Carbenoxolone treatment attenuates symptoms of metabolic syndrome and atherogenesis in obese, hyperlipidemic mice

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The obesity epidemic is a worldwide health problem (5). Of major concern to health care professionals is the accompanying rise in other metabolic syndrome risk factors associated with severe obesity: dyslipidemia, hypertension, and impaired glucose homeostasis, which further predispose patients to cardiovascular disease and diabetes (6). Cushing’s syndrome, characterized by excess circulating endogenously or exogenously derived glucocorticoids, results in metabolic syndrome symptoms, such as central obesity, increased plasma triglycerides (TGs), hypertension, and elevated fasting glucose (38, 55). Glucocorticoids impact body fat distribution and stimulate adipocyte differentiation and lipolysis (19, 21, 48). In hepatocytes, glucocorticoids modulate gluconeogenic and lipogenic processes (14, 53). Because obesity is not frequently associated with excess circulating glucocorticoids, there is speculation that enhanced actions of glucocorticoids in key metabolic tissues, such as adipose tissue and liver, may play a causative role in the altered physiology observed in patients with metabolic syndrome (43).

11β-Hydroxysteroid dehydrogenase (11β-HSD) type 1 (11β-HSD1) is a bidirectional NADP+/NADPH-dependent dehydrogenase/reductase that is highly expressed in key metabolic tissues such as liver and adipose tissue, where it acts to metabolize glucocorticoids and is highly expressed in tissues such as kidney, where it confers protection against excess binding of glucocorticoids to the mineralocorticoid receptor (32, 49). Thus, 11β-HSD1 is the only enzyme capable of regenerating corticosterone from 11-dehydrocorticosterone (11-DHC) in rodents or cortisol from cortisone in humans. As these 11-keto metabolites circulate at high concentrations (24, 57), 11β-HSD1 activity may serve to potentiate local concentrations of active glucocorticoids in a tissue-specific manner, preventing the need for the body to produce excess circulating receptor-competent glucocorticoid isofoms, which may have deleterious effects in other tissues.

Dysregulated 11β-HSD1 activity can profoundly affect metabolic phenotype in mice. Adipose tissue-specific 11β-HSD1 overexpression results in hypertension, increased serum free fatty acid (FFA) and TG levels, insulin resistance, central obesity, and hypertension (30, 31). Liver-specific 11β-HSD1 amplification results in hypertension, a proatherogenic lipoprotein profile, and non-obesity-associated hyperinsulinemia (42). Conversely, global 11β-HSD1 deficiency in mice fed a high-fat diet is associated with decreased weight gain and improved serum HDL lipoprotein profiles, despite increased adrenocortex-derived circulating glucocorticoids (24, 34, 35). Therefore, it appears that 11β-HSD1 activity may potentially impact more than one risk factor for metabolic syndrome. Inasmuch as 11β-HSD1 expression has been reported to be increased in the adipose tissue of obese patients (10, 22, 46), this enzyme may prove to be a promising drug target for patients with the metabolic syndrome.

No study has assessed metabolic outcomes of 11β-HSD1 inhibition in the context of combined obesity, atherogenesis, and hyperlipidemia, nor have the effects of 11β-HSD1 inhibition on lipoprotein metabolism in such a context been
characterized, which would be key to determining effects of such drugs on dyslipidemia in patients with metabolic syndrome. In this study, we administered subcutaneous injections of the nonselective 11β-HSD inhibitor carbenoxolone (CBX) to hyperlipidemic, Western-type diet (WD)-fed moderately obese LDL receptor (LDLR)-deficient (LDLR−/−) and severely obese mice derived from heterozygous agouti (A/a) and homozygous LDLR−/− breeding pairs (A/a; LDLR−/− mice) (11, 12) and examined outcomes on various metabolic syndrome risk factors. We found that systemic inhibition of 11β-HSD1 led to dramatic improvements in body composition, basal metabolic rate, insulin resistance, lipoprotein metabolism, hepatic steatosis, and atherosclerosis in the more severely obese and insulin-resistant mice, suggesting that targeting this enzyme may become a key therapy for patients with multiple metabolic syndrome risk factors.

