 11β-HSD1 is a key mediator of GC regulating insulin sensitivity in adipose tissue through the insulin-signaling cascade. Our findings agree with a recent study showing that an 11β-HSD1 inhibitor blocked the GC-impaired insulin-signaling action in skeletal muscle (31) and that 11β-HSD1-knockout mice enhanced insulin signaling in subcutaneous adipose tissue (23).

The observed effect of 11β-HSD1 shRNA on adipocyte insulin sensitivity that is correlated with an altered lipid metabolic profile implies that insulin signaling action may play an important role in regulating adipose lipolytic pathway. Indeed, humans with IR defect exhibit lipodystrophy and elevated plasma fatty acid levels (19). Akt is a master protein that regulates insulin signaling and affects AMPK signaling, which represents the principal lipolytic pathway in adipose tissue (2). Consistent with this concept, we found that GCs impaired p-Thr^{172} AMPK with reduction of p-Thr^{308} Akt signaling, indicating that GC-induced adipose insulin resistance and lipolysis may be associated with reduction of p-Thr^{308} Akt. This is consistent with dexamethasone effectively reducing p-Thr^{308} Akt and inducing lipolysis in primary rat adipocytes (7). Additionally, our data demonstrated that 11β-HSD1 shRNA blocked GC-mediated suppression of p-Thr^{308} Akt, which paralleled the inhibition of lipase expression with induction of p-Thr^{172} AMPK within adipose tissue. Induction of AMPK itself is sufficient to reduce adipolysis linked to lipid homeostasis (10, 43). Our findings agree with the notion that PI3K/Akt activation causes a net dephosphorylation of lipase HSL and inhibition of lipolysis (11, 37).

It should be noted that 11β-HSD1 was silenced systemically, and our results focused on changes in adipose tissue. Conversely, our injection of the adenovious into the intraperitoneal cavity may lead to selective silencing in the adipose tissue, but we expect that silencing does occur in other tissues. We are planning to use adipocyte-specific GC inactivation mice or generate adipocyte-specific 11β-HSD1-null mice and examine the diabetic phenotype of that mouse to address this important issue.

In summary, we propose that the prereceptor augmentation of adipose 11β-HSD1 expression plays a pivotal role in mediating GC-induced lipolysis with insulin resistance observed in abdominal adipose tissue in mice chronically treated with pharmacologically relevant doses of GCs. We demonstrated that some of the beneficial effects of 11β-HSD1 inhibition by shRNA in GC-induced metabolic syndrome may be mediated

at least in part through inactivation of intracellular GC action related to insulin signaling mechanisms and lipolytic pathways in adipose tissue; it is likely that their efficacy in adipose tissue will provide an additional pharmacological benefit in treating GC-induced adipocyte insulin resistance and related metabolic disorders, conditions that are of clinical relevance to a wide range of patients. Our findings suggest that the use of selective 11β-HSD1 inhibitors may be helpful for the treatment of GC-mediated metabolic dysregulation.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Y.W., C.Y., and Y.L. conception and design of research; Y.W., C.Y., L.L., W.W., H.D., W.F., and Y.L. performed experiments; Y.W., C.Y., L.L., W.W., H.D., W.F., and Y.L. analyzed data; Y.W., C.Y., L.L., W.W., H.D., W.F., and Y.L. interpreted results of experiments; Y.W., C.Y., L.L., H.D., W.F., K.L., M.J., T.C.F., and Y.L. approved final version of manuscript; L.L. and Y.L. drafted manuscript; K.L., M.J., and T.C.F. edited and revised manuscript; Y.L. prepared figures.

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