Recycling Between Cortisol and Cortisone in Human Splanchnic, Subcutaneous Adipose, and Skeletal Muscle Tissues In Vivo

Citation for published version:
https://doi.org/10.2337/db11-1345

Digital Object Identifier (DOI):
10.2337/db11-1345

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Diabetes

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11β-Hydroxysteroid dehydrogenase type 1 (11βHSD1) is a therapeutic target in metabolic syndrome because it catalyses degradative regeneration of cortisol from cortisone in adipose and liver. 11βHSD1 can also catalyze the reverse dehydrogenase reaction in vitro (e.g., if cofactor is limited). We used stable isotope tracers to test the hypothesis that both 11βHSD1-1-reductase and -dehydrogenase activities occur in human metabolic tissues in vivo, 1,5-[3H]2-Cortisone (d2-cortisone) was validated as a tracer for 11β-dehydrogenase activity and its inhibition by licorice, d2-Cortisone (d2-cortisone) and 9,11,12,12-[3H]1-cortisol (d4-cortisol) (to measure 11β-reductase activity) were coinfused and venous samples obtained from skeletal muscle, subcutaneous adipose (n = 6), and liver (n = 4). Steroids were measured by liquid chromatography–tandem mass spectrometry and arteriovenous differences adjusted for blood flow. Data are means ± SEM. 11β-Reductase and -dehydrogenase activities were detected in muscle (cortisol release 19.7 ± 4.1 pmol/100 mL/min, d3-cortisol 5.9 ± 1.8 pmol/100 mL/min, and cortisone 15.2 ± 5.8 pmol/100 mL/min) and splanchnic (cortisol 64.0 ± 11.4 pmol/min, d3-cortisol 12.9 ± 2.1 pmol/min, and cortisone 19.5 ± 2.8 pmol/min) circulations. In adipose, dehydrogenase was more readily detected than reductase (cortisone release 38.7 ± 5.8 pmol/100 g/min). Active recycling between cortisol and cortisone in metabolic tissues in vivo may facilitate dynamic control of intracellular cortisol but makes consequences of dysregulation of 11βHSD1 transcription in obesity and diabetes unpredictable. Disappointing efficacy of 11βHSD1 inhibitors in phase II studies could be explained by lack of selectivity for 11β-reductase. Diabetes 61:1357–1364, 2012

Glucocorticoids are key regulators of fuel metabolism. In recent years, it has become clear that in addition to tight control of circulating cortisol by the hypothalamic-pituitary-adrenal axis, intracellular cortisol levels are controlled by local metabolism (1). For example, recent studies using a stable isotope tracer, 9,11,12,12-[3H]1-cortisol (d4-cortisol) (2), have demonstrated that in vivo hepatic and subcutaneous adipose 11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) regenerates cortisol from inert cortisone, releasing ~900 and 15 pmol of cortisol/100 g tissue/min, respectively (3,4). This 11βHSD1-reductase activity is driven by NADPH cofactor derived from hexose-6-phosphate dehydrogenase (H6PDH), colocalized with 11βHSD1 in the endoplasmic reticulum lumen (5). Altered cortisol generation in tissues such as adipose and liver has been implicated in the metabolic complications of obesity (6,7), and 11βHSD1 inhibition is a potential therapeutic target to lower intracellular cortisol levels in type 2 diabetes (8,9). However, phase 2 trials with small-molecule inhibitors have delivered mixed results (10,11).

One factor that may contribute to unpredictable efficacy of 11βHSD1 inhibitors is the potential reversibility of reactions catalyzed by 11βHSD1. In states of NADPH deficiency, 11βHSD1 can switch direction and exhibit predominant dehydrogenase activity, e.g., when 11βHSD1 is liberated from the intracellular environment or when H6PDH is disrupted by gene targeting in mice (12). Adipose 11β-dehydrogenase activity, inactivating cortisol to cortisone, has been demonstrated in primary cell cultures and during intra-adipose microdialysis in vivo (13,14). As adipose 11βHSD2 expression is negligible and 11βHSD1 expression is abundant (15), this dehydrogenase activity may be attributed to 11βHSD1 and be metabolically protective (16). However, whether it is of sufficient magnitude to influence intracellular cortisol concentrations has not been determined.

