11β-hydroxysteroid dehydrogenase type 1 (11βHSD1) is a therapeutic target in metabolic syndrome because it catalyses reductase 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-reductase and -dehydrogenase activities occur in human metabolic tissues in vivo. 1,5-[2H]Cortisone (d2-cortisone) was validated as a tracer for 11β-dehydrogenase activity and its inhibition by licorice, d2-Cortisone, and 9,11,12,12,13H-cortisone (d4-cortisol) (to measure 11β-reductase activity) were confirmed 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-Reducetase 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 nmol/min, d3-cortisol 12.9 ± 2.1 nmol/min, and cortisone 19.5 ± 2.8 nmol/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 efficacies of 11βHSD1 inhibitors in phase II studies could be explained by lack of selectivity for 11β-reductase.

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-[2H]C-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 cortisone concentration between arterial and portal vein samples (4) suggests that there is also 11β-dehydrogenase activity 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. By 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;
because the rate of appearance of dehydrogenase during steady-state infusion of d4-cortisol (U.K.), and 1,2-[3H]2-cortisone from GE Healthcare UK (Little Chalfont, U.K.), indocyanine green (ICG) from Pulsion Medical (Middlesex, UK), and unlabeled cortisol was obtained from Calbiochem and skeletal muscle tissues.

CORTISOL-CORTISONE RECYCLING IN VIVO

RESEARCH DESIGN AND METHODS

Chemicals and reagents. Reagents were obtained from Sigma (Poole, U.K.), Steraloids (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-[3H]2-cortisone (d2-cortisone), and d4-cortisone 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 × 10⁵) 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 × 10⁵) were incubated (6–24 h, n = 6) with [3H]2-cortisone (5 μmol/L) and cortisone or d2-cortisone (0–1,055 nmol/L). Media was extracted and [3H]2-steroids quantified by HPLC with online β-scintillation counting (Berthold LB500 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 nmol/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, Vaajakoski, 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 ng/h, 0.9% saline, wt/vol) were administered. Venous blood was sampled at intervals (Fig. 1).

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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 (180) 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.

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.
to sample from forearm skeletal muscle (26) and J) in a dorsal vein of the right hand, with the hand warmed to 60°C to sample arterialized blood (27). For confirmation of correct placement of cannulae, O₂ saturation (GEM OPL; Instrumentation Laboratory, Bedford, MA) was confirmed to be >85, <40, and >98% in the femoral, skeletal muscle, and arterialized samples, respectively.

d₂-Cortisone and d₄-cortisol (40% in cortisol) were administered in 0.9% saline, wt/vol, as intravenous boluses of 76.0 µg and 3.5 mg followed by continuous intravenous infusions of 105.3 µg/h and 1.74 mg/h, respectively. Arterialized samples were obtained preinfusion and at hourly intervals for 3 h. From 3 h, four sets of blood samples were obtained simultaneously from all sites at 10-min intervals. Prior to taking of samples from the deep forearm vein, a wrist cuff was inflated to 200 mmHg for 2 min to remove blood flow from the hand. Forearm blood flow was measured by venous occlusion plethysmography (28) (Hokanson, Bellevue, WA) immediately after sampling using the mean of three readings during intermittent occlusion of the upper arm with a cuff inflated to 40 mmHg. Adipose tissue blood flow was measured continuously during the study using a Mediscint γ-counter probe after a subcutaneous injection of 1–2 MBq [¹³C]to cortisol or the umbilicus (29).

**Cortisol/cortisone interconversion in the splanchic circulation.** For facilitation of ethical hepatic vein cannulation, four patients undergoing elective coronary angiography for investigation of coronary artery disease were studied. Two venous cannulae (20G) were sited in peripheral veins. d₂-Cortisone and d₄-cortisol (20% in cortisol) were administered in 0.9% saline, wt/vol, as intravenous boluses of 76.0 µg and 3.5 mg followed by continuous intravenous infusions of 104.8 µg/h and 1.74 mg/h, respectively. A simultaneous infusion of intravenous ICG (30 mg/h constituted in water for injection) was used to estimate liver blood flow. After infusion for at least 3 h, femoral arterial and venous sheaths were inserted and patients underwent coronary angiography with follow-on percutaneous coronary intervention when indicated. Upon completion of the clinical procedure, cannulation of the hepatic vein was undertaken under fluoroscopic guidance via the venous sheath using a Swan-Ganz double lumen catheter (6F; Edward Life Sciences, Irvine, CA). A series of three paired blood samples was drawn simultaneously from hepatic vein and femoral artery.

