Cortisol release from adipose tissue by 11β-hydroxysteroid dehydrogenase type 1 in humans

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ABSTRACT

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, has not been quantified in vivo.

Research Design and Methods: Six healthy men underwent 9,11,12,12-[2H]4-cortisol infusions with simultaneous sampling of arterialised 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 arterialised peripheral vein.

Results: Significant cortisol and 9,12,12-[2H]3-cortisol release were observed from subcutaneous adipose tissue (15.0 (95% confidence intervals 0.4, 29.5) and 8.7 (0.2, 17.2) pmol/min/100g adipose tissue, respectively). Splanchnic release of cortisol and 9,12,12-[2H]3-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 signalling 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 intra-hepatic glucocorticoid signalling.
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 3H2-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 (reviewed 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). Indeed, transgenic overexpression of 11β-HSD1 in adipose tissue in mice results in a 2-3 fold 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 arterio-venous 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]4-cortisol (d4-cortisol) – for this purpose (15). During d4-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 (Figure 1). The dilution of d4-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 where 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, we estimated that a substantial proportion of splanchnic cortisol production occurs in visceral tissues as well as liver (16). However, direct cannulation of veins draining adipose tissue depots during tracer cortisol infusion has not been reported, and portal vein sampling has only been performed in dogs in which cortisol release by visceral tissues was undetectable (18).

Here we report results of deuterated cortisol infusions with selective venous...
cannulation to measure arterio-venous differences across subcutaneous adipose tissue and visceral tissues, to quantify cortisol release by 11β-HSD1 from adipose tissue for the first time in humans.

**RESEARCH DESIGN AND METHODS**

**Participants and protocols.** Participants were male, aged 20-70 years, with body mass index (BMI) 20-45kg/m²; normal full blood count, renal and thyroid function; and no glucocorticoid therapy in the previous 6 months. Six men with normal liver function tests and alcohol intake <21 units/week were recruited for the subcutaneous adipose tissue study. Three subjects had no concurrent medical conditions, two had hypertension and one had Parkinson’s disease. Felodipine, bendroflumethiazide, enalapril, levodopa, entacapone and pramipexole were each taken by 1 subject. Four men with alcoholic cirrhosis and transjugular intra-hepatic portal-systemic shunts (TIPSS) in situ were recruited for the portal vein study. The TIPSS had been inserted at least 1 year previously for portal hypertension. These patients were currently abstinent from alcohol and attending for an annual check of TIPSS patency. Three TIPSS patients had no additional medical conditions; one had type 2 diabetes mellitus. Three were taking proton pump inhibitors; insulin, metoprolol, spironolactone, furosemide and aspirin were each taken by 1 subject. Local ethical approval and written informed consent were obtained.

Subjects were given oral dexamethasone (1mg in TIPSS patients and 1.5mg in healthy men) at 2300h and fasted until attending the Clinical Research Facility at 0800h. Measurements were taken of height, weight, total fat mass and percentage body fat by bioimpedance. Blood was taken for fasting glucose and lipids. 21G cannulae were placed in a right antecubital fossa vein for infusion and a left dorsal hand vein for sampling; the left hand was placed in a box heated to 60°C to achieve arterialisation. 9,11,12,12-[2H]4-Cortisol (d4-cortisol, Cambridge Isotopes, Andover, MA) was infused at 40% molar enrichment with 60% cortisol (Calbiochem, Nottingham, UK) at 1.74mg/h for 210 minutes after a priming 3.5mg bolus. The combination of dexamethasone and unlabelled cortisol administration aimed to maintain circulating cortisol and cortisone concentrations at stable non-stressed physiological levels during steady state measurements. d4-Cortisol was infused at sufficiently high enrichment to ensure detectable levels of d3-cortisol and d3-cortisone, even in hepatic vein. Dexamethasone is not known to interfere with cortisol kinetics.