11βHSD1 is also expressed in liver (3) and skeletal muscle (17)—both important sites of insulin resistance in type 2 diabetes. d4-Cortisol infusion confirms the liver as a major source of extra-adrenal cortisol production by 11β-reductase (3,4,18). A decline in cortisol concentration between arterial and portal vein samples (4) suggests that there is also 11β-dehydrogenase in the splanchnic circulation. This is most likely attributable to 11βHSD2 in the gut; whether there is 11β-dehydrogenase activity in the liver is unknown. In rodents, skeletal muscle 11βHSD1 activity is low compared with adipose and liver (19), while in humans evidence for in vivo skeletal muscle 11βHSD1 activity is equivocal, as no gradients in cortisol or cortisone concentrations were found across forearm muscle (20) but a small amount of reductase activity was detected in the leg using d4-cortisol (18,21). Ex vivo studies have demonstrated skeletal muscle 11βHSD1-reductase activity and a small amount of 11βHSD1-dehydrogenase activity in tissue homogenates (22). Since 11βHSD1 expression in skeletal muscle is higher in diabetes (23), it is important to clarify its function.

d4-Cortisol allows quantification of 11β-reductase because the deuterium in the 11α position is removed by 11β-dehydrogenase to form d3-cortisone, which in turn is regenerated to d3-cortisol by incorporation of a proton;
dilution of d4-cortisol by d3-cortisol exclusively reflects 11β-reductase activity (2). It is not possible to quantify 11β-dehydrogenase during steady-state infusion of d4-cortisol because the rate of appearance ($R_a$) of d3-cortisone cannot be quantified without infusing a tracer into the cortisone pool. For this purpose, we have developed a novel tracer method with 1,2-[3H]2-cortisone (d2-cortisone) and used it in combination with d4-cortisol to measure simultaneous 11β-dehydrogenase and reductase activities in vivo. We used these isotopes in conjunction with arteriovenous sampling to test the hypothesis that there is interconversion of cortisol and cortisone in subcutaneous adipose, splanchic, and skeletal muscle tissues.

**RESEARCH DESIGN AND METHODS**

**Chemicals and reagents.** Reagents were obtained from Sigma (Poole, U.K.), Steroids (Newport, RI), or VWR (Lutterworth, U.K.) unless otherwise specified. Solvents were high-performance liquid chromatography grade from Fisher Scientific (Loughborough, U.K.), d2-Cortisone, 1,2-[2H]2-cortisol (d2-cortisol), and d4-cortisol were obtained from Cambridge Isotope Laboratories (Andover, MA), and unlabeled cortisol was obtained from Calbiochem (Nottingham, U.K.), indocyanine green (ICG) from Pulsion Medical (Middlesex, U.K.), and 1,2-[3H]2-cortisone from GE Healthcare UK (Little Chalfont, U.K.).

**In vitro validation of d2-cortisone tracer.** To establish that d2-cortisone is a substrate for human 11βHSD1, human embryonic kidney (HEK)293 cells (2×10⁶) stably transfected with human 11βHSD1 (24) were incubated (1–24 h, n=6) with d2-cortisone or cortisone (2 μmol/L). Steroids were extracted and analyzed by liquid chromatography–tandem mass spectrometry.

For assessment of any primary isotope effect, HEK293/h11βHSD1 cells (2×10⁶) were incubated (6–24 h, n=6) with [3H]2-cortisone (5 nmol/L) and cortisone or d2-cortisone (0–1,955 nmol/L). Media was extracted and [3H]2-steroids quantified by HPLC with online β-scintillation counting (Berthold LB509 detector; Berthold Technologies, Harpenden, U.K.).

To compare metabolism by other enzymes, human liver cytosol (25.21 mg/mL protein) was incubated (n=6, 37°C, 8 h) with [3H]2-cortisone (10 nmol/L) and either cortisone or d2-cortisone (10 μmol/L), in the presence of a cofactor-generating system (2 mmol/L NADPH), glucose-6-phosphate (5 mmol/L), and glucose-6-phosphate dehydrogenase (0.5 units/250 μL). The reaction was terminated with ethyl acetate (10 vol), and the solvent extracts were analyzed by HPLC, with products identified by comparison of retention times with unlabeled standards detected by absorbance at λ 244 nm for cortisone and 195 nm for reduced metabolites.