**Laboratory analysis.** Blood samples were collected in lithium heparin and plasma stored at ~80°C until analysis. Cortisol, d₄-cortisol, d₃-cortisol, d₂-cortisol, cortisone, and d₂-cortisone were extracted from plasma (1.5–2.0 mL) enriched with d₃-cortisone (500 ng·mL⁻¹) and d₄-cortisol (500 ng·mL⁻¹) using liquid chromatography–tandem mass spectrometry as previously described (4), except that chromatographic separation was optimized using a Restek Biphenvl 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 compounds subsequently underwent analysis on an LTQ Orbitrap FT-MS to confirm the isotopic composition of the product ions chosen. The mass transitions monitored during tandem mass spectrometry (positive electrospray mode, spray voltage 3.25 kV; source temperature 400°C; and collision gas pressure 1.5 mTorr) were 361→163 m/z for cortisol, 363→165 m/z for d₂-cortisone, 365→121 m/z for cortisone and epit-cortisol (internal standard), 365→122 and 365→329 m/z for d₂-cortisol, 365→121 and 367→121 m/z for d₄-cortisol, with collision energies and tube lens voltages, respectively, of 22 and 168 (cortisol), 21 and 115 (d₂-cortisone), 31 and 142 (cortisone), 33 and 13 with a tube lens 118 (d₂-cortisol), 24 and 142 (d₃-cortisol), and 28 and 142 (d₄-cortisol). Concentrations of cortisol, cortisone, and d₂-cortisone were determined using calibration curves. The peak areas of deuterated steroids were corrected for the abundances of naturally occurring isotopomers at baseline. In addition, the peak area of d₄-cortisol was corrected for interference from the m+4 isotope of cortisol and the m+1 isotope of d₃-cortisol. The peak area of d₃-cortisol was corrected for interference from the m+3 isotope of cortisol and the m+1 isotope of d₂-cortisol. Naturally occurring m+2 isotopomers of cortisol were detected at <1% and did not exceed background, so a correction was not applied. Plasma d₂-cortisone, d₃-cortisol, and d₄-cortisol concentrations were calculated by multiplying the concentrations of cortisol by their respective tracer-to-tracer ratios. Serum ICG was measured using HPLC as previously described (4).

**Data analysis, kinetic calculations, and statistics.** Volume of distribution and half-life of d₂-cortisone were calculated following bolus injection using Kineta software (Thermo Scientific, Philadelphia, PA). Clearance was calculated at steady state (as, 60–180 min) using Eq. 1.

\[
\text{Clearance (L/min)} = \frac{\text{rate of infusion (nmol/min)}}{\text{steady-state concentration (nmol/L)}}
\]

(1) Whole-body Rₐ of cortisol, Rₐ d₄-cortisol, and net Rₐ cortisone (nanomoles per minute) in arterial plasma were calculated by dividing the rate of tracer infusion (nanomoles per minute) by the respective tracer-to-tracer ratio.

\[
Rₐ \text{cortisol} = \frac{\text{rate of d₄-cortisol infusion}}{\text{reaction (d₄-cortisol: cortisol)}}
\]

(2) \[
Rₐ \text{d₃-cortisol} = \frac{\text{rate of d₄-cortisol infusion}}{\text{reaction (d₄-cortisol: d₃-cortisol)}}
\]

(3) \[
\text{Net } Rₐ \text{ cortisone} = \frac{\text{rate of d₂-cortisone infusion}}{\text{(d₂-cortisone: cortisone)}}
\]

(4) Subcutaneous adipose (pmol/100 g tissue/min) and muscle tissue (pmol/100 mL tissue/min) production of cortisol, cortisone, and d₃-cortisone were calculated using arterialized and venous samples (superficial epigastric vein for adipose and deep forearm vein for forearm skeletal muscle) and blood flow (L/min) at steady state (180–210 min) using the mean of four samples. Splanchic glucocorticoid production (nanomoles per min) was calculated using the mean of three samples and correcting for hepatic blood flow, calculated from the concentrations of ICG in the hepatic vein and femoral artery as previously described (4). The following equations were used to calculate tissue glucocorticoid release:

\[
\text{Cortisol release} = \frac{\text{(BF} \times \text{[cortisone]})}{\text{cortisone}}
\]

