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Preliminary report: pharmacologic 11β-hydroxysteroid dehydrogenase type 1 inhibition increases hepatic fat oxidation in vivo and expression of related genes in rats fed an obesogenic diet

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Abstract

This study aimed to explore in a model of diet-induced steatosis the impact of pharmacologic 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) inhibition, under conditions of unchanged ingestive behavior, on liver fat oxidation. Male Sprague-Dawley rats were fed an obesogenic diet and were continuously treated or not with an 11β-HSD1 inhibitor (Compound A, 3 mg/[kg d]; Merck Research Laboratories, Rahway, NJ), after which liver expression of oxidative genes and in vivo hepatic fat oxidation were quantified. Treatment with Compound A reduced liver triglyceride concentration (−28%), increased hepatic expression of several genes coding for enzymes of mitochondrial and peroxisomal β-oxidation, and concomitantly enhanced in vivo liver fat oxidation (+38%). The study demonstrates, under conditions that avoided changes in food intake seen in gene knockout or higher-dose pharmacologic models, the efficacy of 11β-HSD1 inhibition to up-regulate hepatic fat oxidation gene expression, which functionally translates into enhanced hepatic lipid oxidation in vivo.

1. Introduction

The importance of 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1)–mediated local amplification is now recognized as a key modulator of glucocorticoid action on metabolism. The 11β-HSD1 enzyme, which locally converts inactive glucocorticoid into bioactive forms such as cortisol in humans and corticosterone in rodents [1], is found in many tissues but displays its highest expression in the liver [2]. Hepatic 11β-HSD1 overexpression in mice is associated with fatty liver and an altered plasma lipid profile [3], whereas 11β-HSD1$^{-/-}$ mice resist diet-induced steatosis and hyperlipidemia [4].

Because the impact of 11β-HSD1 inhibition on hepatic lipid metabolism at the gene expression level has been investigated only in the context of lifelong, whole-body gene invalidation [4], the present study investigated the impact of a more clinically relevant short-term, low-dose pharmacologic inhibition on gene markers of liver lipid metabolism and tested whether treatment effects on oxidative gene expression translated into a congruent change in functional in vivo lipid oxidation. The impact of pharmacologic inhibition of 11β-HSD1 on liver expression of fatty acid oxidation genes and actual in vivo lipid oxidation has not been reported together to date.

2. Materials and methods

2.1. Animals and treatments

Sixteen male Sprague-Dawley rats (65-75 g; Charles River Laboratories, St Constant, Quebec, Canada) were housed, cared for, and handled as described [5], in

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conformance with the Canadian Guide for the Care and Use of Laboratory Animals and as approved by our institutional animal care committee. Rats were fed a purified high-sucrose, high-fat diet [5] that, within a few weeks, increases liver triglycerides (TG) and postprandial lipemia approximately 2-fold compared with a chow-fed reference. Rats were divided into 2 groups of 8 rats each: control and 11β-HSD1 inhibitor (5-{4-[5-(2-trifluoromethylphenyl)-4-methyl-4H-1,2,4-triazol-3-yl]bicyclo[2.2.2]oct-1-yl}-3-(4-fluorophenyl)-1,2,4-oxadiazole; identity as disclosed in Gu et al [6] and designated *Compound A* [CA]; 3 mg/[kg d]; Merck Research Laboratories, Rahway, NJ). This dose of CA does not affect cumulative food intake or weight gain [5]. Although 11β-HSD1 activity was not measured here, robust inhibition was likely achieved because the CA dose used was shown in pilot studies to potently inhibit adipose and liver 11β-HSD1 activity, as detailed elsewhere [5]. The drug was given for 6 weeks in the diet, with frequent adjustment for food intake/body weight. Afterward, after normal night feeding, food was removed at 7:00 AM; and rats were euthanized by decapitation at 1:00 PM.

2.2. Liver RNA isolation and analysis of Pparα, L-Cpt1, Acadvl, Acadl, Acadm, Acads, Aco, Cot, L-Fabp, and Dgat1 messenger RNA

Quantification of messenger RNA (mRNA) levels of key genes of liver lipid metabolism was carried out exactly as described previously for other genes [5], with validated primers that are available upon request. Fatty acid synthase, a key determinant of de novo lipogenesis, was among the original target genes; however, because of technical problems, its mRNA levels could not be determined. Because no reliable housekeeping gene could be found, sample RNA was quantified; and exactly 1 μg RNA was used to generate complementary DNA. Messenger RNA transcript levels are expressed as number of copies per reaction. To control for sample loading, tissue samples were run in duplicate (between-duplicate variation <10%).