MATERIALS AND METHODS

Mice. All mouse procedures were approved by the Vanderbilt University Institutional Animal Care and Use Committee. Mice were allowed food and water ad libitum and were kept under 12:12-h light-dark cycles. Mice were derived from heterozygous agouti (Aya/a) and homozygous LDLR−/− mice (Aya/a; LDLR−/− mice) (11, 12) and non-agouti LDLR−/− littermates.

Experimental design. At 2 mo of age, LDLR−/− and Aya/a; LDLR−/− mice were placed on a 42% fat, 31% sucrose, 15% protein, 0.2% cholesterol Western-type diet (WD; TD.88137, Harlan Teklad) for a total of 10 wk to accelerate atherogenesis and obesity. After they were fed the WD diet for 6 wk, the mice were divided into body weight-matched groups and subcutaneously injected once daily between 5 and 6 PM with 250 μl of PBS vehicle, 25 mg/kg CBX solution (Sigma-Aldrich), or 50 mg/kg CBX solution for 4 wk while...
being maintained on WD. Mice were weighed and total fat and lean mass were assessed weekly using an NMR analyzer (Minispec, Bruker Optics) during the 4 wk of treatment. Unless otherwise noted, at the end of the study, all mice were fasted for 5 h and injected 1 h before they were killed, anesthetized, bled by puncture of the retroorbital venous plexus, euthanized with isoflurane inhalation followed by cervical dislocation, perfused with PBS, and dissected for further tissue analyses. Plasma was separated from whole blood by centrifugation at 4°C.

**Indirect calorimetry.** At the end of the 1st wk of treatment, singly housed, weight-matched female A/α/βLDLR+/– mice were placed in metabolic cages for 24 h of acclimation followed by 24 h of measurement. Whole body O₂ consumption (V˙O₂), CO₂ production (V˙CO₂), and activity were measured continuously for 1 min at 15-min intervals using an indirect calorimetry system (Oxymax Deluxe, Columbus Instruments) with an airflow rate of 0.6 l/min. Oxymax software used the following equations to calculate respiratory exchange ratio (RER) and energy expenditure (heat): RER = V˙CO₂/V˙O₂ and heat = (3.815 + 1.232 × RER) × V˙O₂ (40). Heat was then normalized to lean body mass. Basal metabolic rate for each mouse was calculated by averaging heat from three 45-min intervals of least energy expenditure during the light cycle.

**Plasma analyses.** Plasma TG, total cholesterol (TC), and FFA were measured using TG and cholesterol reagents (Raichem) and the NEFA C kit (Wako), respectively. Blood glucose was measured using a Lifescan One Touch basic glucometer kit (Johnson & Johnson). Insulin and leptin were determined using modified insulin double-antibody RIA kits (Linco Research). Lipoprotein fractionation was achieved by fast protein LC using aliquots of pooled plasma samples from each group of mice. Plasma samples from mice used for VLDL turnover and hepatic TG production experiments were omitted from end-point plasma lipid analysis.

**VLDL turnover.** Plasma from fasted age-matched, WD-fed, overnight-fasted A/α/βLDLR+/– mice was pooled, and VLDL (density <1.019 g/l) was isolated by density gradient ultracentrifugation in a centrifuge (Optima TLX, Beckman Coulter). IODO-GEN precoated reaction tubes (Pierce Biotechnology) were used to incorporate 125I

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Fig. 2. Body weight and composition were affected by CBX. Vehicle- and CBX-treated female (A–C) and male (D–F) mice were weighed and body composition was analyzed throughout the 4-wk injection study. Values are means ± SE of 16–17 female LDLR+/–, 14 female A/α/βLDLR+/–, 8–9 male LDLR+/–, and 9–11 A/α/βLDLR+/– mice. *P < 0.05; #P < 0.01 vs. vehicle.
Cryosections were stained with oil red O. Aortic root from frozen, OCT-embedded hearts were cut according to the time curve from baseline to 2 h.

The time course and was calculated as the slope of the plasma TG vs. clearance. Mice were bled 1 and 2 h after injection, and plasma was analyzed for TG content. Hepatic TG production rate was linear over the entire course and was calculated as the slope of the plasma TG vs. time curve from baseline to 2 h.

