Cortisol Release From Adipose Tissue by 11β-Hydroxysteroid Dehydrogenase Type 1 in Humans

Roland H. Stimson, Jonas Andersson, Ruth Andrew, Doris N. Redhead, Fredrik Karpe, Peter C. Hayes, Tommy Olsson, and Brian R. Walker

OBJECTIVE—11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1) regenerates cortisol from cortisone. 11β-HSD1 mRNA and activity are increased in vitro in subcutaneous adipose tissue from obese patients. Inhibition of 11β-HSD1 is a promising therapeutic approach in type 2 diabetes. However, release of cortisol by 11β-HSD1 from adipose tissue and its effect on portal vein cortisol concentrations have not been quantified in vivo.

RESEARCH DESIGN AND METHODS—Six healthy men underwent 9,11,12,12-[2H] cortisol infusions with simultaneous sampling of arterialized and superficial epigastric vein blood sampling. Four men with stable chronic liver disease and a transjugular intrahepatic porto-systemic shunt in situ underwent tracer infusion with simultaneous sampling from the portal vein, hepatic vein, and an arterialized peripheral vein.

RESULTS—Significant cortisol and 9,12,12-[3H] cortisol release were observed from subcutaneous adipose tissue (15.0 [95% CI 0.4–29.5] and 8.7 [0.2–17.2] pmol·min⁻¹·100 g⁻¹ adipose tissue, respectively). Splanchnic release of cortisol and 9,12,12-[3H] cortisol (13.5 [3.6–23.5] and 8.0 [2.6–13.5] nmol/min, respectively) was accounted for entirely by the liver; release of cortisol from visceral tissues into portal vein was not detected.

CONCLUSIONS—Cortisol is released from subcutaneous adipose tissue by 11β-HSD1 in humans, and increased enzyme expression in obesity is likely to increase local glucocorticoid signaling and contribute to whole-body cortisol regeneration. However, visceral adipose 11β-HSD1 activity is insufficient to increase portal vein cortisol concentrations and hence to influence intrahepatic glucocorticoid signaling. Diabetes 58:46–53, 2009

Cortisol has potent effects in adipose tissue, influencing insulin sensitivity, fatty acid metabolism, adipocyte differentiation, adipokine expression, and body fat distribution (1). Adrenal secretion of cortisol is controlled by the hypothalamic-pituitary-adrenal axis; however, recent evidence suggests that cortisol is also generated from inert cortisone within adipose tissue by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) (2,3). Conversion of cortisone to cortisol occurs in vitro in human adipocytes cultured from visceral and subcutaneous adipose depots (4) and in vivo during infusion of [3H] cortisone into subcutaneous adipose tissue by microdialysis (5). In obesity, 11β-HSD1 mRNA and activity are increased in subcutaneous adipose tissue biopsies (6) and either increased or unchanged in visceral adipose tissue (rev. in 7). 11β-HSD1 inhibitors are being developed to lower intracellular cortisol concentrations in adipose tissue and liver in type 2 diabetes and obesity, with promising preclinical and early clinical results (8).

In addition to influencing intra-adipose cortisol concentrations, it has been suggested that cortisol release into the portal vein from visceral adipose tissue contributes to hepatic insulin resistance associated with central obesity (4). Transgenic overexpression of 11β-HSD1 in adipose tissue in mice results in a two- to threefold increase in portal vein glucocorticoid concentrations without altering systemic levels (9). However, the extent to which cortisol generated by 11β-HSD1 is released into the portal or systemic circulation from visceral or subcutaneous adipose tissue, respectively, in humans is unknown. In arteriovenous samples across subcutaneous adipose tissue, cortisol concentrations do not change, although there is net removal of cortisone (10,11). Similarly, sampling from portal or omental veins during intra-abdominal surgery has not revealed higher cortisol concentrations than in arterial blood (12,13).

Measuring cortisol concentrations in arterial and venous samples may not detect cortisol release by 11β-HSD1 if cortisol is also removed by other enzymes. This occurs, for example, in the liver, where cortisol concentrations are lower in hepatic vein than in arterial blood (14). A tracer technique is required to detect cortisol production in the liver in the face of additional cortisol clearance. We devised a stable isotope deuterated tracer—9,11,12,12-[2H] cortisol (44-cortisol)—for this purpose (15). During 44-cortisol infusion, there is removal of the 11α-2H by 11β-HSD type 2 to form d3-cortisone, which is then regenerated to d3-cortisol by 11β-HSD1 (Fig. 1). The dilution of 44-cortisol by d3-cortisol therefore indicates 11β-HSD1 reductase activity and is independent of removal of both d4-cortisol and d3-cortisol by other enzymes. In tissues in which there is no source of cortisol production other than by 11β-HSD1, the dilution of d4-cortisol by cortisol also indicates 11β-HSD1 activity. Using this technique, we and others have quantified substantial cortisol release into the hepatic vein by 11β-HSD1 in the splanchnic circulation (visceral organs plus liver) (16,17). Moreover, by extrapolating from the rate of cortisol release into hepatic vein during first-pass liver metabolism of an oral dose of cortisone,
Cortisol release from subcutaneous adipose tissue in healthy men. Subjects were served breakfast (30 g cornflakes and 300 ml skim milk) at 0800–0830 h, and 5% dextrose (50 ml/h) was infused intravenously throughout the study. Once the deuterated cortisol infusion was established, a 20G 15-cm catheter was sited in a superficial epigastric vein, as previously described (19). To ensure that blood was collected from subcutaneous adipose tissue and not from deeper structures, O2, saturation was confirmed to be >85%. After 2 h of d4-cortisol infusion, 1–2 MBq 133Xenon (gas) was injected subcutaneously beside the umbilicus, and radioactivity was measured continuously with a NaI detector to assess blood flow (20,21). Blood samples were taken from arterialized hand vein and superficial epigastric vein at intervals (Fig. 2).