**Clinical protocols.** All studies were approved by the local research ethics committee, and written informed consent was obtained. Participants had normal blood indices (hemoglobin, renal function, and glucose) and had not received glucocorticoid treatment by any route for 3 months prior to the studies.

**In vivo pharmacokinetics of d2-cortisone.** For calculation of pharmacokinetics and estimation of a steady-state infusion rate, three healthy lean men received an intravenous bolus of d2-cortisone (141 μg in 0.9% saline, wt/vol 50 mL) over 5 min with venous blood sampled at intervals for 90 min from the contralateral arm.

For measurement of endogenous cortisol production rate at steady state and for testing the effect of inhibition of 11β-dehydrogenase with licorice, three healthy lean men attended on two occasions, once before and a second time after eating 200 g black licorice (Panda licorice chews, 3.8% licorice extract; Panda, Vaaalakoski, Finland) daily for 2 days. A priming dose (76 μg in 0.9% saline, wt/vol) and a 3-h infusion of d2-cortisone (105.3 μg/h, 0.9% saline, wt/vol) were administered. Venous blood was sampled at intervals (Fig. 1).

**Cortisol/cortisone interconversion in subcutaneous adipose and skeletal muscle.** Six healthy lean men attended after an overnight fast in a quiet temperature-controlled (24°C) room. A cannula (18G) was placed anterogradely into a right antecubital fossa vein for infusions. Retrograde 20G cannulae were placed as follows: 1) in a superficial vein on the anterior abdominal wall, under ultrasonic guidance, to sample from subcutaneous adipose (25); 2) in a deep branch of the median cubital vein in the left antecubital fossa of tracer with and without prior licorice administration. All data are means ± SEM for n = 3. For statistical comparisons, see Table 1.

![FIG. 1. In vivo d2-cortisone administration. A: Plasma d2-cortisone concentrations after an intravenous bolus of d2-cortisone without licorice. B: Plasma cortisone concentrations after infusion of tracer with and without prior licorice administration. C: Plasma enrichment of cortisone with d2-cortisone following infusion of tracer with and without prior licorice administration. D: Plasma d2-cortisone concentrations following infusion of tracer with and without prior licorice administration. All data are means ± SEM for n = 3. For statistical comparisons, see Table 1.](image-url)
to sample from forearm skeletal muscle (26); and to sample from forearm skeletal muscle and arterialized blood (27). For cortisol, cortisone, and d2-cortisone were extracted from plasma (1.5 mL) with ethyl ether (50 mL) using liquid chromatography–tandem mass spectrometry as previously described (4), except that chromatographic separation was optimized using a Restek Biphenyl Allure column (5 μm, 10 cm, 4.6 mm, 38°C, and 0.5 mL/min). The most abundant ions for analysis of the deuterated and nondeuterated steroids were selected by tuning on a TSQ Quantum Discovery Mass spectrometer, and conditions for multiple-reaction monitoring were optimized. The most abundant ions for analysis of the deuterated and nondeuterated steroids were selected by tuning on a TSQ Quantum Discovery Mass spectrometer, and conditions for multiple-reaction monitoring were optimized. The most abundant ions for analysis of the deuterated and nondeuterated steroids were selected by tuning on a TSQ Quantum Discovery Mass spectrometer, and conditions for multiple-reaction monitoring were optimized.

**Laboratory analysis.** Blood samples were collected in lithium heparin and stored at −80°C until analysis. Cortisol, d4-cortisol, d3-cortisol, d2-cortisol, cortisone, and d3-cortisone were extracted from plasma (1.5–2.0 mL) enriched with d4-cortisol (500 ng/mL) using liquid chromatography–tandem mass spectrometry as previously described (4), except that chromatographic separation was optimized using a Restek Biphenyl Allure column (5 μm, 10 cm, 4.6 mm, 38°C, and 0.5 mL/min). The most abundant ions for analysis of the deuterated and nondeuterated steroids were selected by tuning on a TSQ Quantum Discovery Mass spectrometer, and conditions for multiple-reaction monitoring were optimized. The most abundant ions for analysis of the deuterated and nondeuterated steroids were selected by tuning on a TSQ Quantum Discovery Mass spectrometer, and conditions for multiple-reaction monitoring were optimized.