### Aortic root lesion area quantification.

Sections (10 μm) of aortic root from frozen, OCT-embedded hearts were cut according to the method of Paigen et al. (41). Cryosections were stained with oil red O (Sigma-Aldrich) for detection of neutral lipids and counterstained with Hoechst 33342 for nuclear and plasmid DNA. Images were captured using a confocal microscope (Zeiss LSM 510, Carl Zeiss, Inc., Jena, Germany). The aortic root lesion area was quantified using image analysis software (ImageJ). The lesion area was expressed as a percentage of the total aortic root area.

### LDLR−/−

**Vehicle**

<table>
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<tr>
<th>n</th>
<th>TC, mg/dl</th>
<th>TG, mg/dl</th>
<th>FFA, meq/l</th>
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<tr>
<td>17</td>
<td>754±35</td>
<td>296±27</td>
<td>1.08±0.12</td>
<td>121±6</td>
<td>0.50±0.08</td>
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<td>757±24</td>
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<td>1,487±81†</td>
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**Males**

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<td>126±9</td>
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Values are means ± SE. Plasma lipids were measured in 5-h-fasted LDL receptor-deficient (LDLR−/−) mice and heterozygous agouti (A/α) mice bred with homozygous LDLR−/− mice (A/αLDLR−/− mice) at the end of the study. TG, triglyceride; TC, total cholesterol; FFA, free fatty acid; CBX, carbofuran. *P < 0.05; †P < 0.01 vs. vehicle.

### Total body weight and end weights of gonadal fat pad, liver, and kidney

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**Males**

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<tr>
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<td>38.5±0.8*</td>
<td>1.86±0.11*</td>
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Values are means ± SE. Total body weight and tissue weights were measured on the last day of the study. *P < 0.05; †P < 0.01 vs. vehicle.
Statistical analyses. Values are means ± SE. GraphPad Prism 4 software was used to assess significance for all data sets, and \( P < 0.05 \) was considered statistically significant. For studies comparing chow diet-fed LDLR/−/− and Ay/a;LDLR/−/− mice, one-way analysis of variance with Bonferroni’s post hoc test was used to determine significance. For comparisons between vehicle-treated LDLR/−/− and Ay/a;LDLR/−/− mice, LDLR/−/− mice treated with vehicle and those treated with 50 mg·kg\(^{-1}\)·day\(^{-1}\) CBX and Ay/a;LDLR/−/− mice treated with vehicle and those treated with 50 mg·kg\(^{-1}\)·day\(^{-1}\), unpaired, two-way Student’s t-test was used to evaluate significance. For studies comparing Ay/a;LDLR/−/− mice treated with vehicle, 25 mg·kg\(^{-1}\)·day\(^{-1}\) CBX, and 50 mg·kg\(^{-1}\)·day\(^{-1}\) CBX, one-way analysis of variance with Dunnett’s post hoc test was used to determine significance.

RESULTS

Measurement of CBX and its effects in liver and adipose tissue. To study effects of 11β-HSD1 inhibition in a mouse model of hyperlipidemia and obesity, we chose to use hyperlipidemic hyperphagic Ay/a;LDLR/−/− mice, which are a mouse model of maturity-onset obesity when fed a chow diet (11, 12). A preliminary analysis of 11β-HSD1 expression in chow diet-fed LDLR/−/− and Ay/a;LDLR/−/− mice indicated that a significant increase in enzyme expression in a representative fat pad was associated with obesity in aged Ay/a;LDLR/−/− mice (Fig. 1A). We observed no such change for liver 11β-HSD1 expression levels (Fig. 1B).

To test whether 11β-HSD1 inhibition affected metabolic syndrome risk factors in the context of obesity and hyperlipidemia, 2 mo-old LDLR/−/− and Ay/a;LDLR/−/− mice (11, 12) were fed WD for 6 wk and subsequently subcutaneously injected with vehicle, 25 mg·kg\(^{-1}\)·day\(^{-1}\) CBX, or 50 mg·kg\(^{-1}\)·day\(^{-1}\) CBX for another 4 wk while maintained on WD. Leshchenko et al. (26) established that intraperitoneal injection of rats with 50 mg/kg CBX resulted in maximal circulating levels of CBX 40–70 min after injection. At 1 h after subcutaneous injection, ultraperformance LC (UPLC)-MS-MS analysis of tissue homogenates from LDLR/−/− and Ay/a;LDLR/−/− mice revealed that ~11–12% and 3–4%, respectively, of the 50 mg/kg dose was detectable in liver and gonadal fat, respectively: 5.9 ± 0.8 and 5.6 ± 1.2 μg/g for 8–10 LDLR/−/− and Ay/a;LDLR/−/− livers, respectively, and 1.7 ± 0.8 and 2.0 ± 0.7 μg/g for 4–7 LDLR/−/− and Ay/a;LDLR/−/− gonadal fat pads, respectively.