Cortisol release into portal and hepatic veins in TIPSS patients. These patients were not given breakfast or infused with dextrose. During the d4-cortisol infusion, dexamethasone was concurrently infused at 240 μg/h. Twenty minutes after beginning the tracer infusion, the right internal jugular vein was cannulated under local anesthesia (5 ml 2% lidocaine), and a 5F pigtail catheter (Cordis, Berks., U.K.) was passed into the TIPSS under X-ray guidance. After confirming patency of the TIPSS, the catheter was positioned in the portal vein for sampling. A 5F vertebral catheter (Merrit Medical, Lanarkshire, U.K.) was then placed in a separate tributary of the hepatic vein for sampling. From 2 h after beginning the tracer infusion, infocyanine green (ICG) (Pulsion Medical, Middlesex, U.K.) was infused into the antecubital vein at 30 mg/h. Blood samples were taken from the portal, hepatic, and arterialized veins at intervals (Fig. 2).

**Laboratory analyses.** Plasma cortisol, d3-cortisol, d4-cortisol, cortisone, and d3-cortisone were measured by liquid chromatography–tandem mass spectrometry (LC-MS/MS). Epi-cortisol (500 ng) was added to 1.5 ml plasma and extracted using 15 ml chloroform. Samples were evaporated and then reconstituted in mobile phase (60% methanol and 40% 5 mmol/l ammonium acetate) before injection into a Thermo Finnigan LC-MS/MS, consisting of a TSQ Quantum Discovery Mass Spectrometer and a Surveyor Liquid Chromatogram using an Allure biphenyl column (50 mm × 4.6 mm × 5 μm; Thams Restek), with column temperature 25°C and mobile phase flow rate 0.5 ml/min. Ionization was achieved by positive electrospray. The precursor and product mass–to–charge ratios used were as follows: cortisol (363–121), d3-cortisol (366–121), d4-cortisol (367–121), cortisone (361–163), and d3-cortisone (364–164). Samples were quantified by the ratio of area under peak of interest to area under peak of internal standard against a standard curve.

Serum ICG was measured by adapting a previous method (22) using diazepam as internal standard. Briefly, acetoniirile (ACN) was added to serum to sediment protein, the supernatant was mixed with ammonium sulfate and centrifuged, and the organic phase was added to water before analysis by high-performance liquid chromatography (HPLC) using a P680 HPLC pump and a PDA-100 photodiode array detector (Dionex, Sunnyvale, CA) with a Nova-pak C18 column (300 mm × 3.9 mm × 5 μm; Thams Restek), with column temperature 25°C and mobile phase flow rate 0.5 ml/min. Ionization was achieved by positive electrospray. The precursor and product mass–to–charge ratios used were as follows: cortisol (363–121), d3-cortisol (366–121), d4-cortisol (367–121), cortisone (361–163), and d3-cortisone (364–164). Samples were quantified by the ratio of area under peak of interest to area under peak of internal standard against a standard curve.

Plasma glucose, lipids, and liver function were measured using enzymatic colorimetric methods on an Olympus Diagnostics analyzer (County Clare, Ireland). Plasma A1C was analyzed by HPLC using a Variant II analyzer (Bio-Rad Laboratories, Hertfordshire, U.K.).

**Kinetic analyses.** Calculations in each subject used the mean of measurements in steady state, between 180 and 210 min of d4-cortisol infusion (Fig. 2). Where possible, kinetic calculations relying on tracer-to-tracee ratios rather than concentrations were favored to minimize variability. The equations are derived from Wolfe and Chinkes (23).

The concentration of ICG in the artery (A) and hepatic vein (HV) in steady state (ss) were used to calculate hepatic blood flow (HBF) (24) using:

\[
\text{HBF (liters/min)} = \frac{\text{ICG extraction rate}}{\left(\frac{\text{ICG}_{\text{HV}}}{\text{ICG}_{\text{A}}}ight) - \left(\frac{\text{ICG}_{\text{HV}}}{\text{ICG}_{\text{A}}}ight) \times \frac{1}{\text{hematocrit}}} \tag{1}
\]

Whole-body cortisol kinetics were calculated in measurements from arterialized samples (15,16) using Eqs. 2 and 3, where “cortisol” denotes unlabeled cortisol. Clearances were calculated by dividing the infusion rate (of cortisol or d4-cortisol) by the steady-state arterial concentration (of cortisol or d4-cortisol) (15).

Rate of appearance of cortisol = \[
\frac{\text{d4-cortisol infusion rate}}{\text{d4-cortisol/cortisol}} - \text{cortisol infusion rate} \tag{2}
\]
The rate of appearance of d3-cortisol is given by

$$\text{Rate of appearance of d3-cortisol} = \frac{\text{d4-cortisol infusion rate}}{\text{d4-cortisol/d3-cortisol}}$$

Subcutaneous adipose tissue production of cortisol and d3-cortisol were calculated from measurements in arterialized (A) and superficial epigastric vein (V) samples using Eqs. 4 and 5. “Tissue delivery” is synonymous with “influx.”

