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# Antisense reduction of 11ß-hydroxysteroid dehydrogenase type 1 enhances energy expenditure and insulin sensitivity independent of food intake in C57BL/6J mice on a Western-type diet

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ABSTRACT

We recently reported that inhibition of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) by antisense oligonucleotide (ASO) improved hepatic lipid metabolism independent of food intake. In that study, 11β-HSD1 ASO-treated mice lost weight compared with food-matched control ASO-treated mice, suggesting treatment-mediated increased energy expenditure. We have now examined the effects of 11β-HSD1 ASO treatment on adipose tissue metabolism, insulin sensitivity, and whole-body energy expenditure. We used an ASO to knock down 11β-HSD1 in C57BL/6J mice consuming a Western-type diet (WTD). The 11β-HSD1 ASO-treated mice consumed less food, so food-matched control ASO-treated mice were also evaluated. We characterized body composition, gene expression of individual adipose depots, and measures of energy metabolism. We also investigated glucose/insulin tolerance as well as acute insulin signaling in several tissues. Knockdown of 11β-HSD1 protected against WTD-induced obesity by reducing epididymal, mesenteric, and subcutaneous white adipose tissue while activating thermogenesis in brown adipose tissue. The latter was confirmed by demonstrating increased energy expenditure in 11β-HSD1 ASO-treated mice. The 11β-HSD1 ASO treatment also protected against WTD-induced glucose intolerance and insulin resistance; this protection was associated with smaller cells and fewer macrophages in epididymal white adipose tissue as well as enhanced in vivo insulin signaling. Our results indicate that ASO-mediated inhibition of 11β-HSD1 can protect against several WTD-induced metabolic abnormalities. These effects are, at least in part, mediated by increases in the oxidative capacity of brown adipose tissue.



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## 1. Introduction

Increased exposure to glucocorticoids (GCs) can lead to the development of obesity, insulin resistance, and the metabolic syndrome [1,2]. Glucocorticoid action in target tissues depends not only on circulating GC concentrations and cellular GC receptor expression, but also on tissue-specific intracellular GC metabolism regulated by $NADP^+$/NADPH-dependent oxidoreductases 11$\beta$-hydroxysteroid dehydrogenases 1 and 2 (11$\beta$-HSD1 and 2) [3-5]. 11$\beta$-Hydroxysteroid dehydrogenase 1 determines intracellular GC function by converting inactive cortisone and 11-dehydrocorticosterone to active cortisol and corticosterone in humans and rodents, respectively; 11$\beta$-HSD2 acts in the opposing direction [6].

Soon after the purification [7] and cloning [8] of 11$\beta$-HSD1, Seckl and colleagues [4,9-11] used genetic approaches to study loss or gain of function of 11$\beta$-HSD1; their studies highlighted the therapeutic potential of inhibiting this enzyme in adipose tissue and liver. In view of the previously published expression pattern of 11$\beta$-HSD1 [2] and the well-characterized tissue distribution of other intraperitoneally administered antisense oligonucleotides (ASOs) [12,13], we postulated that administration of an 11$\beta$-HSD1 ASO would result in relatively specific inhibition of the enzyme in both hepatic and adipose tissue. Indeed, we recently published results showing that ASO-mediated inhibition of hepatic 11$\beta$-HSD1 directly protected mice from a Western-type diet (WTD)–induced steatosis and dyslipidemia by reducing lipogenesis via posttranslational regulation of sterol-response element binding protein-1c and fatty acid synthase, increasing fatty acid oxidation, and causing the assembly and secretion of similar numbers of apolipoprotein B–containing lipoproteins containing less triglycerides per particle [14].

In that same recent report [14], we observed that ASO-mediated inhibition of 11$\beta$-HSD1 resulted in reduced food intake in C57BL/6J mice, requiring comparison of those mice with both ad libitum–fed and food-matched control ASO-treated groups. The present studies, focusing on the effects of ASO-mediated inhibition of 11$\beta$-HSD1 on adipose tissue metabolism, whole-body energy homeostasis, and insulin sensitivity, were also performed with both ad libitum–fed and pair-fed mice that received control ASO. We found that inhibition of 11$\beta$-HSD1, independent of food intake, protected C57BL/6J mice from a WTD-induced obesity and insulin resistance by increasing energy expenditure. Inhibition of 11$\beta$-HSD1 also improved glucose tolerance and insulin sensitivity by enhancing insulin signaling in adipose tissue and skeletal muscle. Enhanced adipose tissue insulin signaling was associated with smaller fat cells and less infiltration of fat tissue by macrophages.

## 2. Research design and methods

### 2.1. Mice, diet, and ASO

Male C57BL/6J wide-type mice from The Jackson Laboratory (Bar Harbor, ME) were housed in ventilated cages in a pathogen-free barrier facility with a 12-hour light/dark cycle (light cycle was 7:00 AM to 7:00 PM). Mice had free access to autoclaved water. The WTD diet was composed of, by calories, 42% fat (anhydrous milk fat), 43% carbohydrate (20% sucrose), and 15% protein and, by weight, 0.15% cholesterol (TD.88137, Harlan Laboratories, Madison, WI, USA). Mice began eating the WTD at 3 weeks of age. We studied 3 groups of mice: an ad libitum control ASO group, a food-matched control ASO group (FMC), and an 11$\beta$-HSD1 ASO group [14]. Both control and 11$\beta$-HSD1 ASO-treated mice were fed ad libitum for 12 weeks, after which these 2 groups of mice were started on treatment with either an in-house universal control ASO (ISIS 141923:5-CCTTCCCTGAAGGTTCCTCC-3) that does not have perfect complementarity to any known gene in public databases or an 11$\beta$-HSD1 ASO (ISIS 146039: 5-TGTTGCAAGAATTTCTCATG-3), respectively. Mice were housed individually throughout the studies; and daily food intake was calculated as the difference between the food provided and the food remaining each week, divided by 7 days. Body weights were recorded once a week. The FMC group started control ASO treatment 1 week later, as this group was delayed 1 week to match food consumption to the 11$\beta$-HSD1 ASO-treated group. For the FMC ASO treatment group, food was provided every afternoon at 5:00 PM. Each ASO was injected for 12 weeks at a dose of 50 mg/kg of body weight intraperitoneally twice a week. All studies were carried out between the 8th and 12th weeks of treatment. During those weeks, we measured energy expenditure, physical activity, fat and lean mass, glucose tolerance, and weight of the specific adipose tissue depots. All procedures were approved by the Institutional Animal Care and Use Committee of Columbia University College of Physicians and Surgeons.

### 2.2. Blood metabolites and hormones

Blood for various plasma metabolites and hormones was obtained after a 4-hour fast. Glucose levels were measured enzymatically (Sigma-Aldrich, St. Louis, MO, USA). Plasma insulin concentrations were measured by radioimmunoassay (Millipore, Billerica, MA, USA). Leptin levels were determined using an enzyme-linked immunosorbent assay (BioVendor, Candler, NC, USA).

### 2.3. Glucose and insulin tolerance tests

Glucose tolerance tests were conducted after an overnight fast. After a baseline blood glucose was measured with a glucometer, mice were injected intraperitoneally with 15% glucose in a 0.9% NaCl solution (1.5 g/kg body weight). Subsequent blood glucose concentrations were measured at 30, 60, 120, and 180 minutes. Insulin tolerance tests were performed after a 6-hour fast. Mice were injected intraperitoneally with human insulin (0.5 U/kg body weight) (Ely Lilly, Indianapolis, IN, USA). Glucose concentrations were determined at 0, 15, 30, 45, 60, 90, and 120 minutes with glucometer. Blood glucose levels at time points after either glucose or insulin injection are expressed as a percentage of the baseline fasting glucose concentration.