To directly assess the impact of CBX on 11β-HSD1 activity, we next measured enzymatic activity in homogenates of gonadal fat pads and livers dissected from Ay/a;LDLR/−/− mice at the end of the study, 1 h after injection. Enzyme activity assays revealed a trend toward a decrease in 11β-HSD1 activity in

Fig. 3. CBX treatment resulted in negative energy balance. A: cumulative food intake was measured for 25 days throughout the drug treatment period in male and female LDLR/−/− and Ay/a;LDLR/−/− mice treated with vehicle or 50 mg·kg\(^{-1}\)·day\(^{-1}\) CBX. Values are means ± SE of 2–3 mice per cage in 5–7 cages. B: food intake efficiency throughout the drug treatment period in male and female LDLR/−/− and Ay/a;LDLR/−/− mice treated with vehicle or 50 mg·kg\(^{-1}\)·day\(^{-1}\) CBX. Values are means ± SE of 2–3 mice per cage in 5–7 cages. C: energy expenditure in weight-matched female Ay/a;LDLR/−/− mice treated with vehicle or 50 mg·kg\(^{-1}\)·day\(^{-1}\) CBX (\( n = 5 \)) at the end of the 1st wk of the drug study. Values are means ± SE. *\( P < 0.01 \) vs. vehicle. #\( P < 0.001 \). \( ^{*}P = 0.0002 \).
adipose tissue homogenates (P = 0.076), with no observable effect of CBX administration on liver 11β-HSD1 activity (Fig. 1, C and D, respectively). For LDLR−/− and A′/α;LDLR−/− mice, UPLC-MS-MS of 11-DHC-to-corticosterone ratios revealed a statistically significant accumulation of 11-DHC substrate over corticosterone product in gonadal fat pads of CBX- vs. vehicle-treated mice, further confirming an inhibitory effect of CBX on adipose tissue 11β-HSD1 reductase activity (Fig. 1E).

CBX did not affect circulating systolic blood pressure. To exclude the possibility that CBX impacted blood pressure and, thus, possibly confounded an end point assessing the extent of atherosclerosis, systolic blood pressure was measured. Systemic administration of CBX had no effect on systolic blood pressure in LDLR−/− or A′/α;LDLR−/− mice (data not shown).

Effects of subcutaneously administered CBX on body weight and composition. Total body weight of LDLR−/− mice was significantly lower than that of A′/α;LDLR−/− mice after 6 wk of WD before CBX treatment (P < 0.0001; Fig. 2, A and D). Male and female WD-fed LDLR−/− mice treated with 50 mg·kg−1·day−1 CBX showed a steady decline in body weight that achieved significance by the end of the study. Male and female WD-fed A′/α;LDLR−/− mice treated with 25 or 50 mg·kg−1·day−1 CBX also showed a similar, dose-dependent reduction in body weight. Body weight changes were reflected in differences in total fat mass (Fig. 2, C and F). Consistent with the observed reductions in total fat mass, circulating leptin levels and gonadal fat pad weight were also dose dependently decreased with CBX treatment by the end of the study (Tables 1 and 2).

CBX treatment results in negative energy balance. Confirming a hyperphagic effect of hypothalamic agouti protein overexpression in agouti mice (17), cumulative food intake data revealed a significant difference between vehicle-treated LDLR−/− and A′/α;LDLR−/− controls: 59 ± 4 and 75 ± 2 g, respectively (P = 0.01; Fig. 3A). However, subcutaneous injection of CBX at the highest dose did not significantly impact cumulative food intake in LDLR−/− or A′/α;LDLR−/− mice after 25 days of treatment. Food intake efficiency was decreased in CBX- compared with vehicle-treated LDLR−/− and A′/α;LDLR−/− mice (Fig. 3B).

