11β-HSD1 inhibition improves triglyceridemia through reduced liver VLDL secretion and partitions lipids toward oxidative tissues

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EXCESS GLUCOCORTICOIDS (GC) promote visceral obesity, hyperlipidemia, and insulin resistance (62), as seen for instance in human Cushing’s syndrome (8, 42). Because these abnormalities parallel those of the metabolic syndrome, it has been suggested that increased GC action may be involved in the pathogenesis of the metabolic complications of obesity (4, 31, 50).

Beyond systemic GC, which are not particularly elevated in obesity (43, 46, 59), increasing evidence suggests the importance of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1)-mediated local amplification in GC action. The enzyme, which converts inactive corticosteroids into bioactive forms such as cortisol in humans and corticosterone in rodents (47, 56), is expressed in many tissues, including the liver (1, 55), adipose tissue (12, 37), heart, and skeletal muscle (10, 13, 60). These tissues together largely determine the fate of circulating lipids. The involvement of 11β-HSD1 activity in determining lipemia is emphasized by studies in transgenic mice overexpressing the enzyme, in which the lipid profile is deteriorated (33, 41). Conversely, 11β-HSD1 gene invalidation as well as recently developed, highly specific pharmacological 11β-HSD1 inhibitors markedly improve the plasma lipid profile in several animal models (23, 35, 61), further supporting a physiologically relevant modulatory role of 11β-HSD1 action on triglyceride (TG) metabolism.

In addition to its effect on plasma levels of lipids, 11β-HSD1 also impacts their metabolic fate. For instance, whole body 11β-HSD1 gene invalidation upregulates the expression of fatty acid oxidation genes in the liver (35), and high-dose pharmacological 11β-HSD1 inhibition prevents lowering of whole body energy expenditure in the face of decreased food intake in obese mice (61). Therefore, a reduction in 11β-HSD1 action appears to influence the metabolic fate of TG at multiple levels. The mechanisms of the hypotriglyceridemic action of selective 11β-HSD1 inhibition (hepatic secretion vs. intravascular clearance) and those of its tissue-specific impact on the metabolic handling of lipids remain largely unknown. In addition, in the context of understanding how 11β-HSD1 inhibition impacts lipid metabolism, the interpretation of previous work is confounded by the anorectic effect of commonly used doses of pharmacological 11β-HSD1 inhibitors (23, 35, 61), with lipemia being obviously highly sensitive to caloric intake. The present study therefore aimed to assess, in rats fed an obesity-promoting diet, the contribution of major determinants (secretion and clearance) of circulating TG levels and their peripheral metabolism under conditions where 11β-HSD1 action has no impact on food intake.

RESEARCH DESIGN AND METHODS

Animals and treatments. Male Sprague-Dawley rats initially weighing 150–175 g were purchased from Charles River Laboratories (St. Constant, QC, Canada) and housed individually in stainless steel cages in a room kept at 23 ± 1°C with a 12:12-h light-dark cycle.
The animals were cared for and handled in conformance with the Canadian Guide for the Care and Use of Laboratory Animals, and protocols were approved by the institutional animal care committee at Laval University. For the first 5 days, rats had free access to tap water and a stock diet (Charles River Rodent Diet no. 5075; Ralston Products, Woodstock, ON, Canada; digestible energy content: 12.9 kJ/g). Rats were then fed a purified high-sucrose, high-fat diet (19.44 kJ/g, 41% energy from carbohydrate, 39% from fat, and 20% from protein) to maximally challenge the hypolipidemic potential of 11β-HSD1 inhibition. One-half of the animals was given the 11β-HSD1 inhibitor (compound A, 3 mg·kg⁻¹·day⁻¹) as an adjunct to their diet for 3 wk. Compound A is a 4-heteroarylbicyclo[2.2.2]octyltriazole; the potency and pharmacodynamic activity of this class of drugs have been published elsewhere (20). It was determined in pilot studies that the dose of compound A used here does not affect food intake or body weight gain. In the present conditions, plasma levels of the drug achieved with this dose (11 ± 2 μM, measured 6 h after food removal following normal night access to food) were nearly identical to those (13 ± 2 μM) of a pilot study in which compound A, given to rats as an adjunct to rodent chow for 7 days, was found to inhibit 97 ± 3% of 11β-HSD1 activity in adipose tissue and liver, as quantified by a [³H]cortisone-to-cortisol conversion assay. These pilot studies have also shown that, with provision of compound A in food, drug levels in plasma measured as an adjunct to their diet for 3 wk. Compound A is a 4-heteroarylbicyclo[2.2.2]octyltriazole; the potency and pharmacodynamic activity of this class of drugs have been published elsewhere (20).