### 2.4. Body composition

Dual-energy x-ray absorptiometry (DEXA) (PIXImus instrument, Lunar PIXImus Corp, Fitchburg, WI, USA) was used to measure fat mass, lean mass, and percentage of body fat.

### 2.5. Tissue preparation, morphometry, and immunohistology

Epididymal (EPI) adipose tissues were obtained at sacrifice, fixed in 10% zinc-formalin fixative (Anatech Ltd, Battle Creek, MI, USA), and paraffin embedded for histological staining. Samples were sliced serially by microtome into 5 $\mu$m in thickness and stained with hematoxylin and eosin. Slides were analyzed using a Nikon Eclipse E400 microscope (Nikon Instruments, Melville, NY, USA), and images were captured with a SPOT Insight color camera (Diagnostic Instruments Inc, Sterling Heights, MI, USA). Cell size and numbers were determined using Image-Pro Plus Version 7.0 software (Media Cybernetics Inc, Bethesda, MD, USA). At least 8 images per mouse were randomly selected to measure the mean cell size.

For immunohistology, 5-$\mu$m sections were mounted on charged glass slides, deparaffinized in xylene, and stained for expression of F4/80 [15] with an anti-F4/80 monoclonal (Caltag Laboratories, Burlingame, CA, USA). At least 8 different high-power fields from sections of each mouse sample were analyzed. The total number of nuclei and the number of nuclei of F4/80-expressing cells were counted for each field.

### 2.6. Energy uptake and expenditure

Energy expenditure and home-cage activity were assessed by using a combined indirect calorimetry Module (TSE Systems Inc, Chesterfield, MO, USA). Energy expenditure, respiratory quotient (RQ), and locomotion were measured every 15 minutes for a total of 72 hours (including 24 hours of adaptation time). Home-cage locomotor activity was determined using a multidimensional infrared light beam system with beams scanning the bottom and top levels of the cage and activity being expressed as beam breaks. Daily food intake was determined over the 72 hours by this system as well.

### 2.7. Quantitative real-time polymerase chain reaction

Total RNA samples were used for cDNA synthesis with oligo-dT primers with a commercial kit from Invitrogen (Grand Island, NY, USA). Quantitative real-time polymerase chain reaction (PCR) was done using SYBR Green PCR Master Mix (Agilent Technologies, Inc, Santa Clara, CA, USA) in triplicate using the Mx3005p Multiplex Quantitative PCR system (Agilent Technologies). Expression of each target gene was quantified by transformation against a standard curve and normalized to $\beta$-actin or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. The primers used in the real-time PCR are shown in Table 1.

### 2.8 In vivo acute insulin stimulation of mice

After 12 weeks ASO treatment, the mice were anesthetized by intraperitoneal injection of ketamine/xylazine mixture; and a total of 5 IU of regular insulin was injected into the inferior vena cava. The liver was removed at 2 minutes, hind limb muscle at 3 minutes, and EPI adipose tissue at 4 minutes. Protein extracts were prepared by using T-per tissue protein extraction buffer (Thermo Scientific, Rockford, IL, USA) containing a complete, EDTA-free protein inhibitor cocktail tablet (Roche Diagnostics Corp, Indianapolis, IN, USA) and were analyzed by Western blot with anti-phospho-AKT (Ser473) antibody and total AKT antibody.

### 2.9. Statistics

Values are expressed as mean ± SE. The significance of the differences in mean values among 3 different treatment groups was evaluated by general linear model with treatment as the model factor and day as a covariate because the FMC group received their ASO treatment 1 week later, followed by post hoc analysis with Bonferroni test. $P < .05$ was considered to be statistically significant.

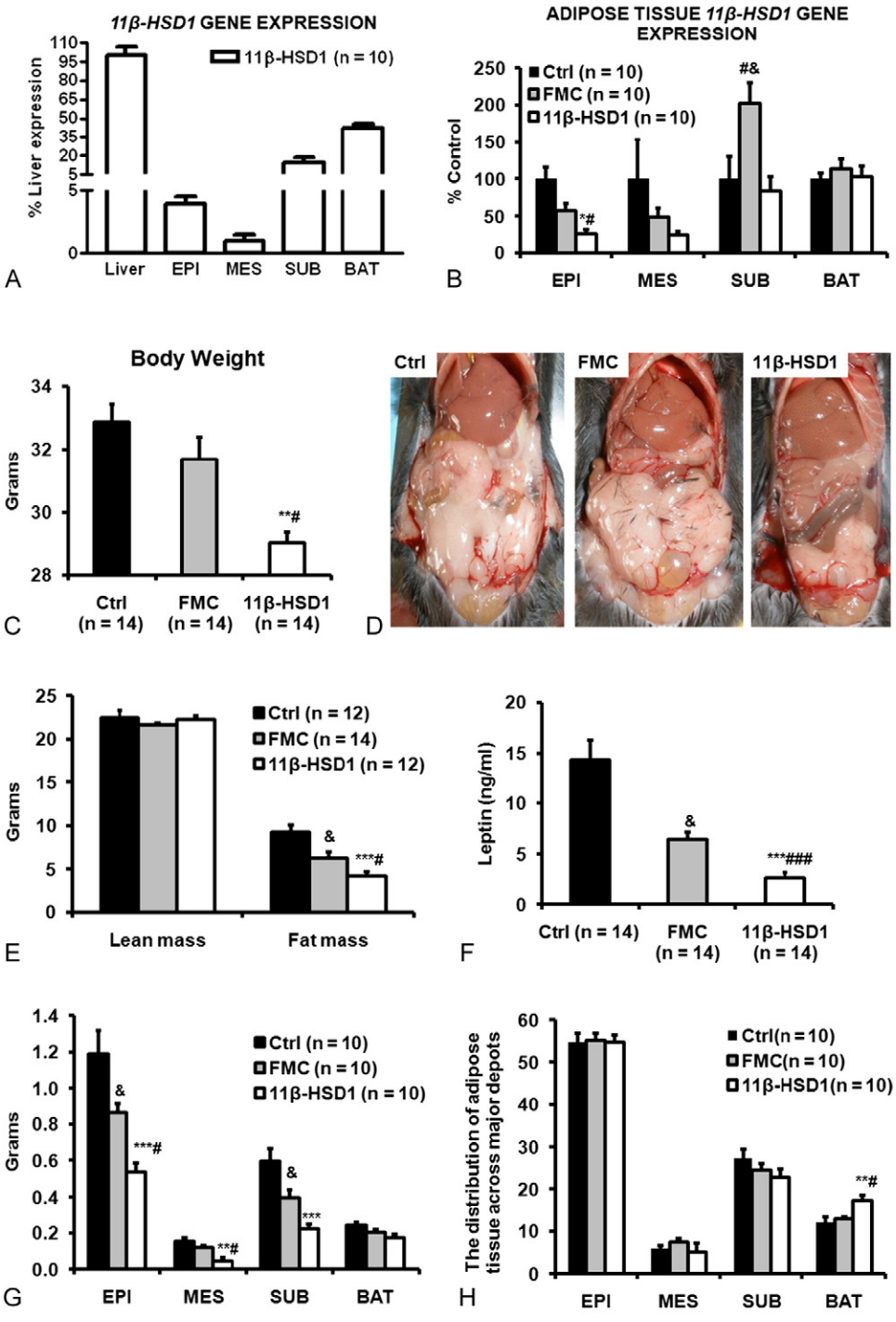
**Table 1 – Primer sequences of genes used for quantitative reverse transcriptase PCR**