**RESULTS**

### Validation of d2-cortisone tracers

d2-Cortisone was a substrate for human 11βHSD1 in vitro. Incubation of HEK293/h11βHSD1 cells with d2-cortisone or cortisone resulted in production of d2-cortisol and cortisone, respectively (0.17 ± 0.05 vs. 0.41 ± 0.15 pmol/10^6 cells/min, P = 0.11). d2-Cortisone and cortisone had comparable effects in competition with [3H]cortisol: for formation of [3H]3α-cortisol in the presence of d2-cortisone or cortisone, Vmax was 0.83 ± 0.34 and 0.73 ± 0.26 pmol/10^6 cells/min (P = 0.75) and apparent Km 1.60 ± 0.84 vs. 0.91 ± 0.39 μmol/L (P = 0.51), respectively. In human hepatic cytosol, [3H]3α-cortisol was converted also to [3H]5β-tetrahydrocortisone; the rates of this reaction, catalyzed by 5β-reductase/3αHSD, were similar for d2-cortisone and cortisone (318 ± 37.1 vs. 947.3 ± 37.9 pmol/mg/h, P = 0.68). [3H]Products were not formed in control samples without cells, cytosol, or cofactor.

Three men aged 35 ± 7 years with BMI 22.8 ± 2.1 kg/m² participated in studies of in vivo pharmacokinetics of d2-cortisone. After bolus administration, elimination of d2-cortisone from blood could be fitted with first-order kinetics and the tracer could be detected for 1.5 h after injection (Fig. 1A). Mean plasma half-life was 57.5 min, volume of distribution 47.0 L, and area under the curve 494 mmol/L per min. Priming dose and steady-state infusion rates were estimated from these values.

During primed infusion of d2-cortisone for 3 h, cortisone, cortisol, d2-cortisone, and d2-cortisol were readily measured in plasma (Fig. 1B and D). Steady-state d2-cortisone
concentrations were achieved after 15 min. The dilution of d2-cortisone by endogenous cortisone resulted in a calculated net \( R_a \) of cortisone of 35.6 ± 8.8 nmol/min (Table 1). Licorice administration had effects consistent with inhibition of renal 11βHSD2 dehydrogenase activity (30), lowering endogenous cortisol concentrations without affecting tracer d2-cortisone concentrations or clearance, and thereby increasing d2-cortisone enrichment and decreasing the calculated \( R_a \) of cortisone (Table 1 and Fig. 1B and C). Given the small number of participants \((n = 3)\), not all of these effects of licorice were statistically significant.

**Cortisol and cortisone recycling in key metabolic tissues in vivo.** For the adipose and skeletal muscle arteriovenous sampling, study participants \((n = 6)\) were aged 30–49 years (mean ± SEM 42 ± 4), with BMI 21–28 kg/m² (24.6 ± 0.9) and fat mass of 10.5–30.5 kg (16.5 ± 2.9), and took no regular medications. For the hepatic vein measurements, whole-body adipose tissue glucocorticoid absorptiometry detected previously (4), these did not achieve statistical significance (Table 3). By multiplication of dual-energy X-ray absorptiometry of cortisone (nmol/min) 35.6

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>Without licorice</th>
<th>With licorice</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{bs} ) cortisol (nmol/L)</td>
<td>64.9 ± 2.6</td>
<td>24.8 ± 4.7</td>
<td>0.02</td>
</tr>
<tr>
<td>( C_{bs} ) d2-cortisone (nmol/L)</td>
<td>9.3 ± 2.1</td>
<td>12.6 ± 1.7</td>
<td>0.25</td>
</tr>
<tr>
<td>Enrichment d2-cortisone (%)</td>
<td>16.9 ± 2.9</td>
<td>34.5 ± 8.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Net ( R_a ) of cortisol (nmol/min)</td>
<td>35.6 ± 5.8</td>
<td>15.1 ± 4.5</td>
<td>0.17</td>
</tr>
<tr>
<td>Clearance d2-cortisone (L/min)</td>
<td>1.04 ± 0.8</td>
<td>0.9 ± 0.2</td>
<td>0.83</td>
</tr>
<tr>
<td>( C_{bs} ) cortisol (nmol/L)</td>
<td>238.0 ± 33.7</td>
<td>177.9 ± 40.5</td>
<td>0.18</td>
</tr>
<tr>
<td>( C_{bs} ) d2-cortisone (nmol/L)</td>
<td>6.2 ± 1.7</td>
<td>18.5 ± 5.8</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Data are means ± SEM for \( n = 3 \). \( C_{bs} \) concentration at steady state. d, deuterium.