Serum and tissue sampling. The 6-h-fasted rats were killed by decapitation; trunk blood was collected and centrifuged (1,500 g, 15 min at 4°C); and serum was stored at −20°C until later biochemical measurements. Liver, red gastrocnemius muscle, heart, and brown adipose tissue (BAT) were excised, weighed, and prepared for further analysis as described below. Samples of liver, red gastrocnemius muscle, heart, and BAT were immediately frozen and stored at −80°C for later quantification of TG content in lipid extracts (17).

Hormone and metabolite determinations. Insulin and corticosterone were determined by RIA using reagent kits from Linco Research (St. Charles, MO) with rat insulin and corticosterone as standards, respectively. Serum glucose concentrations were measured by the glucose oxidase method with the Beckman glucose analyzer. Serum nonesterified fatty acid (NEFA) concentrations were assayed enzymatically with a reagent kit (Wako Chemicals, Richmond, VA). TG concentrations in serum and tissue lipid extracts were measured by an enzymatic method using a reagent kit (Roche Diagnostics, Montreal, QC, Canada) that allows correction for free glycerol.

Very low density lipoprotein-TG secretion rate. Before the end of the 3-wk treatment (4 days), eight control and eight compound A-treated rats were cannulated in the jugular vein and allowed to recover. On the day of the experiment and following the 6-h fast, an initial blood sample (0.15 ml) was withdrawn in an EDTA-containing syringe through the venous cannula, and rats were injected through the cannula with 1 ml/kg Triton WR-1339 (300 mg/ml saline; Sigma-Aldrich, St. Louis, MO), a detergent that prevents intravascular TG catabolism (40). Blood samples (0.15 ml) were then taken 20, 40, and 60 min after the injection. Rats were then injected with a lethal dose of ketamine-xylazine. The following tissues were excised: liver, gastrocnemius muscle, heart, BAT, kidneys, lungs, pancreas, and spleen. No treatment effect was found in kidneys, lungs, pancreas, and spleen, and these data are not reported herein. Tissue lipid uptake was derived from the amount of radioactivity incorporated, as previously described (24). The amount of lipid taken up that was oxidized within tissues, acetate and water being the major end products, was determined by counting radioactivity in the aqueous phase of the tissue extracts (63). Data are reported as percent injected dose of [³H]TG.

Skeletal muscle lipoprotein lipase activity. Because the red gastrocnemius was used entirely for the other assays, LPL activity was measured in alternative oxidative muscles. Immediately upon tissue harvesting, samples (~50 mg) of the soleus and the red portion of the VLM muscle were homogenized, processed, and frozen exactly as described (44). For determination of total extractable lipoprotein lipase (LPL) activity, thawed tissue homogenates were incubated at 28°C with a substrate mixture containing [carboxyl-¹⁴C]triolein, and [¹⁴C]NEFA released by LPL were separated and counted. LPL activity is expressed as micromole NEFA released per gram wet tissue.