| Gene | Forward (5′→3′) | Reverse (5′→3′) |
|---|---|---|
| 11$\beta$-HSD1 | AGTACACCTCGCTTTTGCGT | CTCTCTGTGTCCTTGGCCTC |
| $\beta$-Actin | GTATCCATGAAATAAGTGGTTACAGG | GCAGTACATAATTTACACAGAAGCA |
| GAPDH | GTCGGTGTGAACGGATTTG | AAGATGGTGATGGGCTTCC |
| SREBP1C | GGCACTAAGTGCCCTCAACCT | GCCACATAGATCTCTGCCAGTGT |
| FAS | CCTGGATAGCATTCCGAACCT | AGCACATCTGCAAGGCTACACA |
| ACC1 | GGAGGACCGCATTTATCGA | TGACCAGATCAGAGTGCCT |
| DGAT1 | GTGCACAAGTGGTGCATCAG | CAGTGGGATCTGAGCCATC |
| PPAR$\gamma$2 | AACTCTGGGAGATTCTCCTGTTGA | TGGTAATTTCTTGTGAAGTGCTCATA |
| CIDEa | CTCGGCTGTCTCAATGTCAA | CAGGAACTGTCCCGTCATCT |
| PGC1$\alpha$ | GAGTCTGAAAGGGCCAAACA | ACGGTGCATTCCTCAATTTC |
| Adiponectin | ATCCAACCTGCACAAGTTCC | GTTGCAAGCTCTCCTGTTCC |
| Perilipin | ACACTCTCCGGAACACCATC | CCCTCCCTTTGGTAGAGGAG |
| GLUT4 | CCTTGCCCTGTCAGGTATGT | ACTCTTGCCACACAGGCTCT |
| DIO2 | ATGCAGAAAGGCAGACTCGT | ATGTAACCAGCACCGGAAAG |
| UCP1 | TAAGCCGGCTGAGATCTTGT | GGATTGGCCTCTACGACTCA |
| PGC1$\beta$ | TTGTAGAGTGCCAGGTGCTG | GATGAGGGAAGGGACTCCTC |
| CIG30 | CCTCCTTCCCTTCATCCTTC | GAGACCCTGCTTCCCTATCC |
| OTOP1 | ATGGGGGTAGCACACTCTTG | GCCTGCAATTCTAGCTCCAG |
| COX8b | TGCTGCGGAGCTCTTTTTAT | TGGGGATCTCAGCCATAGTC |
| L-PBE | CTACCTGAGGAGGCTGGTTG | CCATACATGGCAAAATGCAG |

## 3. Results

### 3.1. *11ß-HSD1 ASO treatment decreased total body fat mass by reducing white adipose tissue while sparing brown adipose tissue*

As noted earlier, *11β-HSD1* is expressed in several tissues and organs. We determined the relative expression of *11β-HSD1* in liver and the major adipose tissue depots before ASO treatments. Relative to the liver, expression of 11β-HSD1 was very low in EPI and mesenteric (MES) white adipose tissue (WAT), and moderate in inguinal subcutaneous (SUB) WAT and scapular brown adipose tissue (BAT) (Fig. 1A). When given intraperitoneally, ASOs distribute mainly to the liver but also to adipose tissue [12,13]. 11β-Hydroxysteroid dehydrogenase 1 ASO treatment for 12 weeks reduced the levels of messenger RNA (mRNA) of the enzyme significantly in EPI WAT and tended to reduce the mRNA level in MES WAT compared with both control ASO and FMC ASO mice (Fig. 1B). There was no effect of 11β-HSD1 ASO on gene expression of the enzyme in SUB



WAT compared with the control ASO group, although interpretation of this result was complicated by the significant increase in SUB WAT *11β-HSD1* mRNA in the FMC ASO group. There were no effects of ASO treatment on *11β-HSD1* mRNA in BAT (Fig. 1B). The mRNA changes in EPI and SUB depots were paralleled by changes in 11β-HSD1 protein levels (Supplemental Figure 1); there were inadequate quantities of MES and BAT to perform Western blot analyses on those tissues.

Reductions in 11β-HSD1 were associated with reduced food intake, which was observed immediately after initiation of treatment, requiring an FMC control group [14]. Compared with the ad libitum control and FMC control ASO groups after 12 weeks of treatment, total body weight was significantly lower, by 3.9 and 2.7 g, respectively, in 11β-HSD1 ASO-treated mice (Fig. 1C). Representative photographs clearly showed less EPI fat mass in 11β-HSD1 ASO-treated mice (Fig. 1D). Lean mass was not significantly altered in either the FMC or the 11β-HSD1 ASO groups (Fig. 1E); and weight loss was accounted for by loss of fat mass, which fell approximately 30% and 50% in the FMC and 11β-HSD1 ASO groups, respectively. Plasma leptin levels, used as a marker of fat mass, were significantly reduced in 11β-HSD1 ASO-treated mice compared with both control groups (Fig. 1F). 11β-Hydroxysteroid dehydrogenase 1 ASO treatment was associated with reductions in every WAT depot compared with the control ASO group, and in the EPI and MES depots compared with the FMC group (Fig. 1G). There was a nonsignificant trend toward reduced BAT mass. When changes in adipose tissue depots were expressed as relative changes within each group, there was no evidence of a redistribution of WAT; however, BAT, as a percentage of total fat, increased modestly but significantly in the 11β-HSD1 ASO-treated mice (Fig. 1H). In terms of absolute adipose mass, the largest depot, EPI WAT, had the largest reduction in mass.

### 3.2. 11ß-HSD1 ASO treatment reduced EPI adipose cell size and macrophage infiltration

When we examined EPI WAT, we found both smaller cells (Fig. 2A) and fewer macrophages (Fig. 2B) in 11β-HSD1 ASO-treated mice. Of note, the more modest reduction in adipose tissue mass in the FMC ASO group was not associated with changes in adipocyte size or the number of macrophages.

### 3.3. Effects of 11ß-HSD1 ASO treatment on gene expression varied by adipose depot

Expression of lipogenic genes, lipid droplet genes, and *GLUT4* were not altered in EPI WAT by 11β-HSD1 ASO treatment (Fig. 3A). In MES WAT, there was a consistent trend toward decreased expression of most of these genes; and expression of *PPARγ2* and *GLUT4* was significantly reduced in 11β-HSD1 ASO-treated mice in this fat depot (Fig. 3B). In contrast, in SUB WAT (Fig. 3C), both FMC ASO and 11β-HSD1 ASO groups had marked but similar reductions in this panel of genes. In BAT, expression of thermogenesis-related genes (*DIO2* [16] and *PGC1β* [17]) was stimulated by 11β-HSD1 ASO treatment (Fig. 3D). Genes involved in lipid synthesis (*DGAT1*, *CIG30*) [18] and both mitochondrial- and peroxisomal-based oxidation (*OTOP1*, *COX8B*, and *L-PBE*) [19,20] were also elevated in BAT of 11β-HSD1 ASO-treated mice. *UCP1* expression was not increased in BAT of 11β-HSD1 ASO-treated mice.