**Cortisol release from subcutaneous adipose tissue in healthy men.** Subjects were served breakfast (30grams cornflakes and 300ml skimmed milk) at 0800–0830h and 5% dextrose (50ml/h) was infused intravenously throughout the study. Once the deuterated cortisol infusion was established, a 20G 15cm 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 2h of d4-cortisol infusion, 1-2MBq ¹³³Xenon (gas) was injected subcutaneously beside the umbilicus and radioactivity measured continuously with a NaI detector to assess blood flow (20;21). Blood samples were taken from arterialised hand vein and superficial epigastric vein at intervals (see Figure 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 240mcg/h. Twenty minutes after beginning the tracer infusion, the right internal jugular vein was cannulated under local anaesthesia (5ml of 2% lidocaine), and a 5F pigtail
catheter (Cordis, Berkshire, UK) 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 (Merritt Medical, Lanarkshire, UK) was then placed in a separate tributary of the hepatic vein for sampling. From 2h after beginning the tracer infusion, indocyanine green (ICG) (Pulsion Medical, Middlesex, UK) was infused into the antecubital vein at 30mg/h. Blood samples were taken from the portal, hepatic and arterialised veins at intervals (see Figure 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 (500ng) was added to 1.5ml plasma and extracted using 15ml chloroform. Samples were evaporated then reconstituted in mobile phase (60% methanol, 40% 5mM 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 (Thames Restek; 50mm x 4.6mm x 5μm), with column temperature 25˚C and mobile phase flow rate 0.5ml/min. Ionisation was achieved by positive electrospray. The precursor and product mass/charge ratios used were: cortisol (363→121), d3-cortisol (366→121), d4-cortisol (367→121), cortisone (361→163) and d3-cortisone (364→164). Compounds were quantified by the ratio of (area under peak of interest)/(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, acetonitrile (ACN) was added to serum to sediment protein, the supernatant was mixed with ammonium sulphate and centrifuged, and the organic phase was added to water before analysis by HPLC using a P680 HPLC pump and a PDA-100 photodiode array detector (Dionex, Sunnyvale, CA) with a Nova-pak C18 column (300mm x 3.9mm x 4μm) at a temperature of 35˚C. Analytes were eluted under linear gradient conditions at 1ml/min mobile phase (initially 80% water, 12% ACN, 8% methanol; 0-17min 25% water, 65% ACN, 8% methanol; 17-29min 80% water, 12% ACN, 8% methanol) and detected at λ230 (diazepam) and λ784nm (ICG).

Plasma glucose, lipids and liver function were measured using enzymatic colorimetric methods on an Olympus Diagnostics analyser (Co. Clare, Ireland). Plasma HbA1C was analysed by HPLC using a Variant II analyser (Bio-Rad Laboratories, Hertfordshire, UK).

**Kinetic analyses.** Calculations in each subject used the mean of measurements in steady state (ss), between 180 and 210 minutes of d4-cortisol infusion (Figure 2). Where possible, kinetic calculations relying on tracer:tracee ratios rather concentrations were favoured to minimise 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 were used to calculate hepatic blood flow (HBF) (24) using equation 1.

**Equation 1**

$$\text{HBF (litres/min)} = \frac{\text{ICG extraction rate}}{[\text{ICG}]_A - [\text{ICG}]_H} \times \frac{1}{1 - \text{haematocrit}}$$

Whole body cortisol kinetics were calculated from measurements in arterialised samples (15;16), using equations 2 and 3, where ‘cortisol’ denotes unlabelled 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).

**Equation 2**

$$\text{Rate of appearance of cortisol} = \frac{\text{d4-cortisol infusion rate}}{\text{d4-cortisol/cortisol}} - \text{cortisol infusion rate}$$
Quantification of adipose 11β-HSD1 in humans

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

Equation 4
\[
\text{Tissue d3-cortisol delivery} = \text{blood flow}_m \times [\text{d3-cortisol}]_{ss}
\]

Equation 5
\[
\text{Tissue d3-cortisol delivery} = \text{blood flow}_m \times [\text{d3-cortisol}]_{ss}
\]

Splanchnic cortisol and d3-cortisol production (from visceral tissues and liver combined) were calculated from measurements in arterialised and hepatic vein (HV) samples as previously described (16) using equations 6 and 7, where ‘tissue delivery’ was calculated as above.