Plasma membrane fatty acid binding protein content. Because plasma membrane fatty acid binding protein (FABPpm) could not readily be related with TG-derived fatty acid uptake in the liver because of lipolysis-independent hepatic processing of TG emissions (38), protein mass was not determined in this tissue. Pieces (~10 mg) of thawed red gastrocnemius muscle and heart were homogenized in a ground-glass Dual homogenizer (1.5 ml) with 39 vol ice-cold extracting medium (0.1 M phosphate buffer, 2 mM EDTA, pH 7.2). Homogenates were transferred into 1.5 ml polypropylene microtubes and sonicated six times for 5 s each at 20 W on ice, with pauses of 85 s between pulses. FABPpm protein content was measured by Western blotting. Homogenate protein concentration was determined by the DC PC protein assay (Bio-Rad, Hercules, CA). Homogenate proteins (50 μg) were separated by SDS-PAGE on a 12% resolving gel and transferred electrophoretically to a polyvinylidene difluoride membrane (Bio-Rad). Membranes were blocked, rinsed, and then incubated with a purified polyclonal rabbit anti-FABPpm antibody (1:1,000; see Ref. 58). The secondary incubations were performed with ECL horseradish peroxidase-linked anti-rabbit IgG (1:10,000; GE Healthcare BioSciences, Baie d’Urfé, QC, Canada). Detection with ECL (Plus Western Blotting Detection System; GE Healthcare Biosciences) and exposure to film (Kodak BioMax MR-1 Film; GE Healthcare BioSciences) were then performed. A sample of liver homogenate (50 μg) was used as a reference standard for FABPpm. The integrated density of bands was measured using Labworks image acquisition and analysis software (UVP BioImaging Systems, Upland, CA). Results are expressed as density units relative to the reference standard.

Carnitine palmitoyltransferase 1 activity. Tissue homogenates prepared as described above were used for determination of carnitine palmitoyltransferase 1 (CPT1) activity (maximal velocity) by a spectrophotometric technique as described earlier (14). Enzyme activity is expressed in nanokatals per gram tissue wet weight.
BAT uncoupling protein 1 mRNA. Levels of lipid oxidation products, FABP4 protein, and CPT1 activity in BAT were all below detection levels of the methods described above. Therefore, mRNA levels of uncoupling protein 1 (UCP1), a reliable marker of BAT oxidative/thermogenic capacity, was assessed. Total RNA was prepared from BAT using the Trizol RNA extraction method. RNA concentration was quantitated by absorbance at 260 nm. For cDNA synthesis, Expand reverse transcriptase (Invitrogen, Burlington, ON, Canada) was used following the manufacturer’s instructions, and cDNA was diluted in DNase-free water (1:25) before quantification by real-time PCR. The primers, designed using the Vector NTI program and synthesized by Invitrogen, were the following: 5′-primer TGGTGAAGTTCGACACTTCC; 3′-primer GTGGGCTGCCAATTGAATC. These primers were validated with a sample BAT cDNA. mRNA transcript levels were measured using a Rotor Gene 3000 system (Montreal Biotech, Montreal, QC, Canada). Amplification and detection of the target mRNA was performed with SYBR Green Jumpstart Taq ready mix (Sigma-Aldrich). Levels of mRNA were determined using a standard curve generated with the plasmid corresponding to the target gene and are expressed as number of copies per reaction. To control for sample loading, tissue samples were run in duplicate. Between-duplicate variation never exceeded 10%.

Liver and BAT morphology by light microscopy. Liver and BAT samples were fixed in 10% vol/vol formaldehyde-PBS at 4°C and embedded in paraffin. Thin sections were mounted on glass slides and stained with oil Red O (liver) or hematoxylin/eosin (BAT). Digital images of tissue slices were captured using an Olympus BX60 microscope equipped with a Sony RT Slider Spot Camera (Camsen Group, Markham, ON, Canada) at a magnification of ×20.

Statistical analysis. Data are presented as means ± SE and were analyzed by unpaired Student’s t-test. Variables with nonnormal distributions were log transformed before analysis to ensure homogeneity of variance. Differences were considered statistically significant at P < 0.05.

RESULTS

The low dose of the 11β-HSD1 inhibitor compound A had no significant effect either on cumulative food intake, body weight gain, or food efficiency (Table 1). Treated rats had slightly increased heart weight (+7%, P < 0.04) without change in total protein content (data not shown) and decreased BAT weight (−17%, P < 0.03), whereas no significant difference was observed in liver and gastrocnemius muscle weight. The effects of compound A on hypothalamic-pituitary-adrenal (HPA) axis activation as well as white adipose tissue morphology and metabolism have been reported elsewhere (6).