2.3. Liver lipid oxidation

In a separate protocol, rats were treated or not with CA (3 mg/kg) for 3 weeks (n = 8 per group), cannulated into the jugular vein, and used to quantify in vivo liver oxidation of

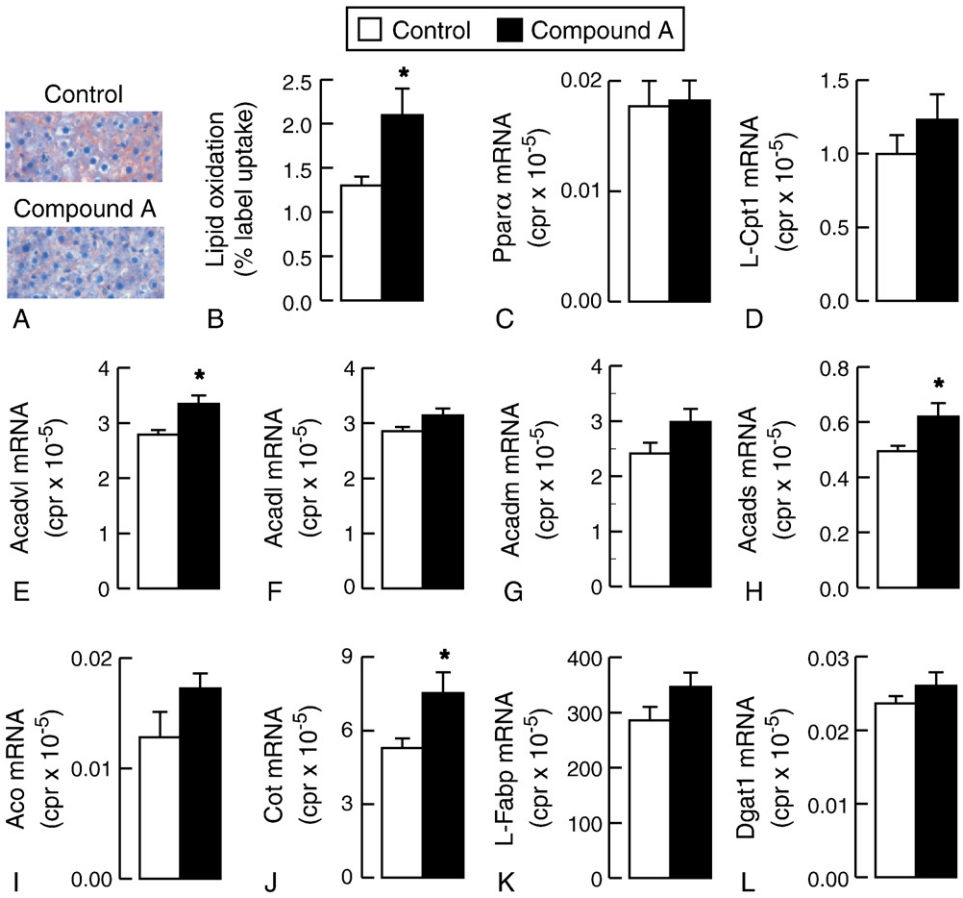

Fig. 1. Liver slices stained with oil red O (lipid content is proportional to intensity of red stain) (A); proportion of incorporated lipids oxidized in liver (B); and mRNA levels of *Pparα* (C), *L-Cpt1* (D), *Acadvl* (E), *Acadl* (F), *Acadm* (G), *Acads* (H), *Aco* (I), *Cot* (J), *L-Fabp* (K), and *Dgat1* (L) in liver of control (open bars) and CA-treated rats (solid bars). Each column represents the mean ± SE of 7 to 8 animals. *Different from control, $P < .05$.

fatty acids derived from an artificial TG emulsion containing ^{3}H-9,10–labeled trioleoylglycerol, exactly as described [5] and according to Hultin et al [7]. Here, however, a more sensitive means of calculation was used to determine *lipid oxidation*, which was defined as the proportion of lipid taken up by the liver that was recovered in the aqueous phase of the tissue extract (oxidation products, mainly acetate and water).

2.4. Statistical analysis

Data are presented as means ± SE, and means were compared by unpaired Student *t* test. Differences were considered statistically significant at $P < .05$.

3. Results

Confirming our previous work [5,8], CA at the dose used here affected neither cumulative food intake (control, 441 ± 15 g vs CA, 453 ± 18 g; not significant) nor body weight gain (control, 130 ± 7 g vs CA, 137 ± 5 g; not significant), significantly decreased hepatic TG concentration (control, 25 ± 2 μmol/g vs CA, 18 ± 2 μmol/g; $P < .03$) and plasma TG (control, 2.2 ± 0.2 mmol/L vs CA, 1.4 ± 0.2 mmol/L; $P < .008$), and tended to reduce plasma nonesterified fatty acids (control, 0.34 ± 0.02 mmol/L vs CA, 0.28 ± 0.02 mmol/L; $P = .054$). Reduced liver lipid content of CA-treated rats is visualized as less intense red in oil red O–stained liver slices (Fig. 1A).