Subcutaneous cortisol production is given by

$$\text{Subcutaneous cortisol production} = \left( \frac{\text{tissue cortisol delivery}}{\text{d4-cortisol/d3-cortisol}} \right) \times \frac{[\text{d4-cortisol}]/[\text{d3-cortisol}]}{[\text{d4-cortisol}]/[\text{d3-cortisol}]}$$

where

FIG. 2. Left panel: subcutaneous sampling; right panel: visceral sampling. Plasma measurements during deuterated cortisol infusion. Data are means ± SE for n = 6 (subcutaneous measurements) and n = 4 (visceral measurements) during deuterated cortisol infusion, with plasma samples from arterialized (■), portal or subcutaneous (◇), and hepatic (▲) cannulae. Plasma cortisol concentrations (A), plasma d4-cortisol enrichment (C), and d4-cortisol-to-d3-cortisol ratio (E) for subcutaneous study. Plasma cortisol concentrations (B), plasma d4-cortisol enrichment (D), d4-cortisol-to-d3-cortisol ratio (F) for visceral study. Statistical comparison of mean values in steady state (180–210 min) is shown in Table 2.
Tissue cortisol delivery = blood flow \times [cortisol]_tissue

Subcutaneous d3-cortisol production = \left( \text{tissue d3-cortisol delivery} \times \frac{[d4-cortisol/d3-cortisol]_A}{[d4-cortisol/d3-cortisol]_V} \right) - \text{tissue d3-cortisol delivery} \tag{5}

where

Tissue d3-cortisol delivery = blood flow \times [d3-cortisol]_tissue

Splanchnic cortisol and d3-cortisol production (from visceral tissues and liver combined) were calculated from measurements in arterialized (A) and hepatic vein (HV) samples as previously described (16) using Eqs. 6 and 7, in which “tissue delivery” was calculated as above.

Splanchnic cortisol production = \left( \text{tissue cortisol delivery} \times \frac{[d4-cortisol/d3-cortisol]_A}{[d4-cortisol/d3-cortisol]_V} \right) - \text{tissue cortisol delivery} \tag{6}

Splanchnic d3-cortisol production = \left( \text{tissue d3-cortisol delivery} \times \frac{[d4-cortisol/d3-cortisol]_A}{[d4-cortisol/d3-cortisol]_V} \right) - \text{tissue d3-cortisol delivery} \tag{7}

Liver production of cortisol and d3-cortisol were calculated from measurements in portal vein (PV) and hepatic vein (HV) using Eqs. 8 and 9. HBF was measured by ICG extraction (Eq. 1) but portal blood flow (PBF) and hepatic arterial blood flow (HABF) were not measured. In healthy individuals, the portal vein provides ~80% and the hepatic artery ~20% of total liver blood flow (25). In cirrhosis, PBF decreases and HABF increases (26,27), such that as little as 10% of HBF may originate from the portal vein (25). To account for this unknown, we have modeled PBF to range between 10 and 80% of HBF and concordantly for HABF to range between 90 and 20% of HBF.

Hepatic cortisol production = \left( \frac{\text{hepatic cortisol delivery} \times (HBF/\text{HBF}) \times [d4-cortisol/d3-cortisol]_A}{[d4-cortisol/d3-cortisol]_V} \right) - \text{hepatic cortisol delivery}

where

Hepatic cortisol delivery = (HBF \times [cortisol]_hepatic) + (PBF \times [cortisol]_PV)

Hepatic d3-cortisol production = \left( \text{hepatic d3-cortisol delivery} \times \frac{(HBF/\text{HBF}) \times [d4-cortisol/d3-cortisol]_A}{[d4-cortisol/d3-cortisol]_V} \right) - \text{hepatic d3-cortisol delivery}

where

Hepatic d3-cortisol delivery = (HBF \times [d3-cortisol]_hepatic) + (PBF \times [d3-cortisol]_PV)

Visceral production of cortisol and d3-cortisol (i.e., release into the portal vein) was calculated using measurements from arterialized blood and portal vein with estimates of PBF as above, using Eqs. 10 and 11, in which tissue delivery was calculated as above.

Visceral cortisol production = \left( \text{visceral cortisol delivery} \times \frac{[d4-cortisol/d3-cortisol]_A}{[d4-cortisol/d3-cortisol]_V} \right) - \text{visceral cortisol delivery}

Visceral d3-cortisol production = \left( \text{visceral d3-cortisol delivery} \times \frac{[d4-cortisol/d3-cortisol]_A}{[d4-cortisol/d3-cortisol]_V} \right) - \text{visceral d3-cortisol delivery}

Net production of cortisone and d3-cortisone across the viscera, liver, and the splanchnic tissues were calculated using Eqs. 12 and 13, in which the relevant measurements of blood flow and venous concentrations were substituted as appropriate.

Visceral cortisone production = \left( \text{visceral cortisone delivery} \times \frac{[cortisone]_V}{[cortisone]_A} \right) \times \text{blood flow}

Visceral d3-cortisone production = \left( \text{visceral d3-cortisone delivery} \times \frac{[cortisone]_V}{[cortisone]_A} \right) \times \text{blood flow}

Statistical analysis. Using variance of steady-state kinetic parameters, a power calculation showed that including four patients in the portal vein sampling study gives >80% power to detect (to P < 0.05) release of 10 nmol/min cortisol into the portal vein. This provides ample power to detect the ~30 nmol/min cortisol, which was estimated to be released into the portal vein from indirect modeling (16).

Using SPSS version 14, comparisons were by paired t tests or repeated measures ANOVA with post hoc Fisher’s least significant differences test, as appropriate. Differences from zero were determined using the one-sample t test. P < 0.05 was considered significant. Data are presented as mean ± SE or, for calculated kinetic parameters, mean (95% CI).