### 3.4. 11ß-HSD1 ASO treatment decreased food intake, increased energy expenditure, and improved whole-body insulin sensitivity

The observation that not only was BAT spared in the 11β-HSD1 ASO-treated group, but expression of oxidation-related genes was increased, led to a detailed examination of energy metabolism in the 3 groups. As reported previously [14], 11β-HSD1 ASO-treated mice consume less food; this was confirmed by measuring average daily food intake and cumulative food intake during the 3-day study in metabolic cages (Fig. 4A, B). The RQ did not differ between control ASO- and 11β-HSD1 ASO-treated groups and was consistent with the composition of the WTD. In contrast, although the FMC ASO mice had similar RQ values during the daylight hours, RQ increased rapidly when food was provided to the cages at 5:00 PM; the changes observed suggested that the FMC ASO mice ate rapidly and used carbohydrates for energy while attempting to store dietary fat (Fig. 4C). Knockdown of 11β-HSD1 was associated with greater energy expenditure, in watts per kilogram lean body mass, compared with FMC ASO (Fig. 4D) and with increased total activity compared with both control ASO and FMC ASO (Fig. 4E). The FMC ASO group had reduced energy expenditure and activity compared with both control ASO and 11β-HSD1 ASO groups during both light and dark periods.

**Fig. 1 – Effects of 11ß-HSD1 ASO treatment on adipose tissue. A, *11ß-HSD1* mRNA distribution was determined using quantitative PCR (QPCR) in liver, EPI, MES, inguinal SUB WAT, and scapular BAT and normalized by reference to both *ß-ACTIN* and *GAPDH* mRNA. *11ß-HSD1* mRNA level in the ad libitum–fed control ASO-treated liver was set as 100%. B, Effect of 11ß-HSD1 ASO treatment for 12 weeks on the distribution of *11ß-HSD1* mRNA was determined using QPCR after ASO treatment in different adipose depots, including EPI, MES, SUB WAT, and BAT, normalized by *ß-ACTIN* mRNA. *11ß-HSD1* mRNA level in the ad libitum–fed control ASO-treated tissues was set as 100%. C, Body weights obtained just before DEXA; mice treated for 12 weeks with 11ß-HSD1 ASO gained less weight (n = 14). D, Photographs of representative mice that had been treated for 12 weeks with 11ß-HSD1 ASO demonstrating less EPI fat. E, Body composition of the mice was determined by DEXA after 12 weeks of ASO treatment. F, Plasma leptin after a 4-hour fast in 12-week ASO-treated mice (n = 14). G, Each major fat depot (EPI, MES, SUB WAT, and BAT) was carefully excised and weighed. H, The distribution of adipose tissue across major depots is presented as a percentage of total fat excised. Data are presented as means ± SEM (n = 10-14). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, 11ß-HSD1 vs control; $^{\#}P < .05$, $^{\#\#\#}P < .001$, 11ß-HSD1 vs FMC; $^{\&}P < .05$, FMC vs control.**

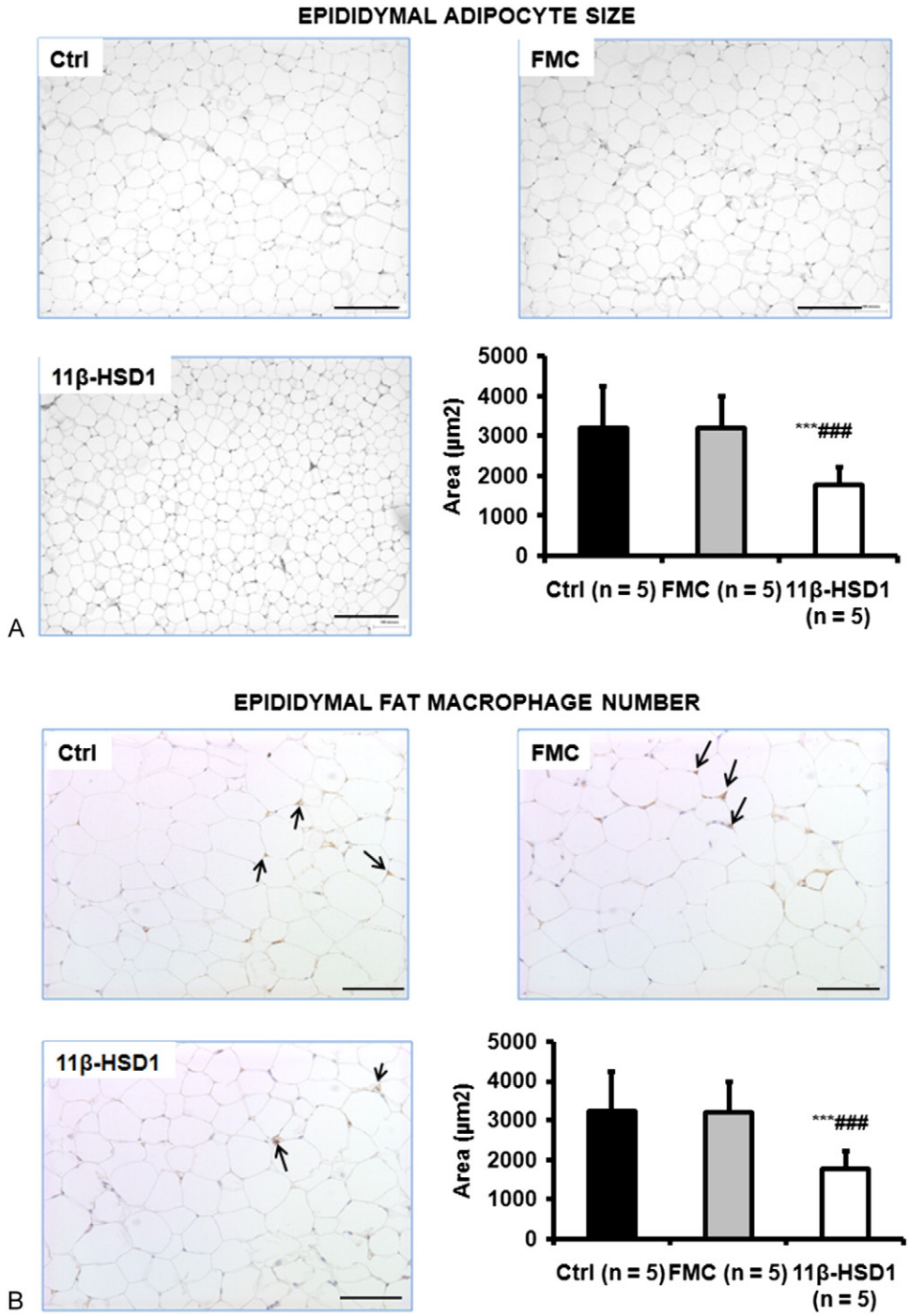



**Fig. 2 – 11β-Hydroxysteroid dehydrogenase 1 ASO treatment reduced EPI adipose cell size and macrophage infiltration. A, Epididymal adipose tissue from mice treated with ASO for 12 weeks was stained with hematoxylin and eosin. A total of 1832, 2237, and 6978 adipose cells were analyzed in control ASO-, FMC ASO-, and 11β-HSD1 ASO-treated mice, respectively. Calibration mark is 200 μm. B, Epididymal adipose tissue samples from each group of mice were stained for the presence of the macrophage-specific antigen F4/80. Macrophage nuclei are stained brown. Arrows indicate representative examples of macrophages. The total nuclei number counted was 2839, 3369, and 4344 for ASO-, FMC ASO-, and 11β-HSD1 ASO-treated mice, respectively. Calibration mark is 100 μm. Data are presented as means ± SEM (n = 5). $^{***}$P < .001, 11β-HSD1 vs control; $^{###}$P < .001, 11β-HSD1 vs FMC.**

