Effect of 11β-hydroxysteroid dehydrogenase-1 inhibition on hepatic glucose metabolism in the conscious dog

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GLUCOCORTICOIDS PLAY AN IMPORTANT ROLE in regulating carbohydrate, fat, and protein metabolism. Whereas cortisol is produced primarily by the adrenal glands, cortisone, an inactive glucocorticoid metabolite, is converted into active cortisol via 11β-hydroxysteroid dehydrogenase type-1 (11β-HSD1) (45, 46) in numerous tissues. This pathway can produce high intracellular cortisol concentrations without necessarily affecting circulating concentrations. 11β-HSD1 is abundant in liver and adipose tissue (7, 35), and local glucocorticoid activation may play a role in insulin resistance in these tissues. In the 60-h-fasted dog, acute treatment with a liver-selective glucocorticoid receptor antagonist reduced basal HGP by about 50% (11), and hepatic insulin sensitivity was increased in patients with type 2 diabetes treated for 1 wk with a nonspecific 11β-HSD1/2 inhibitor (3). These studies confirm that glucocorticoid action plays a role in the regulation of glucose and fat metabolism in the liver and demonstrate that it is a potentially efficacious target of therapeutic intervention.

Cortisol can reduce insulin-mediated glucose uptake in muscle and the liver and attenuates the ability of insulin to suppress HGP. Impaired inhibition of lipolysis by cortisol can also increase circulating nonesterified free fatty acid (NEFA) levels (22, 36), which reduce insulin’s ability to suppress circulating glucose. Cortisol may also affect these and other processes through insulin-independent mechanisms and may promote hyperglycemia during relative or absolute insulin deficiency in individuals with diabetes. Therefore, the aim of this study was to determine the acute effect of 11β-HSD1 inhibition on hepatic glucose and fat metabolism in the conscious dog.

MATERIALS AND METHODS

Animal care and surgical procedures. Experiments were conducted on 16 conscious mongrel dogs of either sex (18–25 kg). Each dog was used for only one experiment and was randomized to treatment with inhibitor or placebo. Control period cortisol data from eight of these 16 dogs were previously published in a preliminary study of cortisol kinetics (5). Housing and diet have been described previously (10). The surgical facility met the standards published by the American Association for the Accreditation of Laboratory Animal Care, and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. All dogs underwent a laparotomy 2 wk before the experiment to implant infusion catheters in the duodenum and the jejunal and splenic veins and sampling catheters in the femoral artery and the portal and hepatic veins (10). Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic artery and portal vein (10). All dogs studied were healthy, as indicated by 1) leukocyte count <18,000/mm3, 2) a hematocrit >35%, 3) a good appetite, and 4) normal stools.

Experimental design. Animals were studied following a 42-h fast. At ~120 min, primed continuous infusions of [3-3H]glucose (50 μCi prime and 0.4 μCi/min continuous; Cambridge Isotope Laboratories, Andover, MA) and [9,11,12,12-2H4]cortisol (0.019 mg/kg prime and
were started for the measurement of endogenous glucose and cortisol to dogs was previously shown to be 71% following a 2.5 mg/kg oral dose. Furthermore, it is not metabolized to any compounds with appreciable groups (50, 51, 52). Glucose was infused through a peripheral vein in the inhibitor group to match the vehicle group experimental period arterial plasma glucose levels.

Immediately after the final sampling time (i.e., 300 min), animals were anesthetized and sections of three liver lobes quickly freeze-clamped in situ and stored at −70°C, as described previously (10). All animals were then euthanized, and the correct positions of the catheter tips were confirmed.

