Novel Adipose Tissue–Mediated Resistance to Diet-Induced Visceral Obesity in 11β-Hydroxysteroid Dehydrogenase Type 1–Deficient Mice

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The metabolic syndrome (visceral obesity, insulin resistance, type 2 diabetes, and dyslipidemia) resembles Cushing's Syndrome, but without elevated circulating glucocorticoid levels. An emerging concept suggests that the aberrantly elevated levels of the intracellular glucocorticoid reamplifying enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) found in adipose tissue of obese humans and rodents underlies the phenotypic similarities between idiopathic and "Cushingoid" obesity. Transgenic overexpression of 11β-HSD-1 in adipose tissue reproduces a metabolic syndrome in mice, whereas 11β-HSD-1 deficiency or inhibition has beneficial metabolic effects, at least on liver metabolism. Here we report novel protective effects of 11β-HSD-1 deficiency on adipose function, distribution, and gene expression in vivo in 11β-HSD-1 nullizygous (11β-HSD-1$^{-/-}$) mice. 11β-HSD-1$^{-/-}$ mice expressed lower resistin and tumor necrosis factor-α, but higher peroxisome proliferator–activated receptor-γ, adiponectin, and uncoupling protein-2 mRNA levels in adipose, indicating insulin sensitization. Isolated 11β-HSD-1$^{-/-}$ adipocytes exhibited higher basal and insulin-stimulated glucose uptake. 11β-HSD-1$^{-/-}$ mice also exhibited reduced visceral fat accumulation upon high-fat feeding. High-fat–fed 11β-HSD-1$^{-/-}$ mice rederived onto the C57BL/6J strain resisted diabetes and weight gain despite consuming more calories. These data provide the first in vivo evidence that adipose 11β-HSD-1 deficiency beneficially alters adipose tissue distribution and function, complementing the reported effects of hepatic 11β-HSD-1 deficiency or inhibition. *Diabetes* 53:931–938, 2004

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11β-HSD-1, 11β-hydroxysteroid dehydrogenase type 1; BAT, brown adipose tissue; PPAR, peroxisome proliferator–activated receptor; TNF, tumor necrosis factor; UCP, uncoupling protein.

Chronic exposure to high circulating glucocorticoid levels (Cushing's syndrome) causes visceral obesity and the associated metabolic abnormalities of insulin resistance, type 2 diabetes, dyslipidemia, and hypertension. Similar metabolic abnormalities occur in human idiopathic obesity and the metabolic syndrome; however, this typically occurs without plasma cortisol excess (1,2).

An emerging concept suggests that amplification of glucocorticoid action within adipose tissue by the intracellular enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD-1) plays a key role in the development of central obesity in humans (3–5) and rodents (6,7), thus providing a molecular basis for the phenotypic similarities between Cushing's and idiopathic obesity. 11β-HSD-1 increases intracellular glucocorticoid levels by converting circulating inert 11-dehydrocorticosterone (cortisone in humans) into active corticosterone (cortisol) through 11β-reductase activity in most intact cells, including adipocytes (8–10). 11β-HSD-1 activity is specifically increased in visceral fat of obese Zucker rats (6), obese Lep^{ob} mice (7), and is elevated in subcutaneous fat of obese humans in most (3–5), though not all (11), studies. Transgenic overexpression of 11β-HSD-1 selectively in white adipose tissue causes visceral obesity, insulin resistance, diabetes, dyslipidemia, and hypertension in mice (7,12). As in many cases of human metabolic syndrome, this occurs without elevation of systemic corticosterone (7). The deleterious metabolic effects of adipose-specific 11β-HSD-1 overexpression are more pronounced in the metabolically active, glucocorticoid receptor–rich (13) visceral adipose tissue where depot-specific hypertrophy occurs (7). It is such visceral fat accumulation, over and above total fat mass, that is strongly and independently correlated with increased morbidity and mortality in humans (14).

In contrast, mice with a targeted disruption of the 11β-HSD-1 gene (11β-HSD-1$^{-/-}$ mice) exhibit enhanced glucose tolerance, attenuated gluconeogenic responses (15), and an improved lipid and lipoprotein profile (16). These effects have been previously ascribed to attenuated glucocorticoid-inducible metabolic functions in liver, despite the modestly elevated plasma levels of corticosterone in 11β-HSD-1$^{-/-}$ mice (15,16), suggesting that 11β-HSD-1 activity is indeed a crucial amplifier of intracellular glucocorticoid action in liver in vivo. Pharmacological inhibition of 11β-HSD-1 in vivo also improves glycemia