Indirect calorimetry revealed a significant increase in energy expenditure in A′/α;LDLR−/− mice treated with 50 mg·kg−1·day−1 CBX compared with vehicle-treated controls that was attributable to a significant increase in light cycle energy expenditure (Fig. 3C). In addition, basal metabolic rate was significantly greater in 50 mg·kg−1·day−1 CBX- than in vehicle-treated A′/α;LDLR−/− mice: 1,187 ± 24 vs. 1,328 ± 34 kcal·kg body mass−1·min−1 (P = 0.0022).

CBX significantly decreased hyperinsulinemia in A′/α;LDLR−/− mice. Because previous studies reported an insulin-sensitizing effect of 11β-HSD1 inhibition in mice and humans (1, 2), we measured end-point glucose and insulin levels in all mice. Although subcutaneous CBX administration had no effect on plasma glucose and insulin in LDLR−/− mice, a significant reduction in fasting insulin, but not glucose, levels was observed in the more severely obese A′/α;LDLR−/− mice (P < 0.05; Table 1).

Hyperlipidemia in A′/α;LDLR−/− mice is attenuated by CBX treatment. Previous reports indicated that 11β-HSD1 may play a role in regulating plasma TG and HDL levels in rodents (9, 20, 28, 29, 34, 42). In our study, neither male nor female LDLR−/− mice showed an effect of CBX treatment on circulating TG, TC, and FFA levels (Table 1). However, CBX-treated male and female A′/α;LDLR−/− mice showed dose-dependent and significant reductions in all measured lipids (P < 0.01 for TC and FFA, P < 0.05 for TG). Analysis of lipoprotein fractions revealed a selective reduction in the VLDL fraction (Fig. 4A).

Fig. 4. Fasting VLDL, postprandial VLDL turnover, and triglyceride (TG) production rate were significantly affected by CBX treatment in A′/α;LDLR−/−, but not LDLR−/−, mice. A: lipoprotein distribution shown as fast protein liquid chromatography-fractionated lipoproteins from pooled plasma samples from 5-h-fasted female mice collected at the end of the study. B: VLDL clearance in plasma collected from nonfasted male mice injected with 125I-VLDL (n = 3–4). C: hepatic TG production determined by injection of overnight-fasted male and female mice with tyloxapol (n = 5–6). Values are means ± SE. *P < 0.05. #P = 0.0008, A′/α;LDLR−/− CBX vs. A′/α;LDLR−/− vehicle.
Because changes in circulating VLDL levels can be a reflection of altered clearance or hepatic secretion, we determined whether these processes were impacted by CBX treatment. Using $^{125}$I-VLDL tracer, we observed no effect on VLDL clearance due to CBX treatment in LDLR$^{-/-}$ mice (Fig. 4B). In $A^{+/a}$;LDLR$^{-/-}$ mice, CBX treatment resulted in a significant increase in VLDL clearance rate at an early time point, 10 min after injection of the VLDL tracer ($P = 0.0008$). Hepatic TG production rate was not impacted by CBX in LDLR$^{-/-}$ mice but was significantly reduced in CBX-treated $A^{+/a}$;LDLR$^{-/-}$ mice compared with vehicle-treated controls ($P < 0.05$; Fig. 4C).

CBX reduces atherosclerosis in $A^{+/a}$;LDLR$^{-/-}$ mice. Elevated plasma cholesterol levels play a well-established, proatherogenic role in humans and mice. Therefore, we sought to determine the effect of CBX treatment on lesion formation in WD-fed LDLR$^{-/-}$ and $A^{+/a}$;LDLR$^{-/-}$ mice. LDLR$^{-/-}$ mice treated with 50 mg·kg$^{-1}$·day$^{-1}$ CBX showed a slight, but nonsignificant, decrease (15% in females and 18% in males) in aortic root lesion area (Fig. 5). However, $A^{+/a}$;LDLR$^{-/-}$ mice showed a dose-dependent and significant decrease in atherosclerotic lesion area with treatment: 12% and 26% in 25 and 50 mg·kg$^{-1}$·day$^{-1}$ CBX-treated females and 12% and 28% in 25 and 50 mg·kg$^{-1}$·day$^{-1}$ CBX-treated males ($P < 0.05$, vehicle vs. 50 mg·kg$^{-1}$·day$^{-1}$ CBX).