**11β-HSD1 inhibitor.** 11β-HSD1 is primarily a reductase, whereas 11β-HSD type 2 (11β-HSD2) generally catalyzes the reverse dehydrogenase reaction (45, 46). Compound 531 is a very selective and potent competitive inhibitor against 11β-HSD1 vs. 11β-HSD2 across several species, including mouse (0.1 vs. 3,310 nM), rat (0.4 vs. 540 nM), dog (0.6 vs. 5,170 nM), monkey (0.4 vs. 23,920 nM), and human (2.7 vs. 11,180 nM) (11β-HSD1 vs. 11β-HSD2 IC50, respectively). Furthermore, it is not metabolized to any compounds with appreciable HSD1/2 inhibitory activity. Oral bioavailability of compound 531 in dogs was previously shown to be 71% following a 2.5 mg/kg oral dose, with a Cmax of 1.6 μg/ml, plasma half-life of 4.8 h, and an area under the curve of 11.75 μg·h−1·ml−1. Near-complete suppression of hepatic d3-cortisol production in the present study indicates near-complete inhibition of 11β-HSD1 activity in the liver (Fig. 1), consistent with previous results in the monkey, where a single oral 10 mg/kg dose reduced hepatic 11β-HSD1 enzymatic activity by 98%.

![Hepatic D3-Cortisol Production](image)

**Fig. 1.** Hepatic d3-cortisol production in conscious dogs treated with vehicle (●) or an 11β-hydroxysteroid dehydrogenase-1 (HSD1) inhibitor (○) during the basal (−30 to 0 min) and experimental (0–300 min) periods (means ± SE; n = 8/group; *P < 0.05 vs. vehicle group).

There was no difference in liver 11β-HSD1 mRNA content between groups at the end of the study (data not shown).

During the study, serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase, and total bilirubin were within the normal range and did not differ between groups or after treatment with compound 531 (data not shown), confirming that the effects of the inhibitor on hepatic glucose metabolism were not due to hepatocellular injury. This is consistent with previous data where, based upon a receptor screen (Cerep, Seattle, WA), compound 531 displayed a biochemical selectivity profile that was unlikely to produce adverse effects mediated by a wide range of receptors and ion channels at pharmacologically relevant plasma concentrations. Two-week safety studies with compound 531 in rats at 100 mg/kg also demonstrated no adverse effects on behavior, food consumption, body weight, clinical chemistry, or gross or microscopic anatomic pathology.

**Analytical procedures.** Hematocrit; plasma glucose, [3H]glucose, insulin, glucagon, cortisol, d4-cortisol, d3-cortisol, and NEFA; and blood alanine, glycine, serine, threonine, lactate, glutamine, glutamate, glyceral, and β-hydroxybutyrate concentrations were determined as described previously (5, 10, 21). RNA extraction, cDNA synthesis, real-time PCR, SDS-PAGE, and Western blotting procedures were performed by standard methods (12).

**Calculations.** Net hepatic substrate balance (NHB) was calculated using the arteriovenous difference method according to the formula NHB = loadout − loadin, where loadout = ([H] × HF) and loadin = ([A] × AF) + ([P] × PF) and where [H], [A], and [P] are the substrate concentrations in hepatic vein, femoral artery, and portal vein blood or plasma, respectively, and HF, AF, and PF are the blood flow in the hepatic vein, hepatic artery, and portal vein, respectively, as determined by the ultrasonic flow probes. A positive balance value represents net output by the liver, whereas a negative value represents net hepatic uptake. Plasma glucose levels were converted to whole blood values, as described previously (30). Net visceral substrate balance (NVS) was calculated using the formula NVS = loadout − loadin, where loadout = ([V] × PF) and loadin = ([A] × AF). Net hepatic and visceral fractional extraction were calculated as NHB ÷ hepatic loadin and NVB ÷ visceral loadin, respectively.

The use of isotopically labeled cortisol in the calculation of hepatic cortisol production has been described in detail previously (2, 6). This approach takes advantage of the fact that d4-cortisol loses a deuterium when it is converted into d3-cortisone by 11β-HSD2. This, in turn, can be converted into d3-cortisone by 11β-HSD1. Therefore, d3-cortisone production provides an index of 11β-HSD1 activity. Hepatic and visceral d3-cortisone uptake were calculated as hepatic or visceral d3-cortisone loadin × hepatic or visceral d3-cortisone fractional extraction, respectively. Hepatic and visceral d3-cortisone production rates were calculated by adding the respective cortisol uptake to cortisol NHB or NVB.

The approximate cortisol, insulin, and glucagon levels in plasma entering the liver were calculated using the formula ([A] × %AF) + ([P] × %PF), where %AF and %PF are the respective percent contributions of arterial and portal flow, respectively, to total hepatic blood flow.