Inhibition of 11β-HSD1 robustly reduced fasting serum TG levels (−42%; Table 2) whereas serum NEFA remained unchanged by compound A. In the present conditions, compound A had no significant effect on fasting serum insulin, glucose, and corticosterone concentrations.

To gain insight into the mechanisms of the hypotriglyceridemic action of 11β-HSD1 inhibition, the rate of hepatic VLDL-TG secretion and that of TG clearance from the circulation, the balance of which determines fasting triglyceridemia, were assessed. Here again, fasting plasma TG levels were significantly lower in treated than control rats. The 3-wk treatment with compound A robustly decreased (−41%) liver TG secretion rate (Fig. 1A), whereas it did not affect global TG clearance (Fig. 1B), indicating that the hypotriglyceridemic action of compound A in the present nutritional conditions was entirely caused by reduced liver TG output in the circulation.

Although the global rate of TG clearance from blood was not altered by 11β-HSD1 inhibition, we next sought to determine

| Table 2. Fasting (6-h) serum concentrations of triglycerides, nonesterified fatty acids, glucose, insulin, and corticosterone in rats treated or not with compound A for 3 wk |
|-------------------|-----------------|-----------------|
|                   | Control         | Compound A      |
| Triglycerides, mM | 3.4±0.5         | 2.0±0.4*        |
| Nonesterified fatty acids, mM | 0.28±0.02 | 0.30±0.02 |
| Glucose, mM       | 8.8±0.1         | 8.8±0.2         |
| Insulin, nM       | 0.43±0.05       | 0.39±0.03       |
| Corticosterone, µM| 0.17±0.06       | 0.18±0.05       |

Data are means ± SE; n = 8 rats. *Different from control, P < 0.05.

Fig. 1. Plasma triglyceride (TG) concentration following Triton WR-1339 injection (A) and TG secretion rate (TGSR, inset in A), percent label remaining in plasma (B) and TG clearance rate (TGCR, inset in B) in rats treated or not with compound A for 3 wk. Each point and column represents the mean ± SE of 5–6 animals. In A, TG levels were significantly (P < 0.05) lower in treated than control rats at all time points. *Different from control, P < 0.05.
whether tissue partitioning of circulating TG was affected by treatment. In our previous study (6), it was determined that compound A did not affect TG-derived fatty acid uptake by various white adipose tissue depots. Therefore, only oxidative tissues were considered herein. Uptake of labeled TG-derived fatty acids indicated that the major effect of 11β-HSD1 inhibition was a redistribution of uptake toward extrahepatic oxidative tissues. Radiolabel uptake was decreased in liver (−19%) and increased in red gastrocnemius muscle (+47%), heart (+39%), and BAT (+46%; Fig. 2, A–D). To gain insight into the mechanisms of such increased uptake, we next considered the availability of LPL, the key enzyme responsible for intravascular TG hydrolysis, as well as that of a major determinant of fatty acid uptake downstream of LPL action. The fatty acid transporter FABP<sub>pm</sub> is present at the plasma membrane where it facilitates fatty acid entry in the cell (53, 54). LPL activity was determined in the soleus and VLM muscles and was found to remain unaffected by treatment [soleus: control, 6.3 ± 1.1 μmol NEFA/g tissue vs. compound A, 6.7 ± 1.3, not significant (NS); VLM: control, 2.2 ± 0.3 μmol NEFA/g tissue vs. compound A, 2.4 ± 0.5, NS]. FABP<sub>pm</sub> protein content was, however, significantly increased in red gastrocnemius (+34%) and heart (2.9-fold) of rats treated with the 11β-HSD1 inhibitor (Fig. 3).

The TG content of oxidative tissues was next assessed to determine whether increased lipid uptake translated into lipid accumulation. As shown in Fig. 4, the 11β-HSD1 inhibitor decreased TG concentration in the liver (−19%; Fig. 4, A and B), with no change in red gastrocnemius (Fig. 4C) and the heart (Fig. 4D). Whereas TG concentration per unit weight of BAT was unchanged by treatment (data not shown), the lower total TG content (−16%; Fig. 4, E and F) of this lipid-rich tissue appeared to largely explain its lower weight (Table 1) seen in rats treated with compound A.