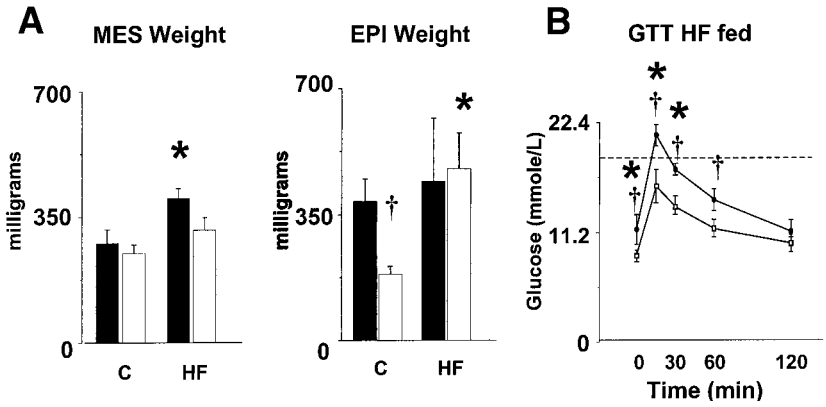

FIG. 1. Effects of 11β-HSD-1 deficiency and high-fat feeding on fat distribution and glucose homeostasis in MF-1 mice. *A*: Mesenteric visceral (MES) and epididymal (EPI) fat pad weights in MF-1 (■) or MF-1–11β-HSD-1$^{-/-}$ (□) mice fed a control (C) or high-fat (HF) diet. *$P < 0.05$, effect of high fat within genotype; †$P < 0.05$ between genotypes. *B*: Glucose tolerance in MF-1 (●) and MF-1–11β-HSD-1$^{-/-}$ (□) mice on a high-fat diet. The horizontal broken line represents the mean peak glucose value for MF-1 mice on the control diet (omitted for clarity). *$P < 0.01$ for effect of high fat in MF-1 mice; †$P < 0.01$ for effect of genotype.

and increases hepatic insulin sensitivity (17–20). However, such studies have used nonspecific drugs, such as carbenoxolone, that do not achieve adipose 11β-HSD-1 inhibition (19) or specific 11β-HSD-1 inhibitor studies (20) that have addressed only hepatic function. Therefore, the potential contribution that adipose tissue 11β-HSD-1 inhibition makes to an improved metabolic phenotype in vivo remains unknown. To address this issue, we have examined adipose distribution and function in 11β-HSD-1 nullizygous mice on both the original intrinsically obesity-resistant (MF-1) strain background and also those newly backcrossed onto the obesity/diabetes-susceptible C57BL/6J strain.

RESEARCH DESIGN AND METHODS

All studies were performed within U.K. Home Office guidelines for scientific procedures on laboratory animals. Male MF1–11β-HSD-1$^{-/-}$ mice and their age-matched, wild-type controls, bred as previously described (15), were housed in standard conditions on a 12/12-h light:dark cycle (lights on at 7:00 A.M.). The targeted 11β-HSD-1 transgene was rederived onto the C57BL/6J strain by embryo transfer and then backcrossed with C57BL/6J mice for 10 generations before the current studies. Adult, age-matched, male, wild-type and 11β-HSD-1$^{-/-}$ mice (n = 6–10) were given a control (11% calories as fat, Research Diets D12328; Research Diets, New Brunswick, NJ) or high-fat diet (58% calories as fat, Research Diets D12331) for 18 weeks, a diet previously optimized for weight gain and insulin resistance (21). An alternative diabetogenic (22) diet that produces elevated LDL cholesterol was also used (wt/wt: protein 20%, carbohydrate 36.4%, fat [lard] 36.4%). Mice were singly housed for the final week of the experiment. Mice were killed at around 8:00 A.M., within 1 min of disturbing each cage.

Intraperitoneal glucose/insulin tolerance test. After 18 weeks on a control or high-fat diet, transgenic and wild-type mice were fasted overnight and then injected intraperitoneally with 2 mg/g D-glucose (25% stock solution in saline) or 1 unit/kg body wt Humulin S (Lilly, Basingstoke, Hampshire, U.K.). Blood samples were taken by tail venesection into EDTA-micro tubes (Sarstedt, Leicester, U.K.) at 0 min (before injection and within 1 min of disturbing the cage) and at 15-, 30-, 60-, and 120-min intervals after the glucose load or insulin bolus. Glucose was measured with the Sigma HK assay (Sigma, Poole, U.K.). For insulin tolerance tests, animals were fasted for 6 h.

Plasma and serum parameters. Serum lipids were measured as previously described (16). The cholesterol and triglyceride distribution among the lipoproteins was determined through fast-protein liquid chromatography fractionation (16). Leptin was measured by enzyme-linked immunosorbent assay (Crystalchem, Downers Grove, IL). Free fatty acids were measured with a Wako nonesterified fatty acid kit (Alpha Laboratories, Hampshire, U.K.). Corticosterone was measured in plasma with an in-house radioimmunoassay (15). Intra-adipose corticosterone levels were determined by radioimmunoassay (ICN Diagnostics, Orangeburg, NY) as described (7).

RNA extraction and analysis. Tissues were snap-frozen in liquid nitrogen and homogenized in Trizol (Life Technologies, Paisley, U.K.). Total RNA was blotted according to standard Northern blot procedure and gene expression analyzed as described (16). Primer sequences were as follows: uncoupling protein (UCP)-2, (forward) 5′-GCATTGCAGGTCTCATCA C, (backward) 5′-CTTGGTGTAGAACTGTTTGAC; peroxisome proliferator–activated receptor (PPAR)γ, (forward) 5′-GAGTGTGACGACAAGATTTG, (backward) 5′-ATAGT GGAAGCCTGATGC; tumor necrosis factor (TNF)-α, (forward) 5′-TGCCTAT GTCTCAGCCTC, (backward) 5′-ACTCCTCCCAGGTATATG; resistin, (forward) 5′-TGTGGGACAGGAGCTAATAC, (backward) 5′-AGACATCTCTGGAG CTACAG; leptin, (forward) 5′-CCAAAACCCTCATCAAGACC, (backward) 5′-GTCCAACTGTTGAAGAATGTCCC; and adiponectin, (forward) 5′-GGATGCT ACTGTTGCAAG, (backward) 5′-CATGTACACCGTGATGTG.