Tracer-determined whole body glucose appearance and utilization were measured using a primed constant infusion of [3-3H]glucose. Data calculation was carried out using the two-compartment model (23) using canine parameters (8). Endogenous glucose appearance was calculated by subtracting the glucose infusion rate from whole body glucose appearance.

Gluconeogenesis is the synthesis and subsequent hepatic release of glucose from noncarbohydrate precursors. Because carbon produced from flux through the gluconeogenic pathway can also be stored in glycogen, we make a distinction between gluconeogenesis and gluconeogenic flux to glucose 6-phosphate (G-6-P) (12). Hepatic gluconeogenic flux to G-6-P was determined by summing net hepatic uptake rates of gluconeogenic precursors (alanine, glycine, serine,
threonine, glutamine, glutamate, glycerol, lactate, and pyruvate); these rates were divided by two to account for the incorporation of three carbon precursors into the six-carbon glucose molecule. Glycolytic flux was estimated by summing the net hepatic output rates (when such occurred) of the substrates noted above (in glucose equivalents) and hepatic glucose oxidation. In earlier studies, glucose oxidation was 0.2 ± 0.1 mg·kg⁻¹·min⁻¹ even when the concentrations of circulating insulin, glucose, and NEFA varied widely (26, 38). Therefore, hepatic glucose oxidation was assumed to be constant (0.2 mg·kg⁻¹·min⁻¹). Net hepatic glycolgenolytic flux was estimated by subtracting gluconeogenic flux from the sum of net hepatic glucose balance (NHGB) and glycolytic flux. Therefore, a positive number indicates net glycogen breakdown, whereas a negative number indicates net glycogen synthesis.

Statistical analysis. Statistical comparisons were carried out using two-way repeated-measures ANOVA (group × time) (SigmaStat). Student-Newman-Keuls post hoc ANOVA was used when significant F ratios were obtained. Confidence intervals (95%) were used to determine whether rates of glucose infusion or cortisol production differed from zero. Significance was determined as P < 0.05.

RESULTS

Hepatic cortisol production. In response to treatment with the 11β-HSD1 inhibitor, hepatic d₃-cortisol production was reduced to nearly zero, indicating complete inhibition of the 11β-HSD1 pathway in the liver (28 ± 2 and 0 ± 1 ng·kg⁻¹·min⁻¹ during the basal period and last hour of the experiment, respectively; Fig. 1). Hepatic cortisol production was unaffected by vehicle treatment (22 ± 2 and 23 ± 3 ng·kg⁻¹·min⁻¹, respectively). Visceral (nonhepatic splanchnic) d₄-production did not differ from zero, change over time, or differ between groups, even when 11β-HSD1 was inhibited, indicating a lack of cortisol production in nonhepatic splanchnic tissues (data not shown).

Cortisol, ACTH, insulin, and glucagon concentrations. Total cortisol (unlabeled + d₃ + d₄) concentrations in arterial plasma and plasma entering the liver remained at basal in both groups throughout the study (Fig. 2). Arterial d₃-cortisol levels did not change in the vehicle group (0.45 ± 0.03 and 0.47 ± 0.03 μg/dl during the basal period and last hour of the experiment, respectively) but decreased as a result of 11β-HSD1 inhibition (from 0.51 ± 0.06 to 0.25 ± 0.01 μg/dl; basal period and last hour, respectively). ACTH levels were not different between groups (vehicle: 24 ± 4 and 17 ± 1 pg/ml; inhibitor: 24 ± 1 and 17 ± 2 pg/ml, during the basal period and last hour, respectively). Arterial and hepatic plasma insulin levels were reduced to nearly zero during the experimental period in both groups as a result of somatostatin infusion (Fig. 2). Hepatic sinusoidal plasma glucagon levels were also reduced, but to a lesser degree due to subbasal portal vein glucagon infusion used in three dogs of each group (Fig. 2).