RESULTS

Subject characteristics. Participants’ anthropometric and biochemical characteristics are shown in Table 1.

Superficial epigastric vein cannulation study. Adrenal cortisol production was suppressed by dexamethasone, with fasting morning plasma cortisol concentrations of 23 ± 9 nmol/l and cortisone concentrations of 9 ± 4 nmol/l. Steady state was achieved between 180 and 210 min of d4-cortisol infusion (Fig. 2).

Mean plasma measurements at steady state are in Table 2. Cortisol and d4-cortisol clearance was 0.56 ± 0.09 and 0.90 ± 0.09 l/min, respectively. Plasma cortisol concentrations were not different between artery and superficial epigastric vein; however, there was a trend for increased d3-cortisol levels in the vein (P < 0.06). d4-Cortisol,
cortisone, and d3-cortisone concentrations were unchanged between artery and vein.

The rates of appearance in arterial blood of cortisol (Eq. 2) and d3-cortisol (a specific measure of whole-body 11β-HSD1 activity; Eq. 3) were 33.8 (95% CI 16.0–51.5) and 28.3 (25.6–31.0) nmol/min, respectively. Adipose blood flow measured 2.5 ± 0.7 ml · min⁻¹ · 100 g⁻¹ adipose tissue. There was significant release across the subcutaneous adipose bed of both cortisol (15.0 [0.4–29.5] pmol · min⁻¹ · 100 g⁻¹ adipose tissue; Eq. 4) and d3-cortisol (8.7 [0.2–17.2] pmol · min⁻¹ · 100 g⁻¹ adipose tissue; Eq. 5) (both P < 0.05 vs. zero).

**Hepatic and portal vein cannulation study.** Fasting morning plasma cortisol and cortisone concentrations were suppressed by dexamethasone, measuring 15 ± 5 and 11 ± 2 nmol/l, respectively. Steady state was achieved between 180 and 210 min of d4-cortisol infusion (Fig. 2).

Mean plasma measurements at steady state are in Table 2. Cortisol and d4-cortisol clearance was 0.27 ± 0.03 and 0.47 ± 0.06 l/min, respectively. In the hepatic vein, d3-cortisol concentrations were increased, and cortisone and d3-cortisone concentrations decreased, consistent with substantial intrahepatic steroid extraction and 11β-HSD1 reductase activity. Conversely, d4-cortisol, which cannot be regenerated by 11β-HSD1, was lower in the hepatic vein, consistent with substantial intrahepatic cortisol metabolism. In the portal vein, cortisol, d3-cortisol and d4-cortisol concentrations were unaltered compared with arterial blood. However, d3-cortisone concentrations were significantly increased, and there was a trend for increased cortisone (P = 0.051) in portal vein, consistent with visceral 11β-dehydrogenase activity.

The whole-body rates of appearance of cortisol (Eq. 2) and d3-cortisol (Eq. 3) in the artery were 36.3 (95% CI 24.4–48.2) and 26.9 (21.0–32.7) nmol/min, respectively. HBF by ICG extraction was 0.40 ± 0.08 l/min. Splanchnic production of cortisol (13.5 [3.6–23.5] nmol/min; Eq. 6) and d3-cortisol (8.0 [2.6–13.5] nmol/min; Eq. 7) was substantial (both P < 0.05 vs. zero). This could be accounted for entirely by the liver, because hepatic cortisol (Eq. 8) and d3-cortisol (Eq. 9) production rates were 13.3 (1.3–25.4) and 7.7 (1.3–14.2) nmol/min, respectively (both P < 0.05 vs. zero and not different from splanchnic production rates) when PBF was estimated as 40% of total HBF (28). No visceral cortisol (Eq. 10) or d3-cortisol (Eq. 11) release into the portal vein was detected (0.0 [−1.7 to 1.7] and 0.1 [−0.7 to 1.1] nmol/min, respectively). Modeling for portal venous flow from 10–80% of HBF did not significantly alter these results (not shown).

Net cortisol and d3-cortisone production across the splanchnic tissues (Eqs. 12 and 13) measured −18.2 (95% CI −33.3 to −3.2) and −9.2 (−15.4 to −3.0) nmol/min, respectively (both P < 0.05 vs. zero). This was accounted for by cortisol and d3-cortisone extraction across the liver (−20.8 [−39.4 to −2.2] and −10.6 [−18.3 to −2.8] nmol/min, estimating portal flow as 40% of HBF), consistent with substantial hepatic cortisone metabolism and 11β-HSD1 reductase activity. However, across the viscera, net cortisol production rate did not differ from zero (2.6 [−1.2 to 6.3] nmol/min; P = 0.12), and there was a trend for net generation of d3-cortisone (1.4 [−0.4 to 3.1] nmol/min; P = 0.09 vs. zero).

**DISCUSSION**

These data quantify for the first time the contributions of subcutaneous adipose tissue, visceral tissues, and liver to whole-body cortisol production by 11β-HSD1 in humans. We confirmed that splanchnic cortisol production is substantial, and we attribute this entirely to 11β-HSD1 activity in the liver. However, although we could not detect release of cortisol by 11β-HSD1 into the portal vein, which drains a number of visceral organs, we found significant cortisol release into veins draining exclusively subcutaneous adipose tissue. Similar results were obtained using the equations derived by Basu et al. (18). These results allow us to put in context the variations in 11β-HSD1 activity described in biopsied tissue, for example, in obesity.