![Figure 2](https://example.com/figure2.png)

**Figure 2.** [3H]TG-derived fatty acid uptake in liver (A), red gastrocnemius (B), heart (C), and brown adipose tissue (BAT, D) of rats treated or not with compound A for 3 wk. Each bar represents the mean ± SE of 5–6 animals. *Different from control, P < 0.05.

Increased fatty acid oxidation constitutes one likely mechanism to explain the fact that increased TG-derived fatty acid uptake did not translate into tissue lipid accumulation. This was directly tested through quantitation of the proportion of labeled lipids administered that was metabolized into watersoluble oxidative products in tissues, as well as through estimation of the activity of CPT1, a key limiting enzyme of mitochondrial lipid oxidation. Lipid oxidative products were modestly increased in liver (+21%, P = 0.06) but more robustly so in red gastrocnemius (+35%, P < 0.03) and heart (+33%, P < 0.04; Fig. 5, A–C), whereas they were below detection level in BAT. Levels of expression of UCP1 were, however, strongly increased in BAT (+48%) in treated rats compared with controls (Fig. 5D). Finally, 11β-HSD1 inhibition increased the activity of CPT1 in the red gastrocnemius (+22%, P < 0.02), and it tended to do so in the liver (+13%, P = 0.12) and heart (+12%, P = 0.10) (Fig. 5, E–G).

DISCUSSION

This study assessed the impact of pharmacological inhibition of 11β-HSD1 on determinants of plasma TG and their metabolic fate under dietary eucaloric conditions. It was found that 11β-HSD1 robustly lowered fasting triglyceridemia because of decreased liver VLDL-TG output, without any change in plasma TG clearance. A greater proportion of TG-derived fatty acids was, however, directed toward oxidative tissues, in which lipids did not accumulate and were in fact slightly lowered because of increased oxidation. Therefore, 11β-HSD1 inhibition beneficially impacts liver lipid metabolism, lipemia, and the peripheral metabolic handling of lipids independent of its anorectic action.

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Red gastrocnemius (A) and heart (B) plasma membrane fatty acid-binding protein (FABP<sub>pm</sub>) content in rats treated or not with compound A for 3 wk. Each bar represents the mean ± SE of 5–6 animals. *Different from control, P < 0.05.
A previous study (6) established that long-term activation of the HPA axis, a well-recognized effect of 11β-HSD1 inhibition (22, 27), did occur even at the low dose of compound A used here; however, terminal levels of corticosterone were unchanged in the present study. Importantly, ingestive behavior was not affected by low-dose 11β-HSD1 inhibition, contrary to the reduction in food intake observed at higher doses, likely because the inhibitor at low dose failed to antagonize GC production in areas of the central nervous system involved in the regulation of food intake. The metabolic improvements achieved with 11β-HSD1 inhibition clearly establish that any upward change in systemic GC resulting from increased HPA axis activity is overridden by local tissue inhibition of GC activation.

The reduced fasting triglyceridemia found here in rats treated with the 11β-HSD1 inhibitor is in line with the improved metabolic profile reported in complete genetic invalidation of the 11β-HSD1 gene (27, 35, 36). One report ascribed the improved plasma lipid profile of 11β-HSD1 knockout mice to an amelioration of hepatic lipid metabolism (35); however, the present study extends this notion by directly demonstrating in vivo that reduced VLDL-TG secretion explains the hypo-triglyceridemic action of pharmacological inhibition of the enzyme. The study also establishes the as yet untested ability of 11β-HSD1 inhibition to directly alter liver TG metabolism independent from its anorectic action in rats fed an obesogenic, metabolically deleterious diet. This finding is particularly relevant because reduced caloric intake is in itself an obvious powerful negative modulator of lipemia, and because, to our knowledge, this confounding factor has not been systematically addressed to date in the context of the impact of pharmacological 11β-HSD1 inhibition on TG metabolism.