Primary adipocyte isolation and glucose uptake. Fat pads were excised and adipocytes isolated from fed C57BL/6J or C57BL/6J–11β-HSD-1$^{-/-}$ mice by collagenase digestion (type 1; Worthington, Lakewood, NJ). The cell suspension was strained and then washed three times in Krebs Ringer (118 mmol/l NaCl, 5 mmol/l $NaHCO_3$, 4.7 mmol/l KCl, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4 \cdot 7\ H_2O$, 25 mmol/l HEPES, 2.5 mmol/l $CaCl_2$, supplemented with 1% BSA, Fraction V, and 200 nmol/l adenosine (Sigma), pH 7.4. Triplicate homogeneous cell suspensions were preincubated for 15 min at 37°C in a shaking water bath with or without insulin (5 nmol/l, Humulin S; Lilly) or 10 μmol/l cytochalasin B (Sigma) to determine basal uptake. Then, 10 μmol/l cold 2-deoxy glucose and 2.5 μCi/ml [^{3}H]2-deoxyglucose tracer (Amersham, Buckinghamshire, U.K.) was added to the cells for a further 3 min. Glucose uptake in the adipocytes was determined on a β-scintillation counter (Wallac, Turku, Finland) after centrifugation of adipocytes through corning oil and homogenizing in 1 ml Triton X-100.

Statistical analyses. Data are expressed as means ± SE. Results were subjected to two-way ANOVA (factors were genotype and diet) or repeated-measures, two-way ANOVA for longitudinal bodyweight gain, glucose, and insulin tolerance tests (factors were genotype and time and diet and time) using a Sigmastat program (Jandel, San Rafael, CA). Where feeding status, genotype, or an interaction between these two factors was found by two-way ANOVA, significant differences between relevant groups were determined with post hoc Tukey multiple comparisons tests. Asterisks represent significant differences due to diet (*, **, and *** are $P < 0.05$, <0.01, and <0.001, respectively). Daggers indicate significant differences between genotypes (†, ††, and ††† are $P < 0.05$, <0.01, and <0.001, respectively).

RESULTS

Adipose 11β-HSD-1 deficiency is associated with favorably altered fat distribution and resistance to diet-induced metabolic disease. Given the potentially causative role of increased adipose tissue 11β-HSD-1 activity in the pathogenesis of visceral obesity and its cardiometabolic consequences (7,12), we assessed the effects of high-fat feeding on body weight, fat distribution, and glucose homeostasis in 11β-HSD-1$^{-/-}$ mice. On the original MF-1 genetic background, neither wild-type nor 11β-HSD-1$^{-/-}$ mice gained weight above controls and organ weights were similar (data not shown) on a high-fat diet, demonstrating that MF-1 mice were intrinsically resistant to high-fat diet–induced weight gain, as are some other mouse strains (21). However, although wild-type mice accumulated metabolically "disadvantageous" (14) visceral fat (Fig. 1*A*) and developed glucose intolerance on a high-fat diet (Fig. 1*B*), 11β-HSD-1$^{-/-}$ mice selectively increased the mass of the epididymal fat depot (Fig. 1*A*) and maintained glucose tolerance within the normal range

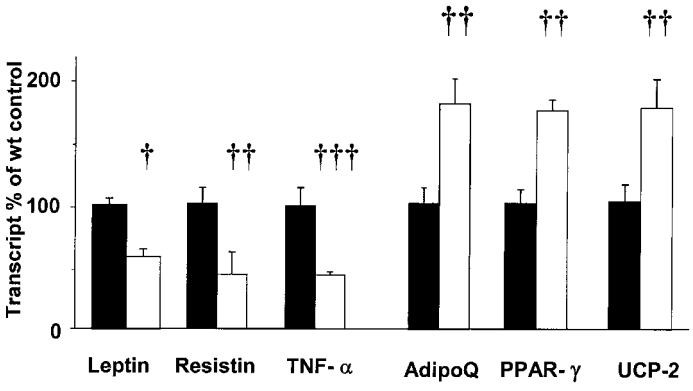

FIG. 2. Pattern of epididymal adipose tissue gene expression in 11β-HSD-1$^{-/-}$ mice. Leptin, resistin, TNF-α, adiponectin (AdipoQ), PPARγ, and UCP-2 mRNA levels in epididymal (EPI) fat of MF-1 (■) or 11β-HSD-1$^{-/-}$ (□) mice. †$P < 0.05$; ††$P < 0.01$; †††$P < 0.001$.

for this strain (Fig. 1*B*). The altered adipose distribution in high-fat–fed 11β-HSD-1$^{-/-}$ mice was not due to altered systemic glucocorticoid levels because the plasma corticosterone levels in 11β-HSD-1$^{-/-}$ mice were slightly elevated, as previously described (15,16), and were unaffected by the high-fat diet (MF-1 control diet: 31 ± 11 nmol/l; 11β-HSD-1$^{-/-}$ control diet: 57 ± 8 nmol/l; MF-1 high-fat diet: 18 ± 4 nmol/l; and 11β-HSD-1$^{-/-}$ high-fat diet: 58 ± 19 nmol/l; significant effect of genotype, $P < 0.01$ by ANOVA). These data suggest that 11β-HSD-1 deficiency produces a "favorably" altered adipose tissue distribution on an intrinsically obesity-resistant genetic background.