Glucose levels and hepatic glucose production. During insulin and glucagon deficiency, there was a gradual and steady rise in the arterial plasma glucose level in the vehicle-treated group (from 112 to 186 mg/dl, basal period to 300 min; Fig. 3). Glucose infusion (0.49 ± 0.25 mg·kg⁻¹·min⁻¹ during the last hour, P < 0.001 between groups; Fig. 3) was required in the inhibitor group to match the arterial glucose levels between groups. This difference was due to an effect on glucose...
production, not utilization. NHGB was similar during the basal period (1.97 ± 0.21 and 1.91 ± 0.26 mg·kg⁻¹·min⁻¹ in the vehicle and inhibitor groups, respectively; Fig. 4). Insulin deficiency in the vehicle-treated group caused NHGB to increase above basal (0.80 ± 0.21 mg·kg⁻¹·min⁻¹, last hour), whereas 11β-HSD1 inhibition attenuated the effect (0.27 ± 0.27 mg·kg⁻¹·min⁻¹, P < 0.001 between groups). Tracer-determined rates of endogenous glucose production (Ra) were similar during the basal period (2.37 ± 0.22 and 2.50 ± 0.15 mg·kg⁻¹·min⁻¹, vehicle and inhibitor groups, respectively; Fig. 4). During the last hour, glucose Ra had increased from basal by 0.57 ± 0.29 in the vehicle group vs. 0.18 ± 0.33 when 11β-HSD1 was inhibited (P < 0.001 between groups). The decreases in whole body glucose utilization and clearance that occurred during insulin deficiency were not affected by 11β-HSD1 inhibition (Fig. 5). Gluconeogenic flux to G-6-P was similar in the two groups (change from basal 0.07 ± 0.16 vs. 0.02 ± 0.13 mg·kg⁻¹·min⁻¹ during the last hour, vehicle and inhibitor groups, respectively; Fig. 6), whereas there was a nonsignificant trend for net hepatic glycogenolysis to increase in the vehicle but not the inhibitor group (0.48 ± 0.26 vs. −0.02 ± 0.23 mg·kg⁻¹·min⁻¹, respectively).

Molecular effects of 11β-HSD1 inhibition in the liver. At the end of the study, phosphoenolpyruvate carboxykinase (PEPCK) mRNA and protein expression were reduced by 57 and 34%, respectively, compared with the vehicle-treated group (Fig. 7). Glucose-6-phosphatase (G-6-Pase) mRNA expression was reduced by 67%. There were no differences in the phosphorylation of Akt, GSK-3β, cAMP response element-binding protein, or peroxisome proliferator-activated receptor-γ coactivator-1α (data not shown).

Metabolite levels and hepatic balance. Plasma NEFA, blood glycerol, β-hydroxybutyrate levels, and net hepatic balance were similar in both groups, suggesting that lipolysis, free fatty acid reesterification, and hepatic β-oxidation were unaffected by 11β-HSD1 inhibition (Table 1). Blood lactate and gluconeogenic amino acid (alanine, threonine, serine, glycine, glutamine, and glutamate) levels and rates of hepatic uptake were also similar and unaffected by treatment (Table 2).

**DISCUSSION**

Cortisol produced by splanchnic tissues may play a role in the dysregulation of glucose and fat metabolism in the diabetic state. Treatment with the selective 11β-HSD1 inhibitor completely inhibited liver d3-cortisol production. Visceral d3-cortisol production was undetectable and was unaffected by treatment with the 11β-HSD1 inhibitor. This is in line with recent studies in obese humans (4, 42) and lean dogs (5), in which the liver was found to be responsible for splanchnic cortisol production. When hepatic cortisol production was reduced to zero, glucose production decreased, whereas nonhepatic glucose uptake and metabolism of fat and protein did not appear to be affected.

Although the liver contributes significantly to cortisol production via 11β-HSD1, it also takes up cortisol at the same rate; therefore, the pathway makes little net contribution to circulating cortisol levels (5). When 11β-HSD1 was inhibited, the circulating d3-cortisol level decreased by 50%. However, this was offset by a small (−20%) increase in the arterial d4-cortisol level, so total cortisol levels did not change. The
latter occurred presumably as a result of decreased flux through the 11β-HSD1 pathway. The hypothalamo-pituitary-adrenal axis sensitively regulates adrenal cortisol production, allowing circulating cortisol levels to be maintained. As a result of d4-cortisol infusion, ACTH levels were suppressed in both groups compared with the expected basal level (50 pg/ml) (11). Since ACTH is not believed to directly regulate hepatic 11β-HSD1 activity (34), the presence of the isotope should not have affected the outcome of this study.