Decreased hepatic steatosis due to CBX treatment. Because end-point liver weights (Table 2) and hepatic TG production rates were dramatically decreased by CBX in $A^{+/a}$;LDLR$^{-/-}$ mice, liver lipid content was assessed in mice treated with vehicle and 50 mg·kg$^{-1}$·day$^{-1}$ CBX. Oil red O staining for neutral lipids indicated an increase in lipid levels in LDLR$^{-/-}$ and $A^{+/a}$;LDLR$^{-/-}$ vehicle-treated controls (Fig. 6A). Hepatic neutral lipid content was decreased by CBX treatment in LDLR$^{-/-}$ and $A^{+/a}$;LDLR$^{-/-}$ mice. Variations in

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**Fig. 5.** Atherosclerotic lesion area was significantly decreased in CBX-treated $A^{+/a}$;LDLR$^{-/-}$, but not LDLR$^{-/-}$, mice. A: representative cross sections of oil red O-stained aortic root lesions from female mice. Magnification ×40. B and C: quantification of aortic root lesion areas for female LDLR$^{-/-}$ ($n = 16$) and $A^{+/a}$;LDLR$^{-/-}$ ($n = 13–14$) mice (B) and male LDLR$^{-/-}$ ($n = 14–15$) and $A^{+/a}$;LDLR$^{-/-}$ mice treated with vehicle ($n = 16$) and CBX ($n = 9$) and 50 ($n = 19$) mg·kg$^{-1}$·day$^{-1}$ CBX (C). Values are means ± SE. *$P < 0.05.$
neutral lipid content were attributable to significant differences in liver TG and unesterified cholesterol levels, as well as a trend toward decreased esterified cholesterol content; CBX treatment had no effect on hepatic phospholipid or FFA composition (Fig. 6B and data not shown).

CBX treatment impacts hepatic gene expression. Hepatic TG and cholesterol content may be modulated by lipogenic processes, cholesterol synthesis, and cholesterol metabolism within the liver. Real-time PCR gene expression analysis revealed striking differences between LDLR−/− and A/α; LDLR−/− vehicle-treated controls. Triacylglycerol hydrolase/carboxylesterase, liver X receptor-α, and cytochrome P-450 (CYP27A1) expression levels were increased 1.5-, 6.2-, and 2.2-fold in A/α;LDLR−/− vehicle-treated relative to LDLR−/− vehicle-treated mice (P < 0.01, P = 0.0002, and P < 0.0001, respectively). Consistent with the reductions in hepatic lipid content, key genes regulating hepatic TG and cholesterol accumulation were downregulated in CBX-treated LDLR−/− and A/α;LDLR−/− mice (Table 3).

DISCUSSION

We have shown that subcutaneous administration enables CBX to target liver and adipose tissue, both key metabolic tissues, ultimately resulting in profound metabolic changes in WD-fed LDLR−/− and A/α;LDLR−/− hyperlipidemic mouse models of obesity. In A/α;LDLR−/− mice, CBX treatment reduced plasma VLDL levels due to a small increase in the rate of VLDL uptake and a more dramatic decrease in the rate of hepatic TG production. The extent of atherogenesis, as assessed at the aortic root, was also significantly reduced in CBX-treated A/α;LDLR−/− mice despite the short (4-wk) treatment period. In LDLR−/− and A/α;LDLR−/− mice, CBX treatment ameliorated hepatic steatosis due to reductions in liver TG and unesterified cholesterol content. This was associated with decreased expression of genes involved in hepatic lipogenesis, cholesterol synthesis, and cholesterol metabolism. These results highlight a role of 11β-HSD1 activity in the regulation of VLDL metabolism and secretion, as well as adiposity, insulin sensitivity, hepatic steatosis, and atherosclerosis, all of which are associated with metabolic syndrome in humans.