Hepatic oxidation of lipid originating from an intravenously injected TG emulsion was quantitated in vivo. Compound A decreased the proportion of label taken up by the liver (control, 58% ± 2% vs CA, 47% ± 3%; $P < .02$), but robustly increased that fraction of incorporated lipid found as lipid oxidative products (+38%, Fig. 1B). To further characterize this prooxidative response of the liver to 11β-HSD1 inhibition in the present model, the expression levels of key genes of the lipid oxidative pathway were determined. Although there was no change in mRNA levels of *Pparα* (Fig. 1C), a master regulator of liver expression of lipid oxidation genes, or in those of *L-Cpt1* (Fig. 1D), the limiting enzyme for fatty acid entry into the mitochondrion, 11β-HSD1 inhibition significantly increased the expression levels of the mitochondrial and peroxisomal β-oxidation genes *Acadvl* (+17%, Fig. 1E), *Acads*, required for the full oxidation of fatty acids (+20%, Fig. 1H), and *Cot* (+30%, Fig. 1J) and tended to raise those of *Acadl* (+9%, $P = .06$, Fig. 1F), *Acadm* (+19%, $P = .09$, Fig. 1G), and *Aco* (+26%, $P = .08$, Fig. 1I). The mRNA levels of *L-Fabp*, a key lipid transport protein linked to fatty acid oxidation, was not significantly altered by the 11β-HSD1 inhibitor (Fig. 1K). The mRNA of *Dgat1* (Fig. 1L), which catalyzes the committed step for TG synthesis, was unaffected by treatment.

4. Discussion

The low-dose 11β-HSD1 inhibition used here was shown to recapitulate, in rats fed an obesogenic diet, the up-regulation of hepatic lipid oxidation genes observed in 11β-HSD1 knockout mice fed a low-fat diet [4]. The study further demonstrates that such effect on gene expression did translate into a functional increase in liver lipid oxidation in vivo, strengthening a previously observed trend [5]. It is important to note that ingestive behavior, a major confounding factor with regard to lipid metabolism, was not affected by low-dose 11β-HSD1 inhibition as is the case with high-fat feeding of 11β-HSD1 knockout mice [9] or with higher doses of inhibitors previously reported [10,11]. This allowed assessment of the direct, food intake–independent effects of 11β-HSD1 inhibition on liver lipid metabolism.

Glucocorticoids inhibit fatty acid β-oxidation [12]. Accordingly, increased liver expression of major genes of fatty acid oxidation, including several PPARα targets, and concomitant reduction in liver lipid content have been observed in 11β-HSD1 knockout mice fed a low-fat diet [4]. The present study extends the above by showing that pharmacologic inhibition recapitulates the consequences of lifelong gene invalidation on the expression of lipid oxidation genes and by providing novel evidence, at the functional level in vivo, of the positive action of 11β-HSD1 inhibition on liver lipid oxidation in the setting of diet-induced obesity without treatment-induced alterations in ingestive behavior. It is worth noting that the CA-induced increase in liver lipid oxidation occurred despite a relative decrease in labeled-lipid uptake. Although direct changes in local uptake mechanisms cannot be excluded, such decrease was likely due to more avid uptake by other oxidative tissues including brown adipose (+85%, $P < .03$), gastrocnemius (+88%, $P < .03$), and heart (+64%, $P < .05$) [5].

Notably, liver lipid content was decreased by CA treatment despite the fact that the drug decreases triglyceridemia through reduced hepatic very low-density lipoprotein–TG secretion [5], which would tend to favor TG retention. This supports the likely importance of lipid oxidation as a determinant of the antisteatotic action of CA. Whereas no effect was observed on the lipid synthesis gene *Dgat1*, the expression of several genes encoding enzymes of mitochondrial (*Acadvl*, *Acadl*, *Acadm*, *Acads*) and peroxisomal (*Aco*, *Cot*) fatty acid β-oxidation was or tended to be increased by 11β-HSD1 inhibition. Several mechanisms may be involved in such up-regulation. Firstly, most of these lipid oxidation genes, including *Acadvl*, *Acadl*, *Acadm* [13], *Acads*, *Aco*, and *Cot* [14], are PPARα targets. Glucocorticoids and PPARα are thought to modulate each other via a negative feedback loop [15] for adaptation to stress and fasting [16]. In the present conditions, the 11β-HSD1 inhibitor did not affect PPARα mRNA levels. However, this action is strongly affected by the nutritional status [4]; and expression of PPARα and its downstream targets may not always be synchronized. Secondly, we have

reported an up-regulation by 11β-HSD1 inhibition of hepatic *Adipo-R2* expression (without change in plasma adiponectin levels) [8], which may enhance the biological activity of adiponectin in the liver. Increased *Adipo-R2* mRNA is associated with increased lipid oxidation and decreased liver fat content, possibly through an adenosine monophosphate-activated protein kinase–mediated pathway [17], as well as with increased liver PPARα signaling [18]. Further studies should aim at resolving the respective contribution of these and other putative mechanisms of action of 11β-HSD1 inhibition on fatty acid oxidation in the liver. Although many oxidative genes are regulated at the expression level, some undergo posttranscriptional modulation (eg, CPT-1 by malonyl coenzyme A); and future research should also seek to substantiate the present mRNA findings at the protein/activity levels.

In summary, pharmacologic inhibition of 11β-HSD1 was shown, under eucaloric conditions, to increase hepatic expression of several lipid oxidation genes, which translated into a functional stimulation of in vivo fatty acid oxidation in the liver. Such action is likely to contribute to the antisteatotic properties of 11β-HSD1 inhibition.

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