The effect of 11β-HSD-1 deficiency on adipose gene expression. We investigated whether the altered fat distribution was associated with changes in adipose tissue function in 11β-HSD-1$^{-/-}$ mice. Specifically, we assessed adipose tissue mRNA levels encoding factors regulating distinct adipose functions such as energy expenditure and fat mass: leptin (23); insulin resistance: resistin (24,25) and TNF-α (26); adipose insulin sensitization and differentiation: adiponectin (27) and PPARγ (28); and energy dissipation: UCP-2 (29,30). On the control diet, 11β-HSD-1$^{-/-}$ mice had reduced epididymal fat mRNA levels encoding leptin, resistin, and TNFα (Fig. 2), whereas adiponectin, PPARγ, and UCP-2 mRNAs were elevated (Fig. 2). Consistent with epididymal fat functioning as the primary source of leptin expression (31), 11β-HSD-1$^{-/-}$ mice also had lower plasma leptin (1.06 ± 0.19 vs. wild type 2.53 ± 0.35 ng/ml, $P < 0.02$).

In visceral fat, PPARγ mRNA was elevated in 11β-HSD-1$^{-/-}$ mice (Fig. 3*A*), though leptin, resistin, TNF-α, adiponectin, and UCP-2 mRNA levels in visceral fat were similar to the wild-type mice on the control diet (not shown). PPARγ ligands, such as thiazolidinediones, cause fat redistribution to peripheral depots (32,33). With high-fat feeding, the elevated PPARγ mRNA levels in control-fed 11β-HSD-1$^{-/-}$ mice were further increased selectively in visceral fat in 11β-HSD-1$^{-/-}$ mice (Fig. 3*A*). This did not occur in wild-type animals or in the epididymal fat depot of 11β-HSD-1$^{-/-}$ mice.

Induction of UCP-2 in adipose tissue on a high-fat diet has been associated with obesity resistance in A/J mice and does not occur in obesity-prone C57BL/6J mice (30). 11β-HSD-1$^{-/-}$ mice showed a high-fat–mediated induction of UCP-2 selectively in visceral adipose tissue that was greater than that observed in wild-type MF-1 mice (Fig. 3*B*). Furthermore, in 11β-HSD-1$^{-/-}$ mice that had been backcrossed for 10 generations onto the diabetes/obesity-prone C57BL/6J genetic background (see below), homozygosity for 11β-HSD-1 deficiency conferred a higher visceral fat UCP-2 mRNA level to the C57BL/6J strain on control diet and promoted an inductive UCP-2 response to high-fat feeding in C57BL/6J mice (Fig. 3*C*) similar to that seen in the obesity-resistant A/J mice (30).

11β-HSD-1 deficiency causes adipose insulin sensitization. Since the adipose gene expression profile indicated insulin sensitization in this tissue, we assessed functional parameters of adipose tissue in the 11β-HSD-1$^{-/-}$ mice. 11β-HSD-1$^{-/-}$ mice had lower fasting plasma fatty acids (0.57 ± 0.1 vs. wild type 0.9 ± 0.14 mmol/l, $P <$ 0.05), a marker of adipose insulin sensitization (34). Fur-

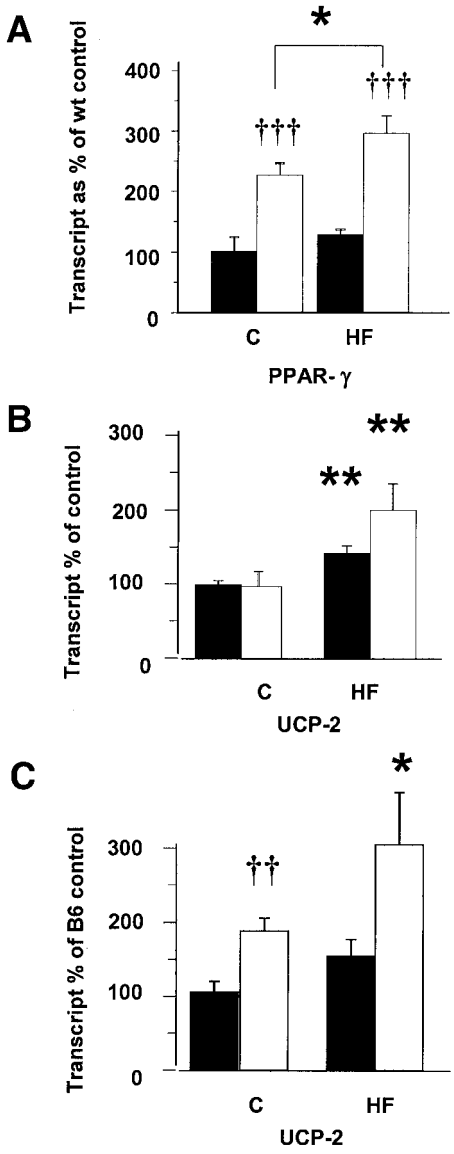

FIG. 3. Pattern of mesenteric visceral adipose tissue gene expression in 11β-HSD-1$^{-/-}$ mice. Shown are PPARγ mRNA (*A*) and UCP-2 mRNA (*B*) levels in MF-1 (■) or MF-1–11β-HSD-1$^{-/-}$ (□) mice fed a control (C) or high-fat (HF) diet. *C*: UCP-2 mRNA levels in C57BL/6J (■) and C57BL/6J–11β-HSD-1$^{-/-}$ (□) mice fed a control (C) or high-fat (HF) diet. ††$P < 0.01$, †††$P < 0.001$ for between genotypes; *$P < 0.05$, **$P <$ 0.01 for effect of high fat within genotypes.