Equation 6
\[
\text{Splanchnic cortisol production} = \left( \frac{\text{tissue cortisol x } [\text{d4-cortisol}]}{[\text{d4-cortisol}]} \right) - \text{tissue cortisol delivery}
\]

Equation 7
\[
\text{Splanchnic d3-cortisol production} = \left( \frac{\text{tissue d3-cortisol x } [\text{d3-cortisol}]}{[\text{d3-cortisol}]} \right) - \text{tissue d3-cortisol delivery}
\]

Liver production of cortisol and d3-cortisol were calculated from measurements in portal vein (PV) and hepatic vein (HV) using equations 8 and 9. Hepatic blood flow was measured by ICG extraction (equation 1) but portal blood flow (PBF) and hepatic arterial blood flow (HABF) were not measured. In health, 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 (28). To account for this unknown, we have modelled PBF to range between 10-80% of HBF, and concordantly for HABF to range between 90-20% of HBF.

Equation 8
\[
\text{Net production of cortisone and d3-cortisone across the viscera, liver and the}
\]

Equation 9
\[
\text{Visceral production of cortisol and d3-cortisol (i.e. release into the portal vein) was calculated using measurements from arterialised blood and portal vein with estimates of PBF as above, using equations 10 and 11, where tissue delivery was calculated as above.}
\]

Equation 10
\[
\text{Visceral cortisol production} = \left( \frac{\text{tissue cortisol x } [\text{d4-cortisol}]}{[\text{d4-cortisol}]} \right) - \text{tissue cortisol delivery}
\]

Equation 11
\[
\text{Visceral d3-cortisol production} = \left( \frac{\text{tissue d3-cortisol x } [\text{d3-cortisol}]}{[\text{d3-cortisol}]} \right) - \text{tissue d3-cortisol delivery}
\]
splanchnic tissues were calculated using equations 12 and 13, in which the relevant measurements of blood flow and venous concentrations were substituted as appropriate.

**Equation 12**

\[ \text{Net tissue cortisol} = (\text{[cortisone]}_A - \text{[cortisone]}_\text{ss}) \times \text{blood flow ss} \]

**Equation 13**

\[ \text{Net tissue d3-cortisone} = (\text{[d3-cortisone]}_A - \text{[d3-cortisone]}_\text{ss}) \times \text{blood flow ss} \]

**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 10nmol/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 modelling (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 ± SEM or, for calculated kinetic parameters, mean (95% confidence intervals).

**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 (Figure 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 (equation 2) and d3-cortisol (a specific measure of whole body \(11\beta\)-HSD1 activity; equation 3) were 33.8 (16.0, 51.5) and 28.3 (25.6, 31.0) nmol/min respectively. Adipose blood flow measured 2.5±0.7 ml/min/100g adipose tissue. There was significant release across the subcutaneous adipose bed of both cortisol (15.0 (0.4, 29.5) pmol/min/100g adipose tissue; equation 4) and d3-cortisol (8.7 (0.2, 17.2) pmol/min/100g adipose tissue; equation 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 minutes of d4-cortisol infusion (Figure 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 intra-hepatic steroid extraction and \(11\beta\)-HSD1 reductase activity. Conversely, d4-cortisol, which cannot be regenerated by \(11\beta\)-HSD1, was lower in the hepatic vein, consistent with substantial intra-hepatic 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 (equation 2) and d3-cortisol (equation 3) in the artery were 36.3 (24.4, 48.2) and 26.9 (21.0, 32.7) nmol/min, respectively. Hepatic blood flow by ICG extraction was 0.40±0.08 litres/min. Splanchnic production of cortisol (13.5 (3.6, 23.5) nmol/min; equation 6) and d3-cortisol (8.0 (2.6, 13.5) nmol/min; equation 7) was substantial (both p<0.05 vs zero). This could be accounted for entirely by the liver, since hepatic cortisol (equation 8) and d3-cortisol (equation 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 portal blood flow was estimated as 40% of total hepatic blood flow (28). No visceral cortisol (equation 10) or d3-cortisol (equation 11) release into the portal vein was detected (0.0 (-1.7, 1.7) and 0.1 (-0.7, 1.1) nmol/min, respectively). Modelling for portal venous flow from 10-80% of hepatic blood flow did not significantly alter these results (not shown).