11β-Hydroxysteroid dehydrogenase 1 ASO treatment was associated with lower plasma glucose and insulin levels compared with control mice but not with FMC ASO mice (Fig. 5A, B). 11β-Hydroxysteroid dehydrogenase 1 ASO treatment did, however, protect the mice from WTD-associated glucose intolerance (Fig. 5C, D) and insulin

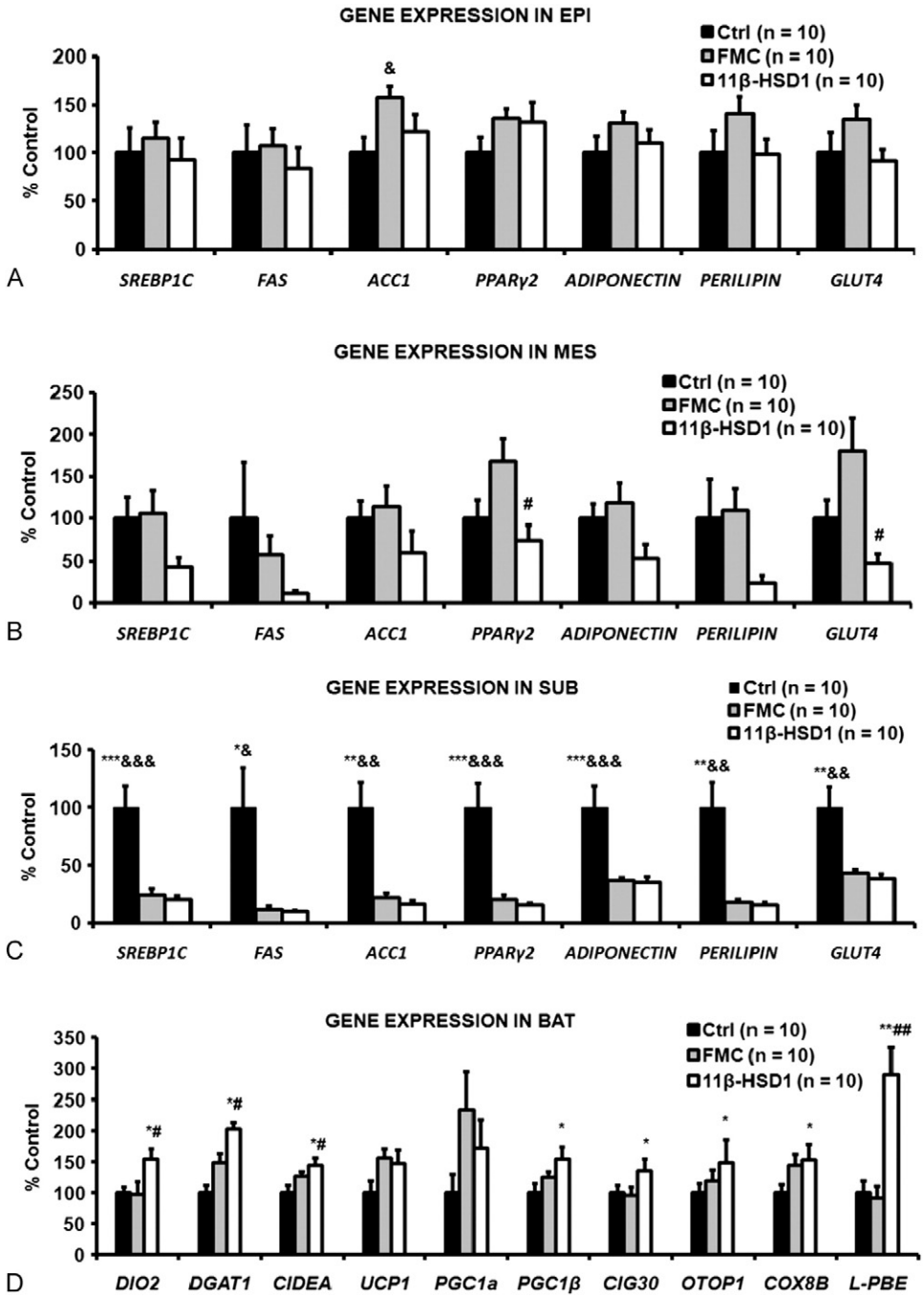



**Fig. 3 – 11β-Hydroxysteroid dehydrogenase 1 ASO treatment has minimal effects on WAT gene expression, but increased expression of thermogenic genes in BAT. Expression of genes in EPI (A), MES (B), SUB WAT (C), and BAT (D) was determined using QPCR and normalized with *β-ACTIN*. Each gene mRNA level in the ad libitum–fed control ASO-treated tissues was set as 100%. Data are presented as means ± SEM (n = 10). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, 11β-HSD1 vs control; $^{\#}P < .05$, $^{\#\#}P < .01$, 11β-HSD1 vs FMC; $^{\&}P < .05$, $^{\&\&}P < .01$, $^{\&\&\&}P < .001$ FMC vs control.**

resistance (Fig. 5E, F). Interestingly, the FMC ASO group had the greatest glucose intolerance and insulin resistance of all 3 groups. We next examined the acute effects on insulin administration on insulin signaling in hind limb muscle, adipose tissue, and liver (Fig. 5G). Levels of phospho-AKT were significantly increased by insulin administration in 11β-HSD1 ASO-treated mice in hind limb muscle and EPI WAT compared with the other groups. There were no differences in the acute effect of insulin on phospho-AKT levels in livers from the 3 groups.

## 4. Discussion

Genetic manipulations that have either ablated 11β-HSD1 in the whole body [4,9,10] or reduced its effects in adipose tissue