We chose to use the nonselective 11β-HSD inhibitor CBX at the onset of our study, because it was the only commercial 11β-HSD inhibitor available at the time. Since the initiation of our study, several specific 11β-HSD1 inhibitors have been characterized and utilized in rodent studies (1, 8, 20). However,
CBX is commonly used in studies assessing the outcomes of modulation of 11β-HSD1 enzyme activity in humans (4, 47, 51, 56, 58). Although use of CBX as a long-term treatment for patients with the metabolic syndrome is limited because of its well-established hypertensive and hypokalemic side effects, resulting from inhibition of renal 11β-HSD2 (45), the results of the present study suggest that specific inhibition of 11β-HSD1 may be particularly beneficial in humans diagnosed with more than one metabolic syndrome risk factor. For the purpose of our study, the lack of effect of CBX on systolic blood pressure eliminated the need for concern regarding possible confounding effects of hypertension on atherogenesis. Moreover, we sought to address metabolic outcomes in liver and adipose tissue, both of which highly express 11β-HSD1 and only minimally, if at all, express 11β-HSD2. Similar to its naturally occurring analog glycyrrhetinic acid, CBX is also a potent gap junction blocker (13), and this activity itself may impact adipogenesis (60), hormonal regulation of hepatocyte function (16, 36, 37), and macrophage response at sites of inflammation (15). Therefore, results reporting CBX effects on metabolism may be subject to the confounding effects of CBX nonspecificity and, thus, must be interpreted with caution.

Route of administration may impact the ability of CBX to reach various adipose tissue depots. Previously published results in rats and humans utilizing orally administered CBX to inhibit 11β-HSD1 indicated that this regimen impacts predominantly liver, with no detectable effect on adipose 11β-HSD1 activity (28, 51). More recently, however, effective targeting of 11β-HSD1 activity in adipose tissue of healthy human subjects has been shown using orally administered CBX (56). For our study, subcutaneous injection was chosen as a route of treatment, as subcutaneous injection was hypothesized to allow more of the CBX to bypass first-pass hepatic metabolism that may occur with oral ingestion, thus potentially enabling more of the drug to target adipose tissue. Indeed, tissue levels of CBX 1 h after injection were ~175- and 60-fold higher than published IC50 values in liver and adipose tissue, respectively (7).

In striking contrast to studies utilizing orally administered CBX to inhibit liver 11β-HSD1, subcutaneous CBX injection resulted in additional metabolic improvements, such as blunted weight gain and reduced fasting insulin, in our unique mouse models (28, 51). The effect of CBX on weight gain was due to selectivity and dose dependently decreased total body fat mass, with no detectable change in lean body mass, revealing that the drug treatment was not causing wasting in the mice. In CBX-treated 11β-HSD1 mice, decreased body fat mass was attributable to negative energy balance due to increased basal metabolic rate. Transgenic modulation of adipose tissue glucocorticoid regeneration in mice has been shown to impact energy expenditure (23, 30), and it is possible that inhibition of adipose tissue 11β-HSD1 activity was key to the effects on energy expenditure, and thus obesity, in our mice. However, effects of CBX-mediated 11β-HSD1 inhibition on other metabolic tissues involved in energy expenditure, such as skeletal muscle and brown adipose tissue, cannot be excluded. For instance, Berthiaume et al. (9) recently reported that, in a rat model of diet-induced obesity, 3 wk of treatment with a specific 11β-HSD1 inhibitor resulted in elevated lipid oxidation product accumulation and/or increased expression of genes involved in fatty acid oxidation in brown adipose tissue, heart, and skeletal muscle.

Hermanowski-Vosatka et al. (20) utilized a specific 11β-HSD1 inhibitor to determine effects on plasma lipids in lean, hyperlipidemic apolipoprotein E−/− mice. During the preparation of our manuscript, two more reports emerged regarding the effects of 11β-HSD1-specific inhibition on white adipose tissue lipolysis and plasma TGs (9, 56). The results from the present study not only confirm that 11β-HSD1 inhibition
impacts fasting plasma FFA, cholesterol, and TG levels, but they also extend the knowledge of the effects of systemic 11β-HSD1 inhibition on plasma lipids in two mouse models of combined diet-induced obesity, hyperlipidemia, and atherosclerosis. Glucocorticoids are well established to stimulate lipolysis in adipose tissue, and increased expression or activity of 11β-HSD1 within white adipose tissue may serve to potentiate such an effect. However, in our study, only A/α; LDLR−/− mice showed a significant effect of CBX treatment on plasma lipids. It is possible that LDLR deficiency itself may have rendered LDLR−/− mice incapable of showing a rescue effect of CBX treatment on elevated plasma lipids, whereas the more severely hyperlipidemic A/α;LDLR−/− mice were capable of normalizing plasma lipid levels to those observed for LDLR−/− mice via other, non-LDLR-mediated, pathways. Alternatively, because fasting insulin and FFAs were dramatically reduced by CBX treatment in A/α;LDLR−/− mice, although neither parameter was affected in LDLR−/− mice, insulin-sensitizing effects of 11β-HSD1 inhibition in adipose tissue may have contributed to changes in plasma FFAs in A/α;LDLR−/− mice.