release was extrapolated to be ~10% of whole-body values. However, there was a statistically significant fall in plasma d2-cortisone enrichment (Table 2) and detectable production of cortisone across subcutaneous adipose tissue (Table 3), consistent with adipose 11β-dehydrogenase activity. The ratio of 11β-reductase activity, as measured by \( R_a \) of cortisol, to 11β-dehydrogenase activity was 1:1.3.

**Skeletal muscle.** Endogenous and tracer steroid concentrations did not change across the forearm circulation/ skeletal muscle except for a fall in d2-cortisone concentration (Table 2). However, there were significant differences in tracer enrichment, indicating dilution of \( d_4 \)-cortisol with both cortisol and \( d_3 \)-cortisol and dilution of d2-cortisone with cortisone (Table 2). Once adjustment was made for forearm blood flow (2.8 ± 0.2 mL/100 mL/min), there was significant release of cortisol, \( d_3 \)-cortisol, and cortisone, indicating both reductase and dehydrogenase activities (Table 3). The ratio of 11β-reductase activity to 11β-dehydrogenase activity was 3:1.

**DISCUSSION**

We developed a novel tracer, d2-cortisone, to quantify cortisone generation for the first time in vivo in humans. This can be attributed to 11β-dehydrogenase activities of 11βHSD2 and/or 11βHSD1, since these are the only enzymes known to catalyze cortisone generation. Substantial 11β-dehydrogenase activity is present not only in whole body (presumably reflecting kidney 11βHSD2 activity [31]) but also in the splanchnic circulation (potentially reflecting 11βHSD2 activity in the gut) and in sites where 11βHSD2 is not expressed significantly, including subcutaneous adipose tissue and forearm skeletal muscle. Although cortisol regeneration by 11β-reductase predominates in the splanchnic circulation, there is balanced recycling between cortisol and cortisone in skeletal muscle and predominant inactivation of cortisol by 11β-dehydrogenase in adipose tissue. These data challenge the concept that 11βHSD1 is exclusively a physiological amplifier of glucocorticoid action, suggesting instead that active recycling between cortisol and cortisone provides the opportunity for an amplified dynamic response to alterations in 11βHSD1. Moreover, they suggest, as has been demonstrated empirically in vitro (32), that 11βHSD1 inhibitors may be required to inhibit reductase activity selectively over dehydrogenase activity in order to lower intracellular cortisol concentrations and be efficacious; failure to achieve this might explain lack of efficacy in phase 2 clinical trials (10).

Previously available methods for measuring 11β-dehydrogenase activity and/or cortisone production are inadequate. Most investigators relied upon measuring urinary free cortisol-to-cortisone ratios as an index of renal 11βHSD2 activity (33,34) or on urinary cortisol-to-cortisone metabolite ratios, which reflect predominantly intrahepatic steroid levels. However, these ratios only reflect net balance between the activities of multiple enzymes and do not quantify rates of turnover between cortisol and cortisone. Others have administered labeled substrates for
11β-dehydrogenase, including 11α-[3H]-cortisol (35), d4-cortisol (2), 11α-[2H]-cortisol (36), and d2-cortisol (37), but the removal of these compounds is not exclusively dependent on 11β-dehydrogenase and $R_a$ of 11β-dehydrogenase product (labeled cortisone or liberated [3H]) cannot be quantified accurately in steady state in the absence of simultaneous measurement of clearance of the product. Here, we used the gold standard approach, in which endogenous cortisone production is inferred from dilution of a "tracer" labeled cortisone, infused in steady state. We selected d2-cortisone because it is available, is distinguishable in assays from metabolites of d4-cortisol, and as a stable isotope can be safely administered and measured with high specificity. Cell-based assays confirmed similar metabolism of cortisone and d2-cortisone.