The absolute rates of appearance of cortisol and d3-cortisol in steady state are sensitive to the prevailing concentrations of cortisone and d3-cortisone, the substrates for 11β-HSD1, which are determined by the rates of exogenous cortisol and d4-cortisol infusion. Moreover, the implications for intra-adipose cortisol concentrations are impossible to estimate, because venous changes in concentration at a given rate of appearance are highly dependent on tissue blood flow. However, with these and other caveats in mind, we can attempt to extrapolate from these data what some of the consequences might be for endogenous cortisol metabolism. If the release of cortisol by adipose tissue in the anterior abdominal wall were mirrored in all adipose depots, then the observed cortisol production rate of 15 pmol · min⁻¹ · 100 g⁻¹ adipose tissue would equate with a whole-body production rate of 4.0 ±

**Table 2**

| Subcutaneous measurements | Visceral measurements |
|--------------------------|-----------------------|
| **Artery** | **Subcutaneous vein** | **Artery** | **Portal vein** | **Hepatic vein** |
| cortisol (nmol/l) | 97 ± 16 | 103 ± 15 | 186 ± 17 | 176 ± 22 | 180 ± 18 |
| d3-cortisol (nmol/l) | 33 ± 4 | 36 ± 4 | 59 ± 4 | 56 ± 4 | 65 ± 6‡ |
| d4-cortisol (nmol/l) | 37 ± 3 | 37 ± 4 | 71 ± 9 | 67 ± 11 | 57 ± 6* |
| cortisone (nmol/l) | 17 ± 2 | 14 ± 2 | 75 ± 6 | 91 ± 6 | 22 ± 16‡ |
| d3-cortisone (nmol/l) | 8 ± 1 | 6 ± 2 | 36 ± 4 | 45 ± 4* | 9 ± 8‡ |
| d4-cortisol-to-cortisol ratio | 0.40 ± 0.03 | 0.38 ± 0.03* | 0.38 ± 0.02 | 0.38 ± 0.02 | 0.32 ± 0.01†‡ |
| d4-cortisol-to-d3-cortisol ratio | 1.13 ± 0.04 | 1.02 ± 0.04‡ | 1.19 ± 0.08 | 1.17 ± 0.10 | 0.87 ± 0.07‡ |

Data are means ± SE for n = 6 (subcutaneous measurements) and n = 4 (visceral measurements) participants. The mean data from samples obtained between 180 and 210 min during the deuterated cortisol infusion was used to calculate steady-state concentrations. Comparisons were made by paired t test (subcutaneous study) or repeated measures ANOVA with post hoc testing with Fisher’s least significant differences test (visceral study). *P < 0.05 and ‡P < 0.001 compared with artery; †P < 0.05 compared with portal vein.
1.5 nmol/min, based on a total adipose mass of 29.4 \pm 7.1 kg measured in our participants using bioimpedance. This represents \sim 12\% of whole-body cortisol regeneration by 11\beta-HSD1. Significant 11\beta-HSD1 activity in subcutaneous adipose tissue is supported by the trend for a fall in cortisone concentrations from artery to vein (Table 2), which, although not statistically significant, was of similar magnitude to previous larger studies (10,11). However, release of cortisol from subcutaneous adipose tissue may be offset by intra-adipose cortisol clearance, e.g., by 5\alpha-reductase type 1 (29), so that arterial and venous cortisol concentrations are unaltered (Table 2), as previously described (10,11). Nevertheless, these data are consistent with the hypothesis that variations in 11\beta-HSD1 have a significant impact on intra-adipose cortisol concentrations (2,3,7).

Cortisol release from subcutaneous adipose tissue into the systemic circulation is unlikely to have effects in other organs, because the feedback control by the hypothalamic-pituitary-adrenal axis will adjust adrenal cortisol secretion to maintain circulating cortisol concentrations. Therefore, the most likely impact of this source of cortisol will be intracrine or paracrine in the local adipose environment. However, release from visceral adipose tissue into the portal vein could deliver cortisol directly to the liver, contributing to the association of central obesity with hepatic insulin resistance and dyslipidemia (4,16). We did not, however, detect release of cortisol from 11\beta-HSD1 in visceral tissues into portal vein, in agreement with a study in dogs (18). Assuming portal venous blood accounts for 40\% of total HBF (28), the mean estimate for visceral cortisol release from these subjects was 0.0 (95\% CI 1.7 to 1.7) nmol/min. Although the number of subjects included was small (n = 4), this provides 97.5\% confidence that visceral cortisol production is <1.7 nmol/min. Because hepatic cortisol delivery was 72.7 nmol/min, we can conclude that any cortisol released by visceral 11\beta-HSD1 would not significantly impact on hepatic cortisol delivery.

To access portal vein samples, we studied patients with alcoholic liver disease and TIPSS. Although sufficiently unwell with portal hypertension to require a TIPSS, these patients were stable and were all overweight or obese. Cortisol metabolism is abnormal in cirrhosis (30–32), although specific measurements of 11\beta-HSD1 activity have not been reported. Compared with healthy volunteers undergoing subcutaneous adipose tissue measurements, our cirrhotic patients had lower whole-body clearance of cortisol and d4-cortisol and hence higher endogenous and deuterated cortisol and cortisone concentrations in steady state (Table 2). Splanchnic cortisol release was less than one-third of that reported previously under similar conditions (16,17,33), even though whole-body cortisol regeneration by 11\beta-HSD1 was not unusually low. This paradox may reflect either upregulation of 11\beta-HSD1 in non-splanchnic tissues or an underestimation of splanchnic cortisol release because of misleading blood flow measurement. Although it is plausible that chronic liver disease may reduce hepatic 11\beta-HSD1 activity, it seems less likely that it would affect extrahepatic 11\beta-HSD1. As previously described in TIPSS patients (34), HBF estimated by ICG extraction was substantially lower than in healthy volunteers. Although none of our patients had reversed flow in the portal vein (hepatofugal flow) (35), a proportion of blood in the portal circulation is shunted away from the portal vein in patients with liver disease via anastomoses with the systemic circulation (36). However, none of these alterations predict that cortisol release into the portal vein should be artifically low.