Type 2 diabetes is characterized by excess HGP resulting from relative or absolute insulin deficiency combined with inappropriately high circulating glucagon levels. Cortisol exerts its effects on glucose metabolism in part through interactions with insulin and glucagon. Although cortisol impairs glucose tolerance by decreasing insulin action and glucose effectiveness (29), the hormone acts synergistically with glucagon to stimulate HGP (13, 17, 19). In patients with type 2 diabetes, nonspecific inhibition of 11β-HSD1/2 during hyperinsulinemic hyperglucagonemic euglycemic clamp conditions reduced whole body glucose production (3). Likewise, hepatic insulin sensitivity was improved by 11β-HSD1 inhibition in hyperglycemic mice (1). In the present study, somatostatin was infused to isolate the direct effect of 11β-HSD1 inhibition from cortisol’s interactive effect with insulin. To investigate whether an effect of the inhibitor might be more pronounced in a state of increased glucose production, a subset of experiments using intraportal glucagon infusion at one-half the basal endogenous secretion rate was performed. Both sets of experiments showed similar suppression of HGP during 11β-HSD1 inhibition regardless of the level of glucagon [in the absence of glucagon (n = 5), net hepatic glucose output was 0.56 mg·kg⁻¹·min⁻¹ less during the last hour in the inhibitor group compared with the vehicle group; during partial glucagon replacement (n = 3), this difference was 0.48 mg·kg⁻¹·min⁻¹]; therefore, the data were pooled.

Insulin and glucagon are the primary regulators of the minute-to-minute control of liver glucose output, and insulin deficiency with or without subbasal glucagon resulted in a progressive rise in HGP in the control group. These conditions also reduced insulin-stimulated glucose uptake at muscle and fat, resulting in a steady decline in glucose utilization and an increase in the arterial glucose level over time. Inhibition of 11β-HSD1 attenuated the rise in HGP without having an effect on glucose utilization, and glucose infusion was required to match glucose levels between groups. The degree of inhibition of HGP was in line with the effect of a hepatoselective glucocorticoid receptor antagonist previously used to reduce cortisol signaling (11). Thus, this study demonstrates the effectiveness of blocking hepatic cortisol production even in the absence of normal insulin action.

G-6-Pase catalyzes the terminal step of glucose formed from gluconeogenesis and glycogenolysis in the liver, and transcription of the gene is increased by glucocorticoids (40). Accordingly, 11β-HSD1 inhibition resulted in a two-thirds reduction in liver G-6-Pase mRNA level. Cortisol also stimulates PEPCK mRNA transcription and enhances the stability of the transcript (32). Indeed, hepatic PEPCK mRNA and protein levels were
Table 1. **Arterial levels and net hepatic uptake of plasma NEFA, blood glycerol, and blood β-hydroxybutyrate**