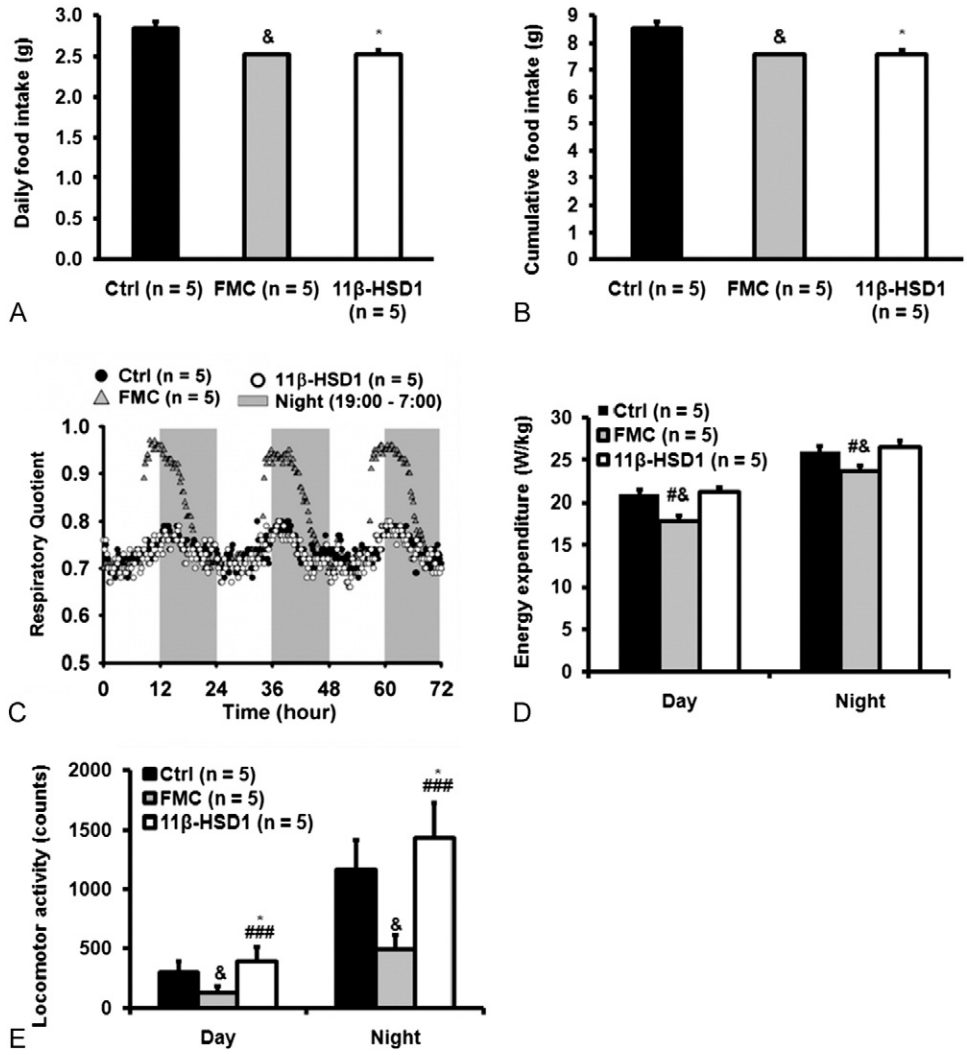



**Fig. 4 – 11*b*-Hydroxysteroid dehydrogenase 1 ASO treatment decreased food intake and increased energy expenditure. Daily food intake (A), cumulative food intake (B), RQ (FMC mice received their daily allotment of food at 5:00 PM) (C), energy expenditure (D), and activity levels (E) were assessed by using a combined indirect calorimetry module. Body weights (mean ± SEM) of control, FMC, and 11β-HSD1 mice at the start of the metabolic cage studies were 32.9 ± 0.6, 31.7 ± 1.0, 29.0 ± 0.4, respectively. $O_2$ consumption and $CO_2$ production were measured every 15 minutes for a total of 72 hours (beginning after a 24-hour adaptation period) to determine the RQ and energy expenditure (watts per kilogram lean body mass). Food intake was measured over the 72-hour period. Locomotor activity was determined using a multidimensional infrared light beam system with beams scanning the bottom and top levels of the cage and activity being expressed as beam breaks. Data are presented as means ± SEM (n = 5). $^{*}P < .05$, 11β-HSD1 vs control; $^{\#}P < .05$, 11β-HSD1 vs FMC, $^{\#\#\#}P < .001$, 11β-HSD1 vs FMC; $^{\&}P < .05$, FMC vs control.**

by overexpression of 11$\beta$-HSD2 in that tissue [21] have been associated with increased insulin sensitivity and improved energy homeostasis. On the other hand, mice overexpressing 11$\beta$-HSD1 in adipose tissue [3] had increased visceral obesity, significant insulin resistance, and abnormalities characteristic of the metabolic syndrome [3]. It is not surprising, therefore, that 11$\beta$-HSD1 was targeted for development of inhibitors as possible therapeutic agents for obesity, type 2 diabetes mellitus, and metabolic syndrome [22-27]. Interpretation of the effects of either genetic or pharmacologic manipulations of 11$\beta$-HSD1 has, however, been confounded by variable disturbances in food intake and body weight [3,10,21]. Some [24,25], but not all [23,26], of the pharmacologic inhibitors of 11$\beta$-HSD1 cause decreased food intake in preclinical models; and this issue has not been addressed in most of those studies. The mechanism underlying the changes in food intake is unclear. Overexpression of 11$\beta$-HSD1 in adipose tissue is associated with increased food intake [3], whereas hepatic overexpression has no effect on food intake [11]. Reduced production of corticosterone in adipose tissue (resulting from overexpression of 11$\beta$-HSD2) is associated with decreased food intake [21], as is hepatic suppression of 11$\beta$-HSD1 [14]. However, total body knockout of 11$\beta$-HSD1 caused hyperphagia [4], suggesting a central nervous system–based mechanism [28]. Of note, reduced food intake in our mice occurred despite reduced levels of leptin.

In the present study, to identify effects of 11β-HSD1 ASO treatment that are independent of food intake, we used a food-matched ASO control to allow characterization of direct effects of 11β-HSD1. We reported previously that the FMC ASO control mice had increased levels of plasma corticosterone, reduced levels of hepatic cholesterol, and increased expression of genes associated with hepatic fatty acid oxidation [14]. In the present experiments, the FMC ASO mice had increased expression of 11β-HSD1 in SUB adipose tissue and unique changes in energy homeostasis compared with the ad libitum control group. Interpretation of our results, therefore, takes into account outcomes in both control groups. It is important to note that corticosterone levels were not different from controls in 11β-HSD1 ASO-treated mice.

Antisense oligonucleotide inhibition of 11β-HSD1 was associated with significant reductions in total body fat independent of reduced food consumption. Fat mass was reduced significantly in all WAT depots, despite reductions in *11β-HSD1* gene expression only in EPI and MES fat. Brown adipose tissue tended to be lower, but was not significantly reduced, in either FMC controls or 11β-HSD1 ASO-treated mice. Of note, the distribution of fat across depots was not altered in WAT; and there was relative sparing of BAT in the 11β-HSD1 ASO-treated group. *11β-HSD1* gene expression was also reduced in lung, kidney, and heart treated with the ASO (Supplemental Figure 2). Studies of the effects of 11β-HSD1 knockdown have not been reported for those organs and should be studied in the future.

*11β-HSD1* knockout mice on the C57BL/6J background had been observed to gain significantly less weight on a high-fat diet despite increased caloric intake, and this was associated with a particular reduction in MES fat [9]. This finding was confirmed in a very recent report from Wamil et al [29] from the same group of investigators. On the other hand, in a model where 11β-HSD2 was overexpressed in adipose tissue (resulting in a "virtual" deficiency of 11β-HSD1), reduced food intake on a high-fat diet was associated with weight loss that was uniform across several WAT depots, although BAT was not affected in that model [21]. Adipose tissue overexpression of 11β-HSD1 resulted in increases in all depots but mostly in the MES depot. [3] It is of interest that although we saw reductions in the mass of EPI, MES, and SUB WAT, *11β-HSD1* expression was only significantly reduced in EPI WAT.