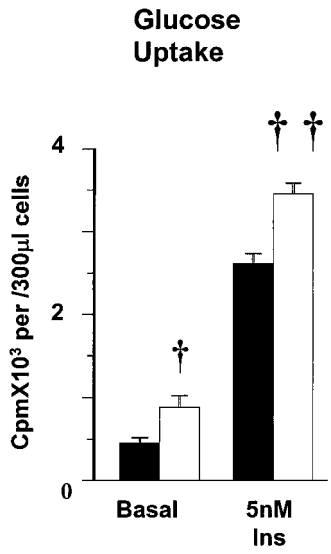

FIG. 4. Effects of 11β-HSD-1 deficiency on adipocyte glucose uptake. Glucose uptake in isolated C57BL/6J (■) or C57BL/6J–11β-HSD-1$^{-/-}$ (□) mouse adipocytes is shown. †P < 0.05, ††P < 0.001 for between genotypes.

ther, isolated primary adipocytes from 11β-HSD-1$^{-/-}$ mice exhibited higher basal and insulin (5 nmol/l)-stimulated glucose uptake than wild-type controls (Fig. 4), directly confirming increased adipose insulin sensitivity.

11β-HSD-1 deficiency in the obesity- and diabetes-prone C57BL/6J strain. Because MF-1 mice exhibited intrinsic obesity resistance, the 11β-HSD-1 null allele was backcrossed onto the obesity- and metabolic disease–prone C57BL/6J strain (10 generations) to allow investigation of the effects of 11β-HSD-1 deficiency upon a well-established model of dietary obesity and its metabolic complications (21,30). C57BL/6J–11β-HSD-1$^{-/-}$ mice maintained normal fertility, health, and survival rates, as previously documented on the MF-1 background (15).

Wild-type C57BL/6J mice given a high-fat diet for 18 weeks became markedly obese (Fig. 5*A*). C57BL/6J–11β-HSD-1$^{-/-}$ mice gained significantly less weight on the high-fat diet (Fig. 5*A*) despite an increased caloric intake relative to C57BL/6J mice (Fig. 5*B*). This might be partly explained by the raised core body temperature (Fig. 5*C*), suggesting a higher metabolic rate in C57BL/6J–11β-HSD-1$^{-/-}$ mice. As with MF-1–11β-HSD-1$^{-/-}$ mice, C57BL/6J–11β-HSD-1$^{-/-}$ mice accumulated significantly less visceral fat (body weight adjusted) (Fig. 5*D*), with a relatively greater fat mass redistributed into the metabolically less disadvantageous epididymal fat with high-fat feeding (Fig. 5*E*). Adipose tissue gene expression patterns in C57BL/6J–11β-HSD-1$^{-/-}$ mice, including those for UCP-2 (Fig. 2*B*–*C*), were similar to MF-1–11β-HSD-1$^{-/-}$ mice, indicating that similar underlying mechanisms drive the phenotype of 11β-HSD-1$^{-/-}$ mice on both strain backgrounds (not shown).

As with the MF-1 strain, a favorable adipose distribution was associated with markedly improved glucose tolerance (Fig. 6*A*) and with greater insulin sensitivity (Fig. 6*B*) in C57BL/6J–11β-HSD-1$^{-/-}$ mice.

C57BL/6J–11β-HSD-1$^{-/-}$ mice have an improved lipid profile on high-fat and cholesterogenic diets. Previous studies revealed that MF-1–11β-HSD-1$^{-/-}$ mice had an improved lipid and lipoprotein profile when fed a standard rodent diet (16). However, our current studies reveal the MF-1 strain to be obesity resistant and to only develop mild glucose intolerance with high-fat feeding. We therefore addressed whether such an improvement in lipid metabolism would be observed in 11β-HSD-1$^{-/-}$ mice rederived onto the diabetes-prone C57BL/6J strain (21,30).