Net cortisone and d3-cortisone production across the splanchnic tissues (equations 12 and 13) measured -18.2 (-33.3, -3.2) and -9.2 (-15.4, -3.0) nmol/min respectively (both p<0.05 vs zero). This was accounted for by cortisone and d3-cortisone extraction across the liver (-20.8 (-39.4, -2.2) and -10.6 (-18.3, -2.8) nmol/min, estimating portal flow as 40% of hepatic blood flow), consistent with substantial hepatic cortisone metabolism and 11β-HSD1 reductase activity. However, across the viscera, net cortisone production rate did not differ from zero (2.6 (-1.2, 6.3) nmol/min; p=0.12), and there was a trend for net generation of d3-cortisone (1.4 (-0.4, 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 attributed 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 (not shown) (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/100g adipose tissue would equate with a whole body production rate of 4.0±1.5 nmol/min, based on a total adipose mass of 29.4±7.1 kg measured in our participants using bioimpedance. This represents approximately 12% of whole body cortisol regeneration by 11β-HSD1. Significant 11β-HSD1 activity in subcutaneous adipose tissue is supported by...
the trend for a fall in cortisol 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 5α-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 11β-HSD1 have a significant impact upon 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, since 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 dyslipidaemia (4;16). We did not, however, detect release of cortisol from 11β-HSD1 in visceral tissues into portal vein, in agreement with a study in dogs (18). Assuming portal venous blood accounts for 40% of total hepatic blood flow (28), the mean estimate for visceral cortisol release from these subjects was 0.0nmol/min (95% confidence intervals -1.7 to +1.7). Although the number of subjects included was small (n=4), this provides 97.5% confidence that visceral cortisol production is less than 1.7nmol/min. Since hepatic cortisol delivery was 72.7nmol/min, we can conclude that any cortisol released by visceral 11β-HSD1 would not significantly impact on hepatic cortisol delivery.

In order 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 11β-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 11β-HSD1 was not unusually low. This paradox may reflect either up-regulation of 11β-HSD1 in non-splanchnic tissues or an underestimation of splanchnic cortisol release due to misleading blood flow measurement. While it is plausible that chronic liver disease may reduce hepatic 11β-HSD1 activity, it seems less likely that it would affect extra-hepatic 11β-HSD1. As previously described in TIPSS patients (34), hepatic blood flow 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 artefactually 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 11β-HSD1 reductase activity, it remains possible that venous drainage from the other visceral tissues dilutes any observable change in d3-cortisol/d4-cortisol ratios in blood from visceral adipose tissue. Hepatic blood flow
Quantification of adipose 11β-HSD1 in humans

was measured at 400ml/min, of which portal vein flow may be up to 320ml/min. This compares with blood flow of just 2.5ml/min/100g 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 3kg (37), then the contribution of visceral fat to portal blood flow may be 75ml/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 unstressed subjects.