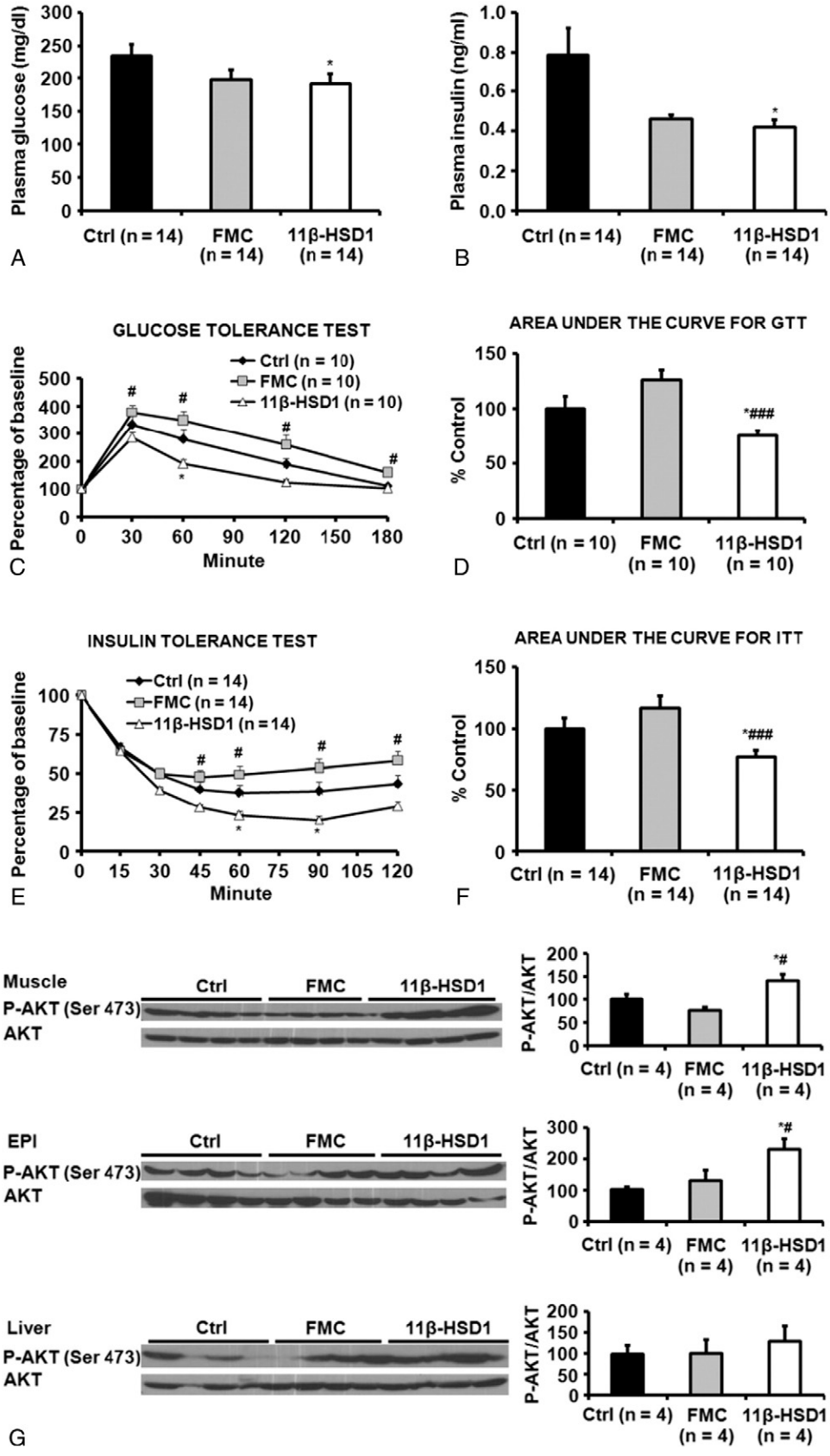
We also observed that EPI fat cells were smaller and there were fewer macrophages present in the 11β-HSD1 ASO-treated mice. Adipocyte size has not been reported in 11β-HSD1 knockout mice, whereas adipose overexpression of 11β-HSD1 was associated with increased size of MES and SUB fat cells [3]. In humans, adipocyte size in different fat depots was correlated with 11β-HSD1 mRNA and activity [30]. Adipocyte size is inversely related to insulin sensitivity, and smaller fat cells attract fewer macrophages [15]. Furthermore, adipose tissue–associated macrophages play crucial roles in linking obesity to insulin resistance and the metabolic syndrome [15,31-33]. Reduced macrophages in EPI WAT of 11β-HSD1 ASO-treated mice suggest that their improved glucose tolerance and insulin sensitivity may have derived, in part, from a reduction in WAT-associated inflammation.

Changes in the expression of several WAT genes associated with lipid synthesis, fat storage, and insulin sensitivity were variable and depot specific. There were reductions in all of these genes in SUB WAT; but this occurred in both the FMC ASO control and 11β-HSD1 ASO mice, indicative of a response to reduced caloric intake rather than any direct effects of 11β-HSD1 ASO treatment. PPARγ and adiponectin mRNA levels were elevated in SUB WAT of mice with adipose tissue overexpression of *11β-HSD2* and reduced 11β-HSD1 activity [21]. We are not sure why those results were opposite ours for those 2 genes; those mice were, however, on the FVB background, whereas ours were C57BL/6J. In the present study, examination of MES WAT revealed trends to reductions in most of the lipid synthesis, fat storage, and insulin-responsive genes only in the mice receiving 11β-HSD1 ASO; FMC ASO control mice did not show any changes in gene expression. In the whole-body knockout of 11β-HSD1 on an MF-1 background and a chow diet, mRNA levels of adiponectin, PPARγ, and UCP-2 were elevated in EPI WAT, whereas only the latter 2 were elevated in MES WAT [10]; strain difference may have also played a role in the differences seen for adiponectin and PPARγ between those studies and the present one. Wamil et al [29] recently reported more detailed gene expression data in the whole-body *11β-HSD1* knockout mouse on the C57BL/6J background. These investigators confirmed their prior findings for *PPARγ* and *Glut4*, whereas we found no difference in the expression of either gene compared with ASO controls in SUB WAT, and lower expression of both genes compared with FMC controls in MES WAT. Unfortunately, marked differences in the experimental protocols preclude any comparison of the 2 studies. In addition, of the genes presented in the recent report [29], only *PPARγ* and *Glut4* are among the panel that we have interrogated in the present investigation.

Reductions of body weight and WAT mass, independent of food intake, led us to study energy metabolism in 11β-HSD1 ASO-treated mice. 11β-Hydroxysteroid dehydrogenase 1 inhibition resulted in a significant increase in absolute total energy expenditure compared with both control groups (data not shown). However, because there were significant differences in fat mass among the 3 groups, we expressed total energy expenditure per kilogram lean body weight; it was increased in 11β-HSD1 ASO-treated vs FMC ASO control-treated mice. In addition, total locomotor activity was higher in 11β-HSD1 ASO-treated mice compared with both control groups. These results suggest that the loss of adipose tissue in 11β-HSD1 ASO-treated mice resulted from the maintenance of "control" levels of energy expenditure despite decreased food intake, with increased locomotion contributing as well. Of note, the FMC group had levels that were lower than both control ASO mice and 11β-HSD1 ASO-treated mice. Morton et al [10] observed increased core temperature in 11β-HSD1 knockout mice on a C57BL/6J background on both chow and high-fat diets, and increased oxygen consumption was demonstrated in mice with 11β-HSD2 overexpression in adipose tissue [21]. Furthermore, chemical inhibition of 11β-HSD1 activity by carbenoxolone was associated with increased energy consumption [34].

Our findings of increased energy expenditure are most likely linked to increased expression of several genes involved in energy expenditure in BAT [19,35]. Thus, although we did

not have clear evidence of increased uncoupling (there were no increases in expression of UCP-1 or PGC1α), we did see genetic evidence for increased mitochondrial and peroxisomal function. There have been no prior reports of the effects of altered 11β-HSD1 activity on BAT gene expression. Overall, our data are consistent with prior studies demonstrating that GCs interfere with the expression of UCP-1 in BAT [36,37] and thermogenesis [38].



Targeted deletion of *11β-HSD1* have significant effects on glucose metabolism, including failure of starvation to induce G6Pase and PEPCK [4] and protection from high-fat diet–induced glucose intolerance and insulin resistance [10]. Similar results were achieved by overexpressing 11β-HSD2 in adipose tissue [21]. In contrast, overexpression of 11β-HSD1 in adipose tissue caused significant glucose intolerance and insulin resistance [3]. As noted earlier, however, alterations in food intake and weight confounded some of those findings. Indeed, we now demonstrate that reductions in fasting glucose and insulin levels in 11β-HSD1 ASO-treated mice were secondary to reduced food intake, results that are concordant with those of Lemke et al [39] where suppression of the hepatic GC receptor affected neither weight nor fasting levels of plasma glucose or insulin. Importantly, however, we demonstrated that 11β-HSD1 ASO treatment does improve glucose tolerance and insulin sensitivity independent of body weight or food consumption.