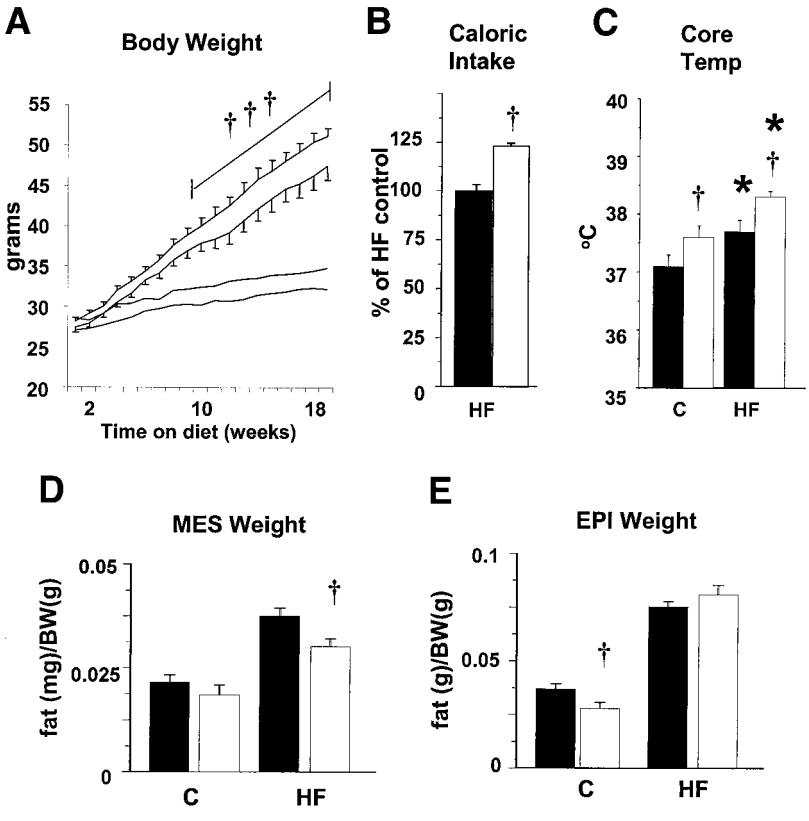

FIG. 5. Effects of 11β-HSD-1 deficiency in the C57BL/6J strain. *A*: Longitudinal body weight in high-fat–fed C57BL/6J (uppermost line with error bars) and C57BL/6J–11β-HSD-1$^{-/-}$ (lower line with error bars, †††P < 0.001 for between genotypes) or control diet–fed C57BL/6J (uppermost line without error bars, omitted for clarity) and C57BL/6J–11β-HSD-1$^{-/-}$ (lower line without error bars) mice. *B*: Caloric intake during the high-fat diet. *C*: Nocturnal (8:00 P.M.) rectal temperature. Mesenteric (MES) (*D*) and epididymal (EPI) (*E*) fat pad–to–body weight ratios of C57BL/6J (■) and C57BL/6J–11β-HSD-1$^{-/-}$ (□) mice fed a control (C) or high-fat (HF) diet are shown. *P < 0.05 for the effect of a high-fat diet within a genotype; †P < 0.05 for the significant difference between genotypes.

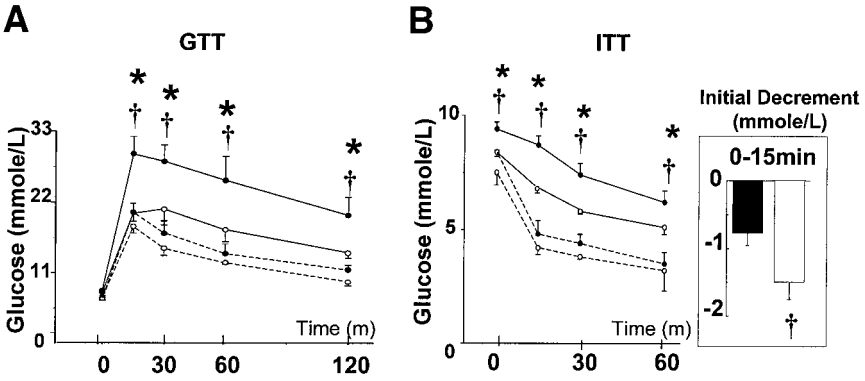

FIG. 6. Effects of 11β-HSD-1 deficiency on glucose and insulin tolerance in C57BL/6J mice. Results from glucose (*A*) and insulin (*B*) tolerance tests (*inset*: decrement in glucose levels at 15 min, †P < 0.05 for comparing genotypes fed a high-fat diet) in C57BL/6J (●) and C57BL/6J–11β-HSD-1$^{-/-}$ (○) mice on a control (broken lines) or high-fat (solid lines) diet are shown. *P < 0.001 for effect of high-fat within genotype; †P < 0.001 for between genotypes.

C57BL/6J–11β-HSD-1$^{-/-}$ mice exhibited reduced high-fat–fed triglycerides (Fig. 7*A*). Control-fed C57BL/6J–11β-HSD-1$^{-/-}$ mice had raised "beneficial" HDL cholesterol levels (Fig. 7*B*). Feeding a highly cholesterogenic lard diet for 6 weeks to wild-type C57BL/6J mice produced a marked switch from atheroprotective HDL- to atherogenic LDL-associated cholesterol (Fig. 7*C*). C57BL/6J–11β-HSD-1$^{-/-}$ mice showed an amelioration of this switch to LDL-associated cholesterol (Fig. 7*D*) and lower control-fed total cholesterol (Fig. 7*E*), as reflected in their contrasting HDL-to-total cholesterol ratio (Fig. 7*F*).

Effects of 11β-HSD-1 deficiency and obesity susceptibility on intra-adipose corticosterone levels. 11β-HSD-1 deficiency presumably acts by reducing intracellular glucocorticoid reactivation. Indeed, intra-adipose (visceral) corticosterone levels were lower in C57BL/6J–11β-HSD-