Steady state plasma d3-cortisone concentrations were significantly higher in portal vein than in artery, and there was a similar trend for cortisol 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 11β-dehydrogenase activity, converting cortisol to cortisone in the viscera. This is likely due to 11β-HSD type 2 activity in the gut, although it is conceivable that 11β-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 modelled 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 ~30nmol/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 upon removal of cortisone by 11β-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 (31kg/m² in the subcutaneous adipose tissue study and 32kg/m² in the portal vein study), although the numbers (n=4-6) were too small to allow meaningful correlations with indices of 11β-HSD1 activity. Previous measurements in biopsies suggest that 11β-HSD1 activity is ~2.5-fold higher per gram of adipose tissue in obese people (BMI ~31kg/m²) than in lean controls with BMI ~9kg/m² lower (6;39). Given the Km of human 11β-HSD1 for cortisone of ~1μM (40), it is reasonable to assume a linear relationship between 11β-HSD1 protein concentrations and cortisol generation rates within the physiological range of cortisone concentrations of ~10-100nmol/l. Therefore, we anticipate that a 10kg/m² increase in BMI might elevate the cortisol production rate in subcutaneous adipose tissue by up to 2.5-fold (i.e. 37.5pmol/min/100g) and that, accounting for an associated ~15kg increase in fat mass, this equates with an increase in whole body adipose cortisol production of ~12.7nmol/min. In obesity, hepatic 11β-HSD1 activity is decreased by around 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.

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REFERENCES


Table 1. Anthropometry and fasting plasma biochemistry.  
*Data are mean ± SEM. BMI = body mass index; HbA1c = glycated haemoglobin A1c.*

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<th>Visceral A-V sampling</th>
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<td>N</td>
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<td>4</td>
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<td>50.9 ± 4.1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.8 ± 3.7</td>
<td>32.3 ± 1.1</td>
</tr>
<tr>
<td>Fat mass (%)</td>
<td>28.9 ± 4.1</td>
<td>30.6 ± 2.6</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>29.4 ± 7.1</td>
<td>32.5 ± 4.3</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>5.5 ± 0.4</td>
<td>7.8 ± 2.0</td>
</tr>
<tr>
<td>Plasma HbA1C (%)</td>
<td>4.3 ± 0.3</td>
<td>6.3 ± 1.2</td>
</tr>
<tr>
<td>Total plasma cholesterol (mmol/l)</td>
<td>5.4 ± 0.3</td>
<td>4.3 ± 0.7</td>
</tr>
<tr>
<td>Plasma triglycerides (mmol/l)</td>
<td>1.7 ± 0.6</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>Serum alanine aminotransferase (U/l)</td>
<td>30.5 ± 6.0</td>
<td>26.3 ± 2.6</td>
</tr>
<tr>
<td>Serum bilirubin (µmol/l)</td>
<td>8.7 ± 1.7</td>
<td>23.5 ± 6.3</td>
</tr>
</tbody>
</table>
Table 2. Steady state concentrations and ratios during deuterated cortisol infusion.
Data are mean ± SEM for n=6 (subcutaneous measurements) and n=4 (visceral measurements)
participants. The mean data from samples obtained between 180 and 210 minutes during the deuterated
cortisol infusion was used to calculate steady state concentrations. Comparisons were made by paired t
tests (subcutaneous study) or repeated measures ANOVA with post-hoc testing with Fisher’s least
significant differences test (visceral study). * p<0.05, ** p<0.01 compared to artery, # p<0.05 compared
to portal vein.

<table>
<thead>
<tr>
<th></th>
<th>Subcutaneous measurements</th>
<th>Visceral measurements</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Artery</td>
<td>Subcutaneous Vein</td>
</tr>
<tr>
<td>cortisol (nmol/l)</td>
<td>97 ± 16</td>
<td>103 ± 15</td>
</tr>
<tr>
<td>d3-cortisol (nmol/l)</td>
<td>33 ± 4</td>
<td>36 ± 4</td>
</tr>
<tr>
<td>d4-cortisol (nmol/l)</td>
<td>37 ± 3</td>
<td>37 ± 4</td>
</tr>
<tr>
<td>cortisone (nmol/l)