After in vivo administration, d2-cortisone had a longer half-life than has been reported for cortisone (58 vs. 28 min) (38). However, the earlier report from the 1950s relied on inferior assay technology. Cortisone production rate was also higher than the rate measured previously with a d4-cortisol tracer (by extrapolation from generation of d3-cortisone) under conditions of non-steady-state kinetics (36 vs. 24 nmol/min, adjusted for substrate concentration and volume of distribution [2]). This likely reflects the advantages of making steady-state measurements based on dilution of tracer by tracee when both tracer and tracee are at physiological concentrations. A limitation of the d2-cortisone tracer is that it only measures net cortisone production and cannot account for recycling of cortisol-cortisone interconversion. Moreover, there was a hint in our data, albeit not statistically significant, that the addition of deuterium to cortisone makes it a worse substrate for 11β-reductase activity. For these reasons, all 11β-dehydrogenase activities reported here may be somewhat underestimated. However, the cortisone production rate was similar in steady state to the cortisol production rate; approximately halved by licorice administration, consistent with the effect of licorice on 11α-[3H]-cortisol half-life (30); and increased by infusion of additional cortisol when d4-cortisol tracer was administered.
CORTISOL-CORTISONE RECYCLING IN VIVO

TABLE 2
Mean steady-state plasma steroid concentrations and ratios during deuterated cortisol and cortisone infusions

<table>
<thead>
<tr>
<th></th>
<th>Adipose and skeletal muscle study</th>
<th>Splanchnic study</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Arterialized vein</td>
<td>Subcutaneous adipose vein</td>
</tr>
<tr>
<td><strong>Cortisol (nmol/L)</strong></td>
<td>263.8 ± 30.6</td>
<td>254.1 ± 30.3</td>
</tr>
<tr>
<td>d3-Cortisol (nmol/L)</td>
<td>60.0 ± 6.0</td>
<td>59.2 ± 5.9</td>
</tr>
<tr>
<td>d4-Cortisol (nmol/L)</td>
<td>76.3 ± 6.9</td>
<td>73.3 ± 8.4</td>
</tr>
<tr>
<td>d4-Cortisol–to–d3-cortisol ratio</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.0</td>
</tr>
<tr>
<td>d4-Cortisol enrichment [d4-cortisol/ (cortisol + d4-cortisol) × 100], %</td>
<td>22.8 ± 1.3</td>
<td>22.6 ± 1.4</td>
</tr>
<tr>
<td>Cortisone (nmol/L)</td>
<td>42.0 ± 5.3</td>
<td>42.0 ± 7.8</td>
</tr>
<tr>
<td>d2-Cortisone (nmol/L)</td>
<td>4.1 ± 0.4</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>d2-Cortisone enrichment [d2-cortisone/ (cortisone + d2-cortisone) × 100], %</td>
<td>7.2 ± 0.6</td>
<td>6.5 ± 0.7†</td>
</tr>
</tbody>
</table>

Data are means ± SEM for the average from each subject in serial samples obtained over 30 min after at least 3 h of tracer infusion in n = 6 men for adipose and skeletal muscle study and n = 4 men for splanchnic study. †P < 0.05, ††P < 0.01, †††P < 0.001 comparing arterialized vs. venous measurements using Student paired t test. d, deuterium.

Altogether, these data validate the use of d2-cortisone as a tracer.

The splanchnic circulation produces more cortisol than cortisone in individuals being investigated for coronary artery disease. Indeed, splanchnic release accounts for the great majority of whole-body d3-cortisol generation by 11β-reductase, as previously described (4,18,39), but accounts for less than one-half of whole-body cortisone generation. However, given the importance of 11βHSD2 in the kidney (35), which was previously thought to be the exclusive source of cortisone (31), it is notable that splanchnic cortisone production is substantial. Its origin is uncertain. 11βHSD1 is expressed in visceral fat and the liver (40,41) and may function as a dehydrogenase. However, although 11βHSD2 expression in adipose and liver is low (15,42,43), it is highly expressed in the epithelia of the gastrointestinal tract (44), and this is likely to be the principal mechanism accounting for cortisone release previously reported in the portal vein (3,4). Given this contribution of visceral 11β-dehydrogenase activity, it appears that liver 11βHSD1 is indeed a predominant reductase, as anticipated. Unlike skeletal muscle and adipose tissue, other enzymes are expressed in human liver that remove cortisol and cortisone, including A-ring reductases. It is possible that differential removal of cortisol or cortisone may influence the substrate concentrations and, hence, the balance between 11β-dehydrogenase and 11β-reductase, respectively.