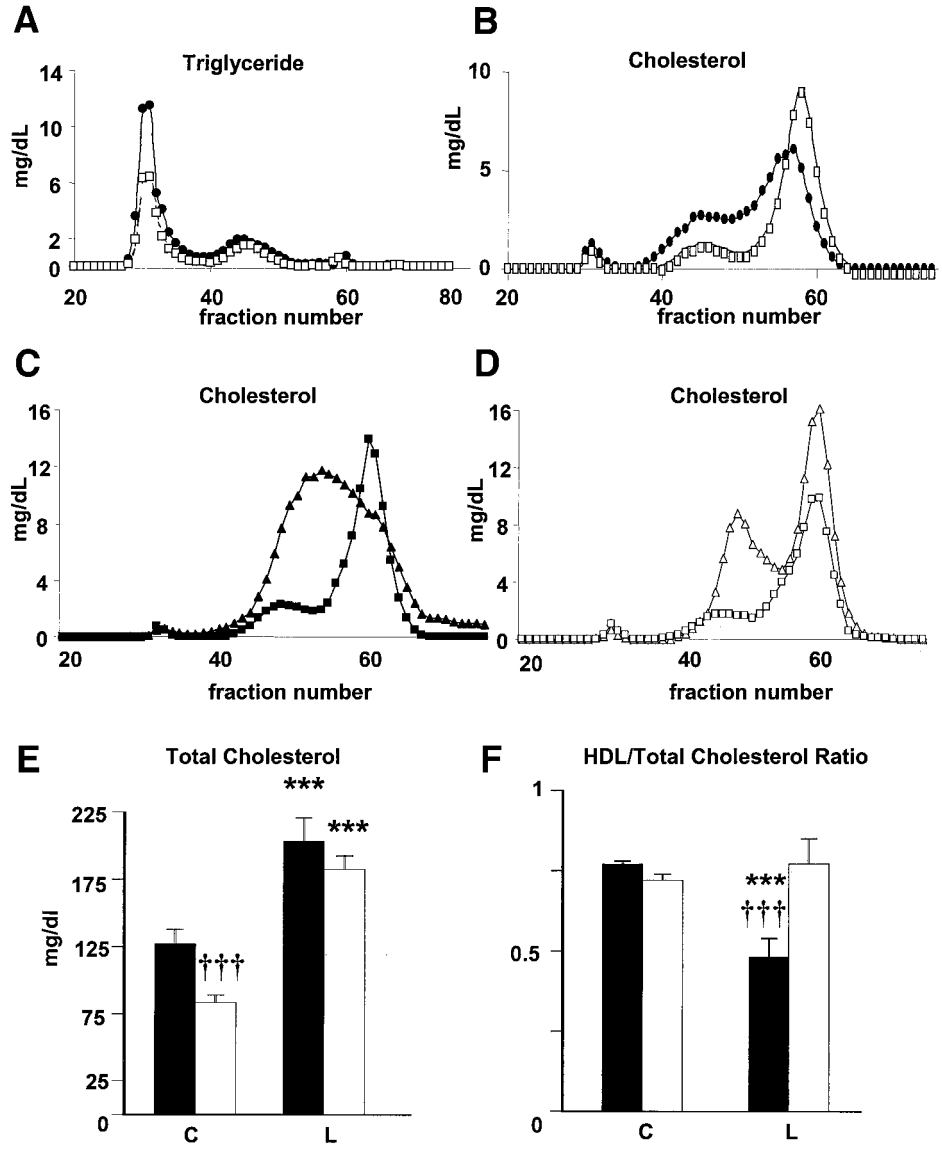

FIG. 7. The effects of 11β-HSD-1 deficiency on triglyceride and cholesterol metabolism in C57BL/6J mice. The fast-protein liquid chromatography profile of serum triglyceride (high-fat diet) distribution (*A*) and serum cholesterol distribution (control diet) (*B*) in C57BL/6J (●) and C57BL/6J–11β-HSD-1$^{-/-}$ (□) mice is shown. *C*: Fast-protein liquid chromatography profile of serum cholesterol distribution in C57BL/6J mice fed a control (■) or lard (▲) diet. *D*: C57BL/6J–11β-HSD-1$^{-/-}$ mice fed a control (□) or lard (△) diet. Total cholesterol levels (*E*) and HDL-to-total cholesterol ratio (*F*) in C57BL/6J (■) and C57BL/6J–11β-HSD-1$^{-/-}$ (□) mice fed a control (C) or lard (L) diet are shown. †††P < 0.001 for between genotypes; ***P < 0.001 for effect of diet within genotype.

$1^{-/-}$ mice on control (159 ± 12 ng/g; C57BL/6J, 477 ± 85; $P < 0.01$) and high-fat diets (404 ± 40; C57BL/6J, 562 ± 46; $P < 0.02$). Similar results were found in epididymal fat (not shown).

DISCUSSION

We describe adipocyte insulin sensitization, a "favorable" pattern of fat distribution and adipose gene expression, an improved metabolic profile, and resistance to dietary weight gain in an obesity/diabetes-prone mouse strain lacking a functional 11β-HSD-1 gene.

The primary mechanism accounting for reduced visceral fat accumulation in 11β-HSD-$1^{-/-}$ mice is presumably relative intracellular glucocorticoid deficiency, given that excess plasma glucocorticoid levels or transgenic overexpression of 11β-HSD-1 in white fat leads to glucocorticoid-induced hypertrophy of visceral adipose tissue (7). These data do not exclude the possibility that 11β-HSD-1 might be acting as a dehydrogenase, such as occurs in human visceral adipose stromal cells in vitro (35,36). Such a hypothesis predicts that 11β-HSD-1 deficiency could exacerbate visceral obesity through local inactivation of glucocorticoids, thus potentially expanding the preadipocyte population (35,36). However, we find reduced visceral fat accumulation and markedly reduced intra-adipose corticosterone levels in 11β-HSD-$1^{-/-}$ mice, despite their slightly higher circulating plasma levels, as well as reduced levels of glucocorticoid-inducible adipose tissue transcripts. This suggests that the predominant direction of 11β-HSD-1 in adipose is 11β reduction and that enzyme activity drives a net amplification of intra-adipose glucocorticoid levels in mice in vivo. Thus, 11β-HSD-1 deficiency is likely to result in reduced visceral fat accumulation by reducing adipose tissue hypertrophy.