<table>
<thead>
<tr>
<th></th>
<th>Basal Period</th>
<th>60</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
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<tr>
<td><strong>Arterial plasma NEFA, μmol/l</strong></td>
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<tr>
<td>Vehicle</td>
<td>933 ± 78</td>
<td>879 ± 114</td>
<td>1,016 ± 122</td>
<td>1,193 ± 119</td>
<td>1,264 ± 120</td>
<td>1,316 ± 117</td>
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<tr>
<td>11β-HSD1 inhibitor</td>
<td>785 ± 89</td>
<td>745 ± 134</td>
<td>879 ± 155</td>
<td>928 ± 106</td>
<td>1,083 ± 102</td>
<td>1,168 ± 87</td>
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<td><strong>Arterial blood glycerol, μmol/l</strong></td>
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<tr>
<td>Vehicle</td>
<td>103 ± 13</td>
<td>113 ± 22</td>
<td>110 ± 17</td>
<td>129 ± 19</td>
<td>141 ± 16</td>
<td>148 ± 20</td>
</tr>
<tr>
<td>11β-HSD1 inhibitor</td>
<td>74 ± 5</td>
<td>84 ± 10</td>
<td>87 ± 12</td>
<td>97 ± 9</td>
<td>103 ± 10</td>
<td>124 ± 12</td>
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<td><strong>Net hepatic glycerol uptake, μmol·kg⁻¹·min⁻¹</strong></td>
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<tr>
<td>Vehicle</td>
<td>1.96 ± 0.23</td>
<td>1.79 ± 0.34</td>
<td>1.97 ± 0.30</td>
<td>2.45 ± 0.35</td>
<td>2.69 ± 0.35</td>
<td>2.86 ± 0.41</td>
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<td>11β-HSD1 inhibitor</td>
<td>1.54 ± 0.25</td>
<td>1.43 ± 0.25</td>
<td>1.39 ± 0.23</td>
<td>1.57 ± 0.15</td>
<td>1.91 ± 0.29</td>
<td>2.32 ± 0.26</td>
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<td><strong>Arterial blood β-hydroxybutyrate, μmol/l</strong></td>
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<tr>
<td>Vehicle</td>
<td>53 ± 7</td>
<td>49 ± 5</td>
<td>69 ± 8</td>
<td>76 ± 14</td>
<td>84 ± 16</td>
<td>106 ± 18</td>
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<td>11β-HSD1 inhibitor</td>
<td>57 ± 9</td>
<td>57 ± 11</td>
<td>64 ± 12</td>
<td>71 ± 15</td>
<td>80 ± 12</td>
<td>97 ± 16</td>
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<td><strong>Net hepatic β-hydroxybutyrate output, μmol·kg⁻¹·min⁻¹</strong></td>
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<tr>
<td>Vehicle</td>
<td>1.51 ± 0.45</td>
<td>1.30 ± 0.29</td>
<td>1.36 ± 0.30</td>
<td>1.99 ± 0.54</td>
<td>2.11 ± 0.46</td>
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<td>11β-HSD1 inhibitor</td>
<td>1.67 ± 0.42</td>
<td>1.43 ± 0.42</td>
<td>1.29 ± 0.38</td>
<td>1.54 ± 0.46</td>
<td>2.26 ± 0.41</td>
<td>1.98 ± 0.33</td>
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</table>

Values are means ± SE; n = 8/group. 11β-HSD1, 11β-hydroxysteroid dehydrogenase-1; NEFA, nonesterified fatty acids. Samples were obtained in dogs treated with vehicle or an 11β-HSD1 inhibitor during the basal (−30 to 0 min) and experimental (0–300 min) periods.

Table 2. **Arterial blood levels and net hepatic uptake of lactate and gluconeogenic amino acids (alanine, serine, glycine, threonine, glutamate, and glutamine)**