Hepatic secretion of apolipoprotein B, particularly VLDL, can be affected by degree of FFA flux (61), and it is likely that reductions in hepatic TG production rate and fasting plasma VLDL in CBX-treated A/α;LDLR−/− mice were a direct consequence of the decreased circulating FFA levels. In LDLR−/− mice, there was no observable effect of CBX treatment on plasma FFA or VLDL. Interestingly, in LDLR−/− and A/α;LDLR−/− mice, a significant and dramatic reduction was observed in the expression of hepatic triacylglycerol hydrolase/carboxylesterase 3, an enzyme involved in the mobilization of TG from hepatocyte lipid droplets during VLDL assembly (18). Moreover, glucocorticoids are well established to increase VLDL secretion by the liver, and it is possible that the effect on hepatic TG production rate in A/α;LDLR−/− mice was partially due to an inhibitory effect of CBX on local glucocorticoid regeneration.

Consistent with decreased hepatic TG and cholesterol content in LDLR−/− and A/α;LDLR−/− mice, we observed reduced liver expression of several genes involved in de novo lipogenesis and cholesterol synthesis and metabolism. CBX-mediated inhibition of adipose tissue glucocorticoid regeneration may have impacted hepatic steatosis. 11β-HSD1 expression in adipose tissue is elevated in obese rats and humans (10, 22, 27, 46). It has been proposed that a resulting increased flux of glucocorticoids regenerated by visceral adipose tissue enters the portal circulation, impacting glucocorticoid-mediated events at sites within the splanchnic bed (3, 30). In this manner, adipose-derived glucocorticoids may modulate the expression of lipogenic genes in hepatocytes. Alternatively, CBX-mediated inhibition of local glucocorticoid regeneration within hepatocytes may also have impacted lipogenic gene expression. In keeping with an inhibitory effect of CBX on glucocorticoid regeneration, reductions in liver expression of CYP7A1, a glucocorticoid-inducible gene (44), were observed in LDLR−/− and A/α;LDLR−/− mice treated with CBX compared with vehicle-treated controls. However, possible confounding effects of CBX on β-reductase activity (25), and thus hepatic cholesterol metabolism, cannot be excluded from our interpretation of these data.

Only A/α;LDLR−/− mice showed significant decreases in aortic root lesion area with CBX treatment, and this effect is most likely due to effects of the drug on plasma lipid levels. However, because VLDL induces lipid loading as well as proinflammatory cytokine expression in macrophages (52, 54), reductions in circulating VLDL may have attenuated atherogenesis not only by impacting lipid accumulation, but also by decreasing inflammation at the site of the lesions. In addition, as speculated by Hermanowski-Vosatka et al. (20), local inhibition of 11β-HSD1 in the arterial wall may also impact atherogenesis. This hypothesis is supported by our data showing a trend in reduction of lesion area, despite the absence of effect on plasma lipids, in CBX-treated LDLR−/− mice. Alternatively, as increased adiposity is associated with elevated circulating proinflammatory cytokines and reduced secretion of the atheroprotective adipokine adiponectin, the effect of differences in adiposity due to CBX treatment on these factors, and thus atherogenesis, cannot be excluded.

Taken together, our results support the hypothesis that 11β-HSD1 activity influences obesity, dyslipidemia, and atherosclerosis, which are risk factors or outcomes of the metabolic syndrome. Our findings hold key implications for future therapies aiming to inhibit 11β-HSD1 in patients with metabolic syndrome, highlighting the importance of inhibitors created to selectively target adipose tissue enzyme activity.

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REFERENCES

CARBENOXOLONE TREATMENT OF MURINE METABOLIC SYNDROME