The epididymal fat depot showed the greatest changes in adipose gene expression in 11β-HSD-$1^{-/-}$ mice. For example, mRNA encoding leptin, a glucocorticoid-inducible gene (37), is lower in the epididymal fat of 11β-HSD-$1^{-/-}$ mice but unchanged in visceral fat. One explanation is that visceral fat expresses higher levels of glucocorticoid receptor (7,13), which might compensate for the relative deficiency of intracellular glucocorticoid levels in 11β-HSD-$1^{-/-}$ mice in this depot. Plasma leptin was also lower in 11β-HSD-$1^{-/-}$ mice, reflecting that peripheral adipose tissue is the predominant adipose tissue expression site of this hormone (31) and confirming the observations on gene expression. Leptin reduces calorie intake and increases energy expenditure (23), thus lower plasma leptin levels found in 11β-HSD-$1^{-/-}$ mice may also explain the relatively higher food intake we observed. Lower body weight gain despite higher food intake in high-fat–fed C57BL/6J–11β-HSD-$1^{-/-}$ mice may be due, in part, to an increased metabolic rate mediated by 11β-HSD-1 deficiency in thermogenic brown adipose tissue (BAT)—a notion supported, though not proven, by the higher core temperature (38) in 11β-HSD-$1^{-/-}$ mice. BAT is a major contributor to energy expenditure and obesity resistance in rodents (39). Thus, because glucocorticoids inhibit thermogenesis in BAT (40,41) and wild-type mice abundantly express 11β-HSD-1 in BAT (N.M.M., unpublished data), it is expected that only the wild-type animals would exhibit 11β-HSD-1–mediated thermogenic constraint by glucocorticoids.

The metabolically "favorable" white adipose tissue distribution and insulin sensitization of adipose tissue in 11β-HSD-$1^{-/-}$ mice could also result from elevated PPARγ levels. PPARγ levels are inversely associated with obesity/insulin resistance (42). PPARγ activation increases adipose tissue insulin sensitivity (32), and, crucially, PPARγ activation by thiazolidinedione ligands increases fat mass selectively in "safer" peripheral depots while reducing intra-abdominal fat (32,33). We observe a similarly altered adipose distribution in the 11β-HSD-$1^{-/-}$ mice in association with their elevated adipose PPARγ levels. However, elevated PPARγ receptor levels alone would not necessarily explain the protected metabolic phenotype of 11β-HSD-$1^{-/-}$ mice. Thus, reduced PPARγ receptor number (PPARγ$^{+/-}$ heterozygote mice) has been associated with insulin sensitization (32) and insulin-resistant obesity can be associated with elevated PPARγ expression (43). We suggest that the protective adipose distribution, insulin sensitization, and gene expression profile of the 11β-HSD-$1^{-/-}$ mice could occur through activation of the elevated PPARγ levels in the adipose tissue of 11β-HSD-$1^{-/-}$ mice due to increased availability of free fatty acid PPARγ agonists (44) with high-fat feeding. Greater PPARγ activation is consistent with the lower leptin (32,45) and resistin (46) expression, and higher UCP-2 mRNA and (29) adiponectin (32) expression found in adipose tissue of 11β-HSD-$1^{-/-}$ mice. As well as redistributing fat to safer peripheral adipose stores, 11β-HSD-1 deficiency may also result in increased energy dissipation within fat. UCP-2 induction is a feature of obesity resistance in A/J mice but is absent in obesity-prone C57BL/6J mice (30). Greater induction of UCP-2 with a high-fat diet in the visceral fat of 11β-HSD-$1^{-/-}$ mice may therefore counter visceral fat accumulation in 11β-HSD-$1^{-/-}$ mice through increased adipose energy dissipation.

In terms of what may drive increased visceral fat PPARγ expression in high-fat–fed 11β-HSD-$1^{-/-}$ mice, this pattern has also been observed with insulin sensitization in the adipose tissue of other models (43). PPARγ expression is positively regulated by insulin (47), and we show that adipocytes from 11β-HSD-$1^{-/-}$ mice have greater insulin sensitivity, which may contribute to their higher adipose PPARγ expression. Reduced adipose TNF-α expression (26) and increased adiponectin (27) in adipose tissue from 11β-HSD-$1^{-/-}$ mice also concurs with improved insulin sensitivity. Furthermore, because TNF-α induces expression of 11β-HSD-1 in adipocytes (9), leading to further insulin resistance, 11β-HSD-1–deficient mice will clearly lack this further constraint on insulin sensitivity. Improved insulin sensitivity in adipose tissue would in turn contribute to favorable metabolic changes in other important organs. For example, elevated adiponectin levels would feed back directly to the liver, where this hormone is known to increase fat oxidation as well as reduce glucose output (48), thus contributing to the improved lipid and lipoprotein profiles observed in 11β-HSD-$1^{-/-}$ mice on both MF-1 (16) and C57BL/6J (present data) strains. In the 11β-HSD-$1^{-/-}$ mice, this additional benefit would complement the direct protective effects ascribed to 11β-HSD-1 deficiency or inhibition in the liver (16–20).

The current data represent the first in vivo evidence for the potential metabolic effects of adipose 11β-HSD-1 inhibition. We anticipate that adipose 11β-HSD-1 deficiency and, by inference, therapeutic inhibition of adipose 11β-HSD-1 will improve insulin sensitivity and glucose uptake, reduce lipolysis, and counteract accumulation of visceral fat and its related metabolic abnormalities.

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