(5) \[
\text{d₃-cortisol release} = \frac{\text{(BF} \times \text{[d₃-cortisol]})}{\text{d₃-cortisol}}
\]

(6) \[
\text{Net cortisone release} = \frac{\text{(BF} \times \text{[cortisone]})}{\text{cortisone}}
\]

(7) where A is arterialized, V is venous, and BF is blood flow.

Data are presented as means ± SEM and were compared using paired Student t tests. Differences from zero were calculated using a single-sample t test. Significance was accepted at P < 0.05.

**RESULTS**

**Validation of d₂-cortisone tracer.** d₂-Cortisone was a substrate for human 11βHSD1 in vitro. Incubation of HEK293/h11βHSD1 cells with d₂-cortisone or cortisol resulted in production of d₂-cortisol or cortisol, respectively (0.17 ± 0.05 vs. 0.41 ± 0.15 pmol/10⁶ cells/min; P = 0.11). d₂-Cortisone and cortisone had comparable effects in competition with [¹³C]H₂-cortisol: for formation of [¹³C]H₂-cortisol in the presence of d₂-cortisone or cortisone, Vₕₜₐₜ = 0.83 ± 0.34 and 0.73 ± 0.26 pmol/10⁶ 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, [¹³C]H₂-cortisol was converted also to [¹³C]H₂-3α,5β-tetrahydrocorticosterone; the rates of this reaction, catalyzed by 5β-reductase/3αHSD, were similar for d₂-cortisone and cortisone (318.8 ± 37.1 vs. 347.3 ± 37.9 pmol/mg/h; P = 0.68). [¹³C]H₂ 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 pmol/L 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 cortisol resulted in a calculated net $R_a$ of cortisol of $35.6 \pm 8.8$ 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 cortisol (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 ($n = 6$ men) were aged 30–49 years (mean $\pm$ SEM 42 $\pm$ 4), with BMI 21–28 kg/m$^2$ (24.6 $\pm$ 0.9) and fat mass of 10.5–30.5 kg (16.5 $\pm$ 2.9), and took no regular medications. For the hepatic vein study, participants ($n = 4$) were aged 43–70 years (60 $\pm$ 6), with BMI 24–26 (25.3 $\pm$ 0.6), being investigated for ischemic heart disease and taking statins ($n = 4$) and β-adrenergic receptor blockers ($n = 3$).

**Whole body.** Plasma d4-cortisol concentrations and enrichment of plasma with d4-cortisol and d3-cortisol were in steady state by 3 h, while plasma d2-cortisone concentrations and enrichment were in steady state by 1 h (Fig. 2). From tracer enrichments at steady state (Table 2), whole-body cortisol, d3-cortisone, and cortisone production were calculated (Table 3). Cortisol production was similar in the two studies and as previously reported (14). d3-cortisol production was approximately twice as high in the adipose and muscle study than in the splanchnic study, consistent with infusion of a higher enrichment of d4-cortisol tracer (40 vs. 20%) in the former to ensure detection in small volumes obtained from adipose veins. Cortisone production was somewhat higher when d4-cortisol was infused with d2-cortisone, consistent with the additional source of cortisol as substrate for 11β-dehydrogenase activity from the cortisol infusion.

**Adipose.** Across adipose tissue, there were no arteriovenous differences in endogenous steroid or tracer concentrations (Table 2). Although there were trends for dilution of d4-cortisol with both cortisol and d3-cortisol, and once corrected for blood flow (5.7 $\pm$ 1.3 mL/min/100 g adipose) the mean cortisol and d3-cortisol release was consistent with a magnitude of reductase activity similar to that detected previously (4), these did not achieve statistical significance (Table 3). By multiplication of dual-energy X-ray absorptiometry–derived fat mass and local adipose tissue measurements, whole-body adipose tissue glucocorticoid release was extrapolated to be $\sim$10% of whole-body values.