The portal vein drains blood from other organs, including gut, pancreas, and spleen. Although our results suggest that none of these organs releases cortisol by 11\beta-HSD1 reductase activity, it remains possible that venous drainage from the other visceral tissues dilutes any observable change in d3-cortisol-to-d4-cortisol ratios in blood from visceral adipose tissue. HBF was measured at 400 ml/min, of which portal vein flow may be up to 320 ml/min. This compares with blood flow of just 2.5 ml \cdot min^{-1} \cdot 100 g^{-1} in subcutaneous adipose tissue. If adipose blood flow were the same in the visceral as in the subcutaneous adipose depot and the visceral depot weighed 3 kg (37), then the contribution of visceral fat to PBF may be 75 ml/min, as little as a quarter of total flow. This dilution effect could only be overcome by cannulating an omental vein during d4-cortisol infusion, which is unlikely to be achievable during steady-state tracer cortisol infusion in unstimulated subjects.

Steady-state plasma d3-cortisone concentrations were significantly higher in portal vein than in artery, and there was a similar trend for cortisone concentrations (Table 2). Cortisol, d3-cortisol, and d4-cortisol concentrations showed opposite trends. Although not confirmed by statistically significant differences in visceral d3-cortisone production, these results suggest 11\beta-dehydrogenase activity, converting cortisol to cortisone in the viscera. This is likely due to 11\beta-HSD type 2 activity in the gut, although it is conceivable that 11\beta-HSD1 may be functioning in the dehydrogenase direction in visceral adipose tissue (38).

In a previous study, we measured splanchnic cortisol production in steady state and modeled the relative contribution of liver and visceral tissues by measuring first pass conversion of oral cortisone into cortisol in the hepatic vein (16). We estimated that up to two-thirds of splanchnic cortisol production occurs in visceral tissues and that portal vein cortisol concentrations were likely to be \sim 30 nmol/l higher than arterial concentrations. The current data do not support these estimates, at least in patients with cirrhosis. This discrepancy is most likely due to portal vein cortisone concentrations being higher than we predicted; our model was based in part on removal of cortisone by 11\beta-HSD1 in visceral adipose tissue, as occurs in subcutaneous adipose tissue (see above). Revisiting our model in light of the new finding of higher cortisone concentrations in the portal vein, we have confirmed that it predicts much higher steady-state cortisol regeneration in the liver and hence a much lower contribution from visceral adipose tissue.

What implications do these observations have for patients with obesity? The mean BMI in our participants was in the obese range (31 kg/m\textsuperscript{2} in the subcutaneous adipose tissue study and 32 kg/m\textsuperscript{2} in the portal vein study), although the numbers (n = 4–6) were too small to allow meaningful correlations with indexes of 11\beta-HSD1 activity. Previous measurements in biopsies suggest that 11\beta-HSD1 activity is \sim 2.5-fold higher per gram of adipose tissue in obese people (BMI \sim 31 kg/m\textsuperscript{2}) than in lean controls with BMI \sim 9 kg/m\textsuperscript{2} lower (6,39). Given the K\textsubscript{m} of human 11\beta-HSD1 for cortisone of \sim 1 \mu mol/l (40), it is reasonable to assume a linear relationship between 11\beta-HSD1 protein concentrations and cortisol generation rates within the physiological range of cortisone concentrations of \sim 10–100 nmol/l. Therefore, we anticipate that a 10-kg/m\textsuperscript{2} increase in BMI might elevate the cortisol produc-
tion rate in subcutaneous adipose tissue by up to 2.5-fold (i.e., 37.5 pmol \cdot min^{-1} \cdot 100 g^{-1}) and that, accounting for an associated ~15 kg increase in fat mass, this equates with an increase in whole-body adipose cortisol production of ~12.7 nmol/min. In obesity, hepatic 11β-HSD1 activity is decreased by ~50% (6,39,41). The predicted increase in cortisol release from adipose tissue may cancel out the decrease in cortisol release from the liver in obesity, potentially explaining the lack of change in whole-body d3-cortisol production rate (5,33).

These data support the concept that 11β-HSD1 is a key determinant of intra-adipose cortisol concentrations but appear to refute the concept that 11β-HSD1 substantially elevates cortisol concentrations in the portal vein.

ACKNOWLEDGMENTS

This work was funded by the British Heart Foundation, the Translational Medicine Research Institute, the Northern Sweden County Council, the Sweden Research Council, and the Sweden Heart and Lung Foundation and Heart Centre.

Within the last two years, T.O. has received consultant fees from Wyeth Pharmaceuticals and lecture fees from GlaxoSmithKline. Within the past two years, B.R.W. has consulted for AstraZeneca, Dainippon Sumitomo, Merck, Johnson & Johnson, Incyte, Ipsen, Roche, Vitae, Wyeth, and Zydus Research Centre, received lecture fees from Abbott and Bristol Myers Squibb, and received research funding from Wyeth. B.R.W. is also an inventor with relevant patents held by the University of Edinburgh. No other potential conflicts of interest relevant to this article were reported.