In adipose tissue, results were not as anticipated, with substantial 11β-dehydrogenase activity. This confirms previous ex vivo (13) and in vivo findings with microdialysis (14) but using a technique that does not risk dissociation of 11βHSD1 from the colocalized H6PDH and, hence, loss of NADPH cofactor supply. Remarkably, the dehydrogenase activity exceeded 11β-reductase activity in adipose tissue in steady state. The magnitude of 11β-reductase activity as judged by d3-cortisone release might be underestimated owing to the recently discovered slow turnover of the intra-adipose glucocorticoid pool in humans (45) so that accumulation of d3-cortisone substrate to steady state in adipose may take longer than the duration of infusion. However, this is not a confounder of the measurement of cortisol released from adipose 11β-reductase, which was also slower than the release of cortisone. The source of dehydrogenase activity in adipose is likely to be 11βHSD1, as adipose 11βHSD2 transcript levels are very much lower than 11βHSD1 and may not be sufficient to produce measurable active protein (13,42,43,46). Adipose tissue 11βHSD1 has been a focus of research since the description of its upregulation in human obesity (7) and the potent effects of its manipulation in mice (47), but it has been assumed to be an exclusive 11β-reductase. However, an artificial increase in adipose dehydrogenase activity by transgenic overexpression of 11βHSD2 in adipocytes is protective against the adverse metabolic effects of high-fat feeding in mice (16). The current data suggest that there could be switching between reductase and dehydrogenase activities of 11βHSD1 in adipose tissue and that it can no longer be assumed that intra-adipose glucocorticoid concentrations are linearly related to 11βHSD1 protein levels.

Skeletal muscle has only been recognized relatively recently as a potentially important site for 11βHSD1 activity. Previous in vivo studies measuring arteriovenous gradients

TABLE 3
Calculated steady-state kinetic parameters for cortisol and cortisone generation in adipose tissue, skeletal muscle, and splanchnic tissues

<table>
<thead>
<tr>
<th></th>
<th>Adipose and skeletal muscle study</th>
<th>Splanchnic study</th>
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<tbody>
<tr>
<td></td>
<td>Whole body</td>
<td>Subcutaneous adipose</td>
</tr>
<tr>
<td></td>
<td>(nmol/min)</td>
<td>(pmol/100 g/min)</td>
</tr>
<tr>
<td>Ra cortisol</td>
<td>62.6 ± 8.4†</td>
<td>29.3 ± 21.1</td>
</tr>
<tr>
<td>Ra d3-cortisol</td>
<td>24.9 ± 1.0††</td>
<td>11.4 ± 7.9</td>
</tr>
<tr>
<td>Net Ra cortisone</td>
<td>65.1 ± 6.9††</td>
<td>38.7 ± 13.0†</td>
</tr>
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</table>
in total cortisol and cortisone concentrations failed to demonstra-
te foream 11βHSD1-reductase activity (20,48,49), de-
spite skeletal muscle 11βHSD1 expression (17). However, 
this may be explained by balanced recycling between 
cortisol and cortisone, as revealed here with stable iso-
tope tracers. Using d4-cortisol, 11β-reductase activity 
has been detected in the leg of obese subjects (18,21). 
We show that there is both 11β-reductase and 11β-
dehydrogenase activity in human forearm, albeit of lower 
magnitude than in adipose tissue. Since skeletal muscle 
biow blood accounts for most of forearm blood flow when 
the hand is excluded from the circulation, the 11β-
reductase activity can most likely be attributed to 11βHSD1 
in the rhabdomyocytes (17). The 11β-dehydrogenase activity 
may be due to reversibility of 11βHSD1 in rhabdomyocytes, 
as in adipose tissue, but might also be due to 11βHSD2 
expressed within skeletal muscle. 11βHSD2 has been 
reported in skeletal muscle vascular and interstitial cells 
and in rhabdomyocytes (22), although other investigators 
have not replicated these findings in needle biopsy muscle 
samples (23,50).