**DISCUSSION**

We developed a novel tracer, d2-cortisone, to quantify cortisol 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 cortisol 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 cortisol 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

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Concentrations of cortisol and d2-cortisone tracer and calculated kinetic parameters before and after licorice administration</th>
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<tbody>
<tr>
<td></td>
<td>Without licorice</td>
</tr>
<tr>
<td>$C_{ss}$ cortisol (nmol/L)</td>
<td>64.9 $\pm$ 2.6</td>
</tr>
<tr>
<td>$C_{ss}$ d2-cortisone (nmol/L)</td>
<td>9.3 $\pm$ 2.1</td>
</tr>
<tr>
<td>Enrichment d2-cortisone (%)</td>
<td>16.9 $\pm$ 2.9</td>
</tr>
<tr>
<td>Net $R_a$ of cortisol (nmol/min)</td>
<td>35.6 $\pm$ 8.8</td>
</tr>
<tr>
<td>Clearance d2-cortisone (L/min)</td>
<td>1.04 $\pm$ 0.8</td>
</tr>
<tr>
<td>$C_{ss}$ cortisol (nmol/L)</td>
<td>238.0 $\pm$ 33.7</td>
</tr>
<tr>
<td>$C_{ss}$ d2-cortisone (nmol/L)</td>
<td>6.2 $\pm$ 1.7</td>
</tr>
</tbody>
</table>

Data are means $\pm$ SEM for $n = 3$. $C_{ss}$ concentration at steady state.
11β-dehydrogenase, including 11α-[3H]-cortisol (35), d4-cortisol (2), 11α-[3H]-cortisol (36), and d2-cortisol (37), but the removal of these compounds is not exclusively dependent on 11β-dehydrogenase and Ra 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 than cortisone 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.

![FIG. 2. Unlabeled and tracer glucocorticoids in plasma from arterialized samples and veins draining skeletal muscle and subcutaneous adipose tissue. Data are means ± SEM for n = 6 volunteers. Statistical comparisons were conducted on average values in each subject (Table 2). A: Cortisol concentrations. B: d4-cortisol-to-cortisol ratio. C: d4-cortisol-to-d3-cortisol ratio. D: Cortisone concentrations. E: d2-cortisone-to-cortisone ratios. ◆, arterialized vein; ▲, superficial epigastric vein; □, deep forearm vein.](diabetesjournals.org)
TABLE 2
Mean steady-state plasma steroid concentrations and ratios during deuterated cortisol and cortisone infusions

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Adipose and skeletal muscle study</th>
<th>Splanchnic study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Arterialized vein</td>
<td>Subcutaneous adipose vein</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</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>22.6 ± 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.

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 it 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-cortisol 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 11β-reductase 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>Tissue Type</th>
<th>Adipose and skeletal muscle study</th>
<th>Splanchnic study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole body (nmol/min)</td>
<td>Subcutaneous adipose (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 cortisol</td>
<td>65.1 ± 6.9†††</td>
<td>38.7 ± 13.0†</td>
</tr>
</tbody>
</table>

Data are means ± SEM for n = 6 men in the adipose and skeletal muscle study and n = 4 in the splanchnic study. †P < 0.05, ††P < 0.01 vs. zero using single-sample t tests.
in total cortisol and cortisone concentrations failed to demonstrate forearm 11βHSD1-reductase activity (20, 48, 49), despite skeletal muscle 11βHSD1 expression (17). However, this may be explained by balanced recycling between cortisol and cortisone, as revealed here with stable isotope 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 blood flow 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 glucocorticoid metabolism in key metabolic tissues in humans. The number of participants is relatively small and so, although sufficient for paired comparisons of reductase and dehydrogenase 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 process, 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 cortisone 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 inactivation. This insight calls for a reappraisal of our understanding of the consequences of tissue-specific disruption of 11βHSD1 in obesity and the basis for targeting 11βHSD1 as a treatment for metabolic syndrome.

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K.A.H. performed clinical and laboratory studies, data analysis, and wrote the manuscript. K.N.M. and J.I. performed 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 interpretation. D.E.N. assisted with study design and performed clinical studies. R.A. conceived the studies, assisted with study design and data analysis, and edited the manuscript. 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. R.B.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES


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