We thank Jill Harrison, Scott Denham, Natalie Homer, Kim MacBeth, Inger Arnesjo, Britt-Inger Norberg, Alistair Millar, Jean Antonelli, Lesley Breen, and the staff of the Welcome Trust Clinical Research Facility and its Mass Technical Advice.

Welcome Trust Clinical Research Facility and its Mass Technical Advice.

This work was funded by the British Heart Foundation, the Translational Medicine Research Institute, the Northern Sweden County Council, the Sweden Research Council, and the Sweden Heart and Lung Foundation and Heart Centre.

REFERENCES

1. Macfarlane DP, Forbes S, Walker BR: Glucocorticoids and fatty acid metabolism in humans: fuelling fat redistribution in the metabolic syndrome. J Endocrinol 197:189–201, 2008
2. Seck JR, Walker BR: 11β-Hydroxysteroid dehydrogenase type 1: a tissue-specific amplifier of glucocorticoid action. Endocrinology 142:1371–1376, 2001
3. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Laverty GG, Cooper MS, Hewison M, Stewart PM: 11Beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. Endor Rev 25:831–866, 2004
4. Bujalska IJ, Kumar S, Stewart PM: Does central obesity reflect 'Cushing's disease of the omentum'? Lancet 349:1210–1213, 1997
5. Sandeep TC, Andrew R, Homer NZ, Andrews RC, Smith K, Walker BR: Increased in vivo regeneration of cortisol in adipose tissue in human obesity and effects of the 11β-hydroxysteroid dehydrogenase type 1 inhibitor carbocortolone. Diabetes 54:572–578, 2005
6. Rask E, Olsson T, Soderberg S, Andrew R, Livingstone DEW, Johnson O, Walker BR: Tissue-specific dysregulation of cortisol metabolism in obesity. J Clin Endocrinol Metab 86:1418–1421, 2001
7. Walker BR: Glucocorticoids and cardiovascular disease. Eur J Endocrinol 158:545–559, 2007
8. Hughes KA, Webster SP, Walker BR: 11-beta-hydroxysteroid dehydrogenase type 1 (11beta-HSD1) inhibitors in type 2 diabetes mellitus and obesity. Expert Opin Investig Drugs 17:451–496, 2008
9. Masuzaki H, Paterson J, Shinaya H, Morton NM, Mullins JJ, Seck JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. Science 294:2166–2170, 2001
10. Katz JR, Mohamed-Ali V, Wood PJ, Yudkin JS, Coppack SW: An in vivo study of the cortisol-cortisone shuttle in subcutaneous abdominal adipose tissue. Clin Endocrinol 50:63–68, 1999
11. Wake DJ, Sisson RH, Tan GD, Homer NZ, Andrew R, Karpe F, Walker BR: Effects of peroxisome proliferator-activated receptor (PPAR) [alpha] and [gamma] agonists on 11beta-hydroxysteroid dehydrogenase type 1 in subcutaneous adipose tissue in men. J Clin Endocrinol Metab 92:1848–1856, 2007
12. aldahi W, Mun E, Goldfine AB: Portal and peripheral cortisol levels in obese humans. Diabetologia 47:833–836, 2004
13. Tarantino G, Lobello R, Scopacasa F, Contaldo F, Pasanisi F, Cirillo M, De Caroli M, Conca P, Terracciano M, Gennarini R, Ariello M, Mazzarella C, Grimaldi E, Macchia V: The contribution of omental adipose tissue to adiopine concentrations in patients with the metabolic syndrome. Clin Invest Med 30:E192–E199, 2007
14. Walker BR, Campbell JC, Fraser R, Stewart PM, Edwards CRW: Mineralocorticoid excess and inhibition of 11β-hydroxysteroid dehydrogenase in patients with ectopic ACTH syndrome. J Endocrinol (Oxf) 27:483–492, 1992
15. Andrew R, Smith K, Jones GC, Walker BR: Distinguishing the activities of 11beta-hydroxysteroid dehydrogenases in vivo using isotopically labelled cortisol. J Clin Endocrinol Metab 87:277–285, 2002
16. Andrew R, Westerbacka J, Wahren J, Yki-Jarvinen H, Walker BR: The contribution of visceral adipose tissue to splanchic cortisol production in healthy humans. Diabetes 54:1394–1370, 2005
17. Basu R, Singh RJ, Basu A, Chittilapilly EG, Johnson CM, Toffolo G, Cobelli C, Rizza RA: Splanchic cortisol production occurs in humans: evidence for conversion of cortisone to cortisol via the 11-beta hydroxysteroid dehydrogenase (11beta-hsd) type 1 pathway. Diabetes 53:2051–2059, 2004
18. Basu R, Edgerton DS, Singh RJ, Cherrington A, Rizza RA: Splanchic cortisol production in dogs occurs primarily in the liver: evidence for substantial hepatic specificity of 11beta-hydroxysteroid dehydrogenase type 1 activity. Diabetes 55:3013–3019, 2006
19. Coppack SW, Fisher RM, Gibbons GF, Humphreys SM, McDonough MJ, Potts JL, Fryan KN: Postprandial substrate deposition in human forearm and adipose tissues in vivo. Clin Sci (Lond) 70:339–348, 1986
20. Larsen OA, Lassen NA, Quaade F: Blood flow through human adipose tissue determined with radioactive xenon. Acta Physiol Scand 66:337–345, 1966