In conclusion, these data obtained with novel tools 
provide key new insights into the physiology of glucocor-
ticoid metabolism in key metabolic tissues in humans. 
The number of participants is relatively small and so, although 
sufficient for paired comparisons of reductase and de-
hydrogenase activities within individuals, is insufficient 
to estimate biological variability, for example in relation to 
 obesity or variations in regulators of 11βHSD1 expression. 
In addition, the hepatic vein samples were obtained in 
different participants from the skeletal muscle and adipose 
samples, so comparison between tissues must be cautious. 
However, we speculate that since recycling between active 
cortisol and inert cortisone is an energy-consuming pro-
cess, it is likely to have a physiological advantage. More 
work is required to understand whether glucocorticoid 
recycling occurs within each cell by 11βHSD functioning 
as both reductase and dehydrogenase (autocrine recycling) 
or whether recycling occurs in neighboring cells (paracrine 
recycling); this requires in vivo studies beyond the reach of 
current methodologies. As in other "futile recycling" in 
metabolic pathways, recycling between cortisol and corti-
sone within tissues may confer a more dynamic response to 
variation either in 11βHSDs themselves or in substrate 
availability. Moreover, in the basal state it appears that liver 
metabolism is set in favor of cortisol regeneration, while 
adipose metabolism may be set in favor of cortisol in-
activation. This insight calls for a reappraisal of our un-
derstanding of the consequences of tissue-specific disruption 
of 11βHSD1 in obesity and the basis for targeting 11βHSD1 
as a treatment for metabolic syndrome.

ACKNOWLEDGMENTS

This study was supported by a British Heart Foundation 
(BHF) Programme Grant, a BHF Centre of Research 
Excellence Exchange Visit Grant, and a Society for Endo-
crinology Laboratory Visit Grant.

This study was also supported by an award from the 
Translational Medicine Research Collaboration (Grant CVMD/ 
EU016), a consortium made up of the Universities of Aber-
deen, Dundee, Edinburgh, and Glasgow; the four associated 
National Health Service (NHS) Health Boards (Grampian, 
Tayside, Lothian, and Greater Glasgow & Clyde); Scottish 
Enterprise; and Pfizer (formally Wyeth Pharmaceuticals).
B.R.W. is an inventor on relevant patents owned by the 
University of Edinburgh and has consulted for several companies 
developing selective 11βHSD1 inhibitors. No other potential 
conflicts of interest relevant to this article were reported.

K.A.H. performed clinical and laboratory studies, data 
analysis, and wrote the manuscript. K.N.M. and J.I. per-
formed clinical studies and data analysis. N.L.C. assisted 
with study design and performed clinical studies. R.H.S. 
and R.M.R. assisted with study design and data analysis and 
terpretation. D.E.N. assisted with study design and per-
formed clinical studies. R.A. conceived the studies, assisted 
with study design and data analysis, and edited the manu-
script. F.K. assisted with study design and performed clinical studies. B.R.W. conceived the studies, assisted with study design and data analysis, and edited the manuscript. B.R.W. 
is the guarantor of this work and, as such, had full access to 
tall the data in the study and takes responsibility for the 
integrity of the data and the accuracy of the data analysis.

Parts of this study were presented in abstract form at The 
Endocrine Society’s Annual Meeting, San Diego, California, 

The authors are grateful to Dr. Michael Hansen (Pfizer) 
for helpful discussions; Jane Cheeseman and Louise 
Dennis (both Oxford Centre for Diabetes, Endocrinology 
and Metabolism) for clinical support; Sandy Humphreys, 
Marjorie Gilbert (both Oxford Centre for Diabetes, En-
docrinology and Metabolism), Carolyn Cairns (Endo-
crinology Unit, University of Edinburgh), and Scott Denham 
(Wellcome Trust Mass Spectrometry Core Laboratory, Edin-
burgh) for laboratory technical support; Dr. Alistair Millar for 
pharmacy support (NHS Lothian); Dr. Roger Brown (Endo-
crinology Unit, University of Edinburgh) for donation of 
archived tissues and advice on enzymology; Dr. David Watson 
and Dr. RuAngelie Edrada-Ebel (both Strathclyde Institute for 
Pharmacy and Biomedical Science, University of Strathclyde) 
for FT-MS; and the Wellcome Trust Clinical Research Facility 
and its Mass Spectrometry Core Laboratory, Edinburgh, U.K., 
for the use of facilities.

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