Glucocorticoids are involved in the transcriptional control of several genes involved in the regulation of hepatic glucose production, including *PGC1α* [40], and are potent functional antagonists of insulin action. Indeed, inhibition of hepatic GC receptors resulted in lower expression of *PEPCK* and *PGC1α* [39]. We also observed reductions in hepatic *PGC1α* and *PEPCK* mRNA in 11β-HSD1 ASO-treated mice [14] but did not, in the present studies, demonstrate significant increases in insulin signaling in the liver of those animals. Overall, our results indicate that inhibition of hepatic 11β-HSD1 activity, most likely by reducing gluconeogenesis, has modest effects on glucose tolerance and insulin sensitivity, a conclusion consistent with the mild effects of hepatic 11β-HSD1 overexpression on glucose tolerance and insulin sensitivity [11].

By contrast, inhibition of 11β-HSD1 in adipose tissue has a much greater impact on glucose metabolism. We demonstrated significantly better insulin action in adipose tissue in the 11β-HSD1 ASO-treated mice, and this is concordant with findings of increased glucose uptake by isolated adipocytes in the studies by Morton et al [10]. Wamil et al [29] recently reported increased insulin signaling in SUB WAT but not in MES WAT isolated from whole-body knockout mice on a high-fat diet for 4 weeks; it is difficult to compare those results to ours because we examined only EPI WAT in much older mice on a WTD for much longer.

The increased insulin signaling likely resulted from reduced adipocyte size [41] and/or fewer macrophages [15] in WAT of 11β-HSD1 ASO-treated mice. Smaller fat cell size and lower macrophage infiltration suggested that 11β-HSD1 ASO treatment might be associated with a more favorable cytokine/adipokine profile. Wamil et al [29] recently reported few macrophages in both SUB and MES WAT depots in the whole-body 11β-HSD1 knockout mice. However, we found that expression of inflammatory genes, including *TNFa*, *Il-1a*, and *Il-1b*, in EPI fat was similar in 11β-HSD1 and control ASO-treated mice, as were plasma levels of TNFα and IL-6 (data not shown). In general, these findings are in accord with those of Wamil et al [29]. We also found significantly greater insulin signaling in hind limb muscle of 11β-HSD1 ASO-treated mice, results consistent with demonstration that steroid-induced insulin resistance in cultured myotubes and explants of skeletal muscle was reversed by a small molecule inhibitor of 11β-HSD1 [42]. Our finding is intriguing because of the relatively low expression of 11β-HSD1 in skeletal muscle and the fact that ASO does not distribute significantly to that tissue. It is possible that lower ambient, particularly postfeeding, insulin levels resulted in improved insulin signaling in skeletal muscle.

The strength of our study was the use of a specific 11β-HSD1 ASO to examine effects on reduced intracellular cortisol production on aspects of adipose tissue metabolism in mice fed a high-fat, high-sucrose diet. The weaknesses derive from the action of 11β-HSD1 ASO, administered intraperitoneally, on several tissues and organs in addition to adipose tissue, particularly the liver; this was addressed in part by examining, in detail, the effects of the ASO on hepatic lipid metabolism [14]. Decreased food intake resulting from 11β-HSD1 ASO treatment was another weakness; we used pair-fed controls as well as ad libitum–fed controls to address that problem.

In conclusion, this report demonstrates that ASO-mediated inhibition of 11β-HSD1 in visceral adipose tissue

**Fig. 5 – 11ß-Hydroxysteroid dehydrogenase 1 ASO treatment improved total body glucose tolerance, insulin sensitivity, and insulin signaling in adipose tissue and hind limb muscle. Plasma glucose (A) and serum insulin (B) levels after a 4-hour fast in 8- to 12-week ASO-treated mice. (C) Mice (n = 10 per group) were injected intraperitoneally with a solution of 15% glucose (1.5 g/kg body weight) after an overnight fast, and blood samples were obtained for measurement of glucose before and for 3 hours afterward. Plasma glucose levels are expressed as percentages of initial glucose. The baseline glucose levels were 127.6 ± 8.5, 84.4 ± 4.0, and 108.6 ± 7.7 mg/dL and the 60-minute glucose levels were 346.3 ± 40.7, 291.4 ± 29.4, and 206.7 ± 22.6 mg/dL in control ASO-, FMC ASO-, and 11ß-HSD1 ASO-treated mice, respectively. D, Area under the curve above baseline for glucose tolerance test was obtained as described in "Research Design and Methods" and normalized to control mice. E, Mice (n = 14 per group) were fasted for 6 hours before the intraperitoneal injection of insulin (0.5 IU/kg body weight). Plasma glucose levels at time points before and for 2 hours after insulin injection were measured. Plasma glucose levels are expressed as percentages of initial glucose. The baseline glucose levels were 181.1 ± 13.8, 156.3 ± 8.0, and 159.3 ± 11.8 mg/dL and the 60-minute glucose levels were 64.6 ± 8.7, 77.1 ± 8.6, and 35.3 ± 4.1 mg/dL in control ASO-, FMC ASO-, and 11ß-HSD1 ASO-treated mice, respectively. F, Area under the curve from baseline for insulin tolerance test was determined as described in "Research Design and Methods" and normalized to control mice. G, Acute insulin stimulation in muscle, EPI adipose tissue, and liver. After 12 weeks ASO treatment, mice were anesthetized by intraperitoneal injection of ketamine/xylazine mixture and injected with 5 IU of regular insulin via the inferior vena cava. The liver was removed 2 minutes, hind limb muscle 3 minutes, and EPI adipose tissue 4 minutes after injection. Protein extracts were analyzed by immunoblotting with an anti-phospho-AKT (Ser473) specific antibody (upper panel) or antibody to total AKT (lower panel). Data are presented as means ± SEM (n = 4-14). $^{*}P < .05$, 1ß-HSD1 vs control; $^{\#}P < .05$, $^{\#\#\#}P < .001$, 11ß-HSD1 vs FMC.**

improves WTD-induced obesity, defective energy homeostasis, glucose tolerance, and insulin resistance, independent of reduced food intake and weight loss, in the WTD model of obesity in C57BL/6J mice. Inhibition of 11β-HSD1 in adipose tissue directly reduced fat mass and the number of macrophages in adipose tissue while activating BAT. The latter effect was associated with increased energy expenditure and activity, leading to weight loss independent of changes in food consumption. Reductions in adipose tissue 11β-HSD1 also mediated improvements in glucose tolerance and insulin signaling/sensitivity. Overall, our findings indicate that inactivation of GCs by 11β-HSD1 in adipose tissue should have beneficial effects in humans even if food intake and weight are unaffected. The potential for translation of our findings, and the findings of other preclinical investigations, to humans is supported by several therapeutic trials that have been reported as well as recent reports of a correlation between 11β-HSD1 expression in visceral adipose tissue and plasma insulin levels [43] as well as the association of single-nucleotide polymorphism for the 11β-HSD1 gene with increased expression of the mRNA in adipose tissue as well as increased production of cortisol in vivo [44].

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## Conflict of Interest

GL, AH-O, and HNG have no disclosures. RMC and MJG are employees of ISIS Pharmaceuticals.

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