21. Samra JS, Fryan KN, Giddings JA, Clark ML, MacDonald IA: Modification and validation of a commercially available portable detector for measurement of adipose tissue blood flow. Clin Physiol 15:241–248, 1995
22. Burns E, Triger DR, Tucker GT, Bax ND: Inodopamine green elimination in patients with liver disease and in normal subjects. Clin Sci (Lond) 89:155–160, 1991
23. Wolfe RL, Chinks D: Arterio-venous balance technique to measure amino acid kinetics. In Isotope Tracers in Metabolic Research. Principles and Practice of Kinetic Analysis. 2nd ed. New York, John Wiley and Sons, 2005, p. 381–420
24. Lee CL, Mendenhall CL, Lesko W, Howard MM: Estimation of hepatic blood flow with indocyanine green. J Clin Invest 41:1119–1177, 1962
25. Zoli M, Magalotti D, Bianchi G, Gueli C, Orlandini C, Grimaldi M, Marchesini G: Total and functional hepatic blood flow decrease in parallel with ageing. Age Ageing 28:29–33, 1999
26. Richter S, Mucke I, Mengel MD, Vollmar B: Impact of intrinsic blood flow regulation on cirrhosis maintenance of hepatic arterial buffer response. Am J Physiol Gastrointest Liver Physiol 279:G454–G462, 2000
27. Lautta WW: Regulatory processes interacting to maintain hepatic blood flow constancy: vascular compliance, hepatic arterial buffer response, hepatorenal reflex, liver regeneration, escape from vasoconstriction. Hepatol Res 37:891–901, 2007
28. Menzel J, Schober O, Reimer P, Domuschke W: Scintigraphic evaluation of hepatic blood flow after intrahepatic portosystemic shunt (TIPS). Eur J Nucl Med 24:655–641, 1997
29. Wake DJ, Strand M, Rask E, Westerbacka J, Livingstone DE, Soderberg S, Andrew R, Yki-Jarvinen H, Olsson T, Walker BR: Intra-adipose sex steroid metabolism and body fat distribution in idiopathic human obesity. Clin Endocrinol (Oxf) 66:440–446, 2007
30. Tyler FH: The effect of liver disease on adrenal cortical function. Am J Clin Nutr 5:377–380, 1957
31. Kawai S, Ichikawa Y, Homma M: Differences in metabolic properties of adrenocortical hormones in renal failure. New Horizons for Hormone Research. 15:241–248, 1995
32. Stewart PM, Burra P, Shackleton CHL, Sheppard MC, Elias E: 11β-Hydroxysteroid dehydrogenase deficiency and glucocorticoid status in humans. Diabetes, Vol. 58, January 2009
patients with alcoholic and non-alcoholic liver disease. J Clin Endocrinol Metab 76:748–751, 1993
33. Basu R, Singh RJ, Basu A, Chittilapilly EG, Johnson MC, Toffolo G, Cobelli C, Rizza RA: Obesity and type 2 diabetes do not alter splanchnic cortisol production in humans. J Clin Endocrinol Metab 90:3919–3926, 2005
34. Rodriguez-Laiz JM, Banares R, Echenagusia A, Casado M, Camunez F, Perez-Roldan F, de Diego A, Cos E, Clemente G: Effects of transjugular intrahepatic portasystemic shunt (TIPS) on splanchnic and systemic hemodynamics, and hepatic function in patients with portal hypertension: preliminary results. Dig Dis Sci 40:2121–2127, 1995
35. Rector WG Jr, Hoefs JC, Hossack KF, Everson GT: Hepatofugal portal flow in cirrhosis: observations on hepatic hemodynamics and the nature of the arterioporal communications. Hepatology 8:16–20, 1988
36. Bosch J, Pizcueta P, Feu F, Fernandez M, Garcia-Pagan JC: Pathophysiology of portal hypertension. Gastroenterol Clin North Am 21:1–14, 1992
37. Westerbacka J, Yki-Jarvinen H, Vehkavaara S, Hakkinen A-M, Andrew R, Wake DJ, Seckl JR, Walker BR: Body fat distribution and cortisol metabolism in healthy men: enhanced 5β-reductase and lower cortisol/cortisone metabolite ratios in men with fatty liver. J Clin Endocrinol Metab 88:4924–4931, 2003
38. Bujalska IJ, Walker EA, Tomlinson JW, Hewison M, Stewart PM: 11Beta-hydroxysteroid dehydrogenase type 1 in differentiating omental human preadipocytes: from de-activation to generation of cortisol. Endocr Res 28:449–461, 2002
39. Rask E, Walker BR, Soderberg S, Livingstone DE, Eliasson M, Johnson O, Andrew R, Olsson T: Tissue-specific changes in peripheral cortisol metabolism in obese women: increased adipose 11beta-hydroxysteroid dehydrogenase type 1 activity. J Clin Endocrinol Metab 87:3330–3336, 2002
40. Shafqat N, Elleby B, Svensson S, Shafqat J, Jornvall H, Abrahmsen L, Oppermann U: Comparative enzymology of 11beta-hydroxysteroid dehydrogenase type 1 from glucocorticoid resistant (guinea pig) versus sensitive (human) species. J Biol Chem 278:2030–2035, 2003
41. Stewart PM, Boulton A, Kumar S, Clark PMS, Shackleton CHL: Cortisol metabolism in human obesity: impaired cortisone: cortisol conversion in subjects with central adiposity. J Clin Endocrinol Metab 84:1022–1027, 1999