The amount of VLDL-TG secreted by the liver is modulated by several factors, one of the most important being the amount of lipids available for VLDL assembly. Modulation of hepatocyte fatty acid channeling to storage or secretory lipid pools and its integration with the synthetic and oxidative fatty acid pathways indeed appear to be key determinants of the ultimate VLDL production rate (30). GC exert several actions on hepatic lipid metabolism, including stimulation of lipogenesis (19, 28) and promotion of VLDL-TG secretion (25, 45). Here we show that, consistent with reduced liver GC availability, 11β-HSD1 inhibition significantly reduced liver TG content, extending earlier findings obtained in 11β-HSD1 knockout mice (35). Reduced liver uptake of fatty acids derived from the labeled TG emulsion used here may have contributed to the lowering of hepatic lipid content; however, liver uptake data should be interpreted with caution because several mechanisms independent of TG hydrolysis are involved in the hepatic clearance of such emulsions (38). Also, altered de novo lipogenesis is unlikely to have contributed to the reduction in liver lipid content. Expression levels of key lipogenic and fatty acid esterification genes, including ACS1, FAS, SCD1, GPAT, and DGAT1, were not affected by treatment in the present study (data not shown). In addition, previous work in the 11β-HSD1 knockout mouse showed either no change (fed and 24-h-fasted states) or an increase (refeeding after 24-h fast, indicating higher insulin sensitivity) in the expression levels of major lipogenic genes (35). Another potential contributor to reduced liver lipid content and VLDL-TG secretion is reduced provision of NEFA from visceral fat to the liver. Although systemic NEFA levels were unaltered by 11β-HSD1 inhibition in the present study, our previous (6) and other studies (2, 3, 23, 35, 61) have found a modest reduction (~20%) in plasma NEFA. Conversely, adipose overexpression of 11β-HSD1 was associated with an increase in systemic NEFA that was even more marked in portal blood (33). Given the stimulatory action of GC on adipose lipolysis, these findings suggest a possible contribution of decreased adipose-derived NEFA supply to the reduction in liver lipids associated with 11β-HSD1 inhibition. Alternatively, the present study clearly supports the likely importance of 11β-HSD1 inhibition-induced lipid oxidation in the reduction in steatosis. The expression of major genes of fatty acid oxidation, including several peroxisome proliferator-activated receptor-α targets, was previously shown to be increased in chow-fed 11β-HSD1 knockout mice (35). The present study confirms and extends these findings by directly demonstrating at the functional level the positive action of 11β-HSD1 inhibition on liver lipid oxidation, in the setting of diet-induced obesity and without alterations in ingestive behavior.

Clearance of TG from plasma did not appear to contribute to the effects of 11β-HSD1 inhibition on triglyceridemia in the

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present conditions. Although with little effect by themselves, GC potentiate the positive modulation of adipose LPL by insulin (5, 18, 39). Our previous study, however, did not reveal any change in adipose tissue TG-derived lipid uptake or LPL activity by 11β-HSD1 inhibition under the present conditions (6). In addition, complete removal of GC by adrenalectomy has little impact on LPL in oxidative tissues such as skeletal muscle (15, 32), in line with the present study with 11β-HSD1 inhibition. Although LPL is obviously not the sole determinant of TG clearance, these findings constitute a plausible explanation for the lack of contribution of TG clearance to the hypolipidemic action of 11β-HSD1 inhibition.