<table>
<thead>
<tr>
<th></th>
<th>Basal Period</th>
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<th>240</th>
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<td><strong>Arterial blood lactate, μmol/l</strong></td>
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<tr>
<td>Vehicle</td>
<td>469 ± 108</td>
<td>507 ± 126</td>
<td>540 ± 141</td>
<td>528 ± 131</td>
<td>525 ± 117</td>
<td>514 ± 78</td>
</tr>
<tr>
<td>11β-HSD1 inhibitor</td>
<td>355 ± 40</td>
<td>448 ± 92</td>
<td>520 ± 114</td>
<td>520 ± 94</td>
<td>473 ± 62</td>
<td>579 ± 95</td>
</tr>
<tr>
<td><strong>Net hepatic lactate uptake, μmol·kg⁻¹·min⁻¹</strong></td>
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<tr>
<td>Vehicle</td>
<td>5.38 ± 0.96</td>
<td>3.45 ± 1.74</td>
<td>4.29 ± 1.53</td>
<td>5.30 ± 0.96</td>
<td>5.56 ± 0.50</td>
<td>6.62 ± 0.68</td>
</tr>
<tr>
<td>11β-HSD1 inhibitor</td>
<td>4.90 ± 1.57</td>
<td>1.79 ± 1.07</td>
<td>2.85 ± 1.06</td>
<td>4.00 ± 0.82</td>
<td>5.30 ± 1.10</td>
<td>6.24 ± 1.52</td>
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<tr>
<td><strong>Arterial blood gluconeogenic amino acids, μmol/l</strong></td>
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</tr>
<tr>
<td>Vehicle</td>
<td>1,895 ± 80</td>
<td>2,091 ± 76</td>
<td>2,294 ± 122</td>
<td>2,382 ± 136</td>
<td>2,548 ± 196</td>
<td>2,533 ± 257</td>
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<tr>
<td>11β-HSD1 inhibitor</td>
<td>1,679 ± 97</td>
<td>1,845 ± 88</td>
<td>2,173 ± 111</td>
<td>2,289 ± 141</td>
<td>2,450 ± 234</td>
<td>2,625 ± 229</td>
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<tr>
<td><strong>Net hepatic gluconeogenic amino acid uptake, μmol·kg⁻¹·min⁻¹</strong></td>
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<tr>
<td>Vehicle</td>
<td>4.29 ± 0.31</td>
<td>3.11 ± 0.31</td>
<td>4.06 ± 1.02</td>
<td>3.12 ± 0.76</td>
<td>3.64 ± 0.70</td>
<td>4.24 ± 0.77</td>
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<tr>
<td>11β-HSD1 inhibitor</td>
<td>3.19 ± 0.29</td>
<td>2.64 ± 0.65</td>
<td>2.47 ± 0.63</td>
<td>2.77 ± 0.65</td>
<td>2.83 ± 0.91</td>
<td>3.01 ± 0.53</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8/group. Samples were obtained in dogs treated with vehicle or an 11β-HSD1 inhibitor during the basal (−30 to 0 min) and experimental (0–300 min) periods.
gen phosphorylase levels (39) and to increase glucagon binding to hepatocytes (9), although the latter did not correlate with glucagon-stimulated glycogenolysis. In rat fetal hepatocytes, cortisol was necessary for the glycogenic effect of insulin, but the glucagon-dependent regulatory pathway did not depend on cortisol (33). During insulin deficiency in the dog, the glucogenolytic rate increased significantly in animals with chronically (5-day) elevated cortisol (15); however, an acute (3-h) increase in cortisol had no effect on glycogenolysis or glucose-geneexpression (14). Therefore, although the present study suggests that 11β-HSD1 inhibition is associated with decreased gluconeogenic enzyme expression and reduced net hepatic glycogenolysis, further investigation will be required to definitively determine the mechanism by which HGP was reduced. The effects of cortisol are complex, and hormone-hormone interactions, diet, obesity, length of fast, age, and metabolic dysfunction may play a role in the response to 11β-HSD1 inhibition.

Beyond effects on glucose metabolism in the liver, 11β-HSD1 inhibition may also improve dyslipidemia. Whereas mice overexpressing 11β-HSD1 developed visceral obesity and hyperlipidemia (24, 31), knockout or inhibition of the enzyme reduced fasting plasma triglycerides, NEFA, and cholesterol and prevented progression of atherosclerosis in mice (1, 16, 27). In addition, after treatment with an 11β-HSD1/2 inhibitor, prednisone-induced lipolysis was reduced in healthy humans, as indicated by decreased subcutaneous adipose tissue release of glycerol, with no effect on serum NEFA, possibly due to rapid reesterification of released fatty acids or nonspecific effects of 11β-HSD2 inhibition on the kidney (44). In the present study, 11β-HSD1 inhibition did not alter arterial plasma NEFA or glycerol levels, suggesting that rates of lipolysis and NEFA reesterification were not affected. In addition, hepatic fat oxidation also appeared to be unaffected by inhibition of 11β-HSD1 since net hepatic β-hydroxybutyrate output was similar in both groups. However, it is possible that chronic treatment with the inhibitor would produce effects on lipolysis and fat metabolism.

In summary, treatment with an 11β-HSD1 inhibitor resulted in complete inhibition of the enzyme’s activity in the liver. Inhibition of 11β-HSD1 resulted in reduction of HGP, whereas fat metabolism was not affected during 5 h of treatment. These data support the concept that 11β-HSD1 may be a suitable therapeutic target for the treatment of the excess hepatic glucose production observed in patients with type 2 diabetes.

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