Although TG clearance remained unaltered, 11β-HSD1 inhibition led to a posthydrolysis increase in TG-derived fatty acid uptake by extrahepatic oxidative tissues, including skeletal muscle, heart, and BAT. Tissue uptake of fatty acids is a complex process involving passive diffusion and facilitated transport by several plasma membrane proteins (9). Because neither TG clearance nor serum NEFA levels were altered by treatment, we sought to determine whether 11β-HSD1 inhibition affected FABPpm, a binding protein that facilitates the uptake of a large fraction of fatty acids by several organs, including oxidative tissues (7, 21, 52, 53). Although below detection levels in BAT, FABPpm protein content was indeed increased in skeletal muscle and heart of compound A-treated rats relative to controls, suggesting its involvement in the concomitant stimulation of TG-derived fatty acid uptake. Several other fatty acid transport proteins (e.g., FAT/CD36 and FATP1) may conceivably also play a role therein. In a separate study, FAT/CD36 protein in oxidative tissues was found to be unchanged by 11β-HSD1 inhibition (Berthiaume and Deshaies, unpublished observations); however, this does not rule out possible alterations at the intracellular distribution level. Such transport proteins certainly deserve further investigation, as are the detailed mechanisms by which modulation of GC impacts fatty acid transport.

Notably, increased lipid uptake did not result in lipid accumulation in target tissues because of a concomitant increase in their oxidation. In this context, a close relationship between FABPpm content and tissue oxidative capacity has been described (7, 57) and is further suggested by the present findings. Furthermore, the activity of CPT1, a rate-limiting enzyme in the mitochondrial β-oxidation pathway (34), tended to be increased in several oxidative tissues. Increased whole body energy expenditure is a feature of the 11β-HSD1 knockout mouse (36), and the present findings corroborate and broaden this notion by demonstrating that pharmacological 11β-HSD1 inhibition favors fatty acid oxidation in several tissues. At the low dose of 11β-HSD1 inhibitor used here it appears, however, that such metabolic changes were too modest to result in a frank, detectable elevation in whole body energy expenditure, as indirectly witnessed by unchanged food efficiency.

Although increased lipid oxidation could conceivably result from indirect endocrine influences from modified metabolism of adipose tissue or liver, the well-established antiooxidative action of GC in various tissues suggests a direct involvement of local 11β-HSD1 inhibition. In skeletal muscle, lipid oxidation is known to be reduced by dexamethasone (26, 29). Muscle oxidative capacity is a better predictor of insulin sensitivity than its TG and fatty acyl-CoA content (11) and may therefore contribute to the improvement in whole body insulin sensitivity associated with 11β-HSD1 gene knockout or pharmacological inhibition (2, 3, 23, 35, 61). With respect to the heart, the ability of cardiomyocytes to use both circulating corticosterone and 11-dehydrocorticosterone as a source of GC suggests that the heart is under tonic GC control (48). In the present study, heart TG content tended to be slightly reduced by 11β-HSD1 inhibition in conjunction with increased lipid oxidation; however, whether the latter is beneficial to cardiac metabolism regardless of the prevailing metabolic conditions remains to be established (16). Finally, BAT is a well-established target of GC, where the steroids reduce UCP1 expression and thermo-
genesis (49, 51). Although lipid oxidation products could not be detected in BAT in the present conditions, TG content was reduced and UCP1 mRNA was nearly doubled in rats treated with compound A, suggesting increased thermogenesis. These findings, observed in the absence of change in food intake, agree well with the 11β-HSD1−/− mouse model (61). Taken together, these observations support the notion that 11β-HSD1 inhibition prevents extra-adipose fat accumulation through stimulation of fatty acid oxidation and that, in rodents, it establishes conditions that favor energy dissipation through BAT thermogenesis.

In summary, pharmacological inhibition of 11β-HSD1 led to a robust reduction in triglyceridemia that was the result of decreased hepatic TG output in the circulation. In addition, 11β-HSD1 inhibition favored TG-derived fatty acid uptake by oxidative tissues, possibly facilitated by the concomitant increase in FABPpm abundance. Tissue lipid accumulation was, however, prevented because of increased fatty acid oxidation. Therefore, in a rat model of diet-induced obesity, 11β-HSD1 inhibition exerts beneficial actions both on triglyceridemia and on the metabolic handling of lipids in oxidative tissues. The findings underline the importance of GC action in the deterioration of the metabolic profile associated with diet-induced obesity and demonstrate that 11β-HSD1 inhibition can lead to metabolic improvement independent of its impact on the central regulation of ingestive behavior.

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DISCLOSURES

R. Thieringer is employed by Merck Research Laboratories, the manufacturer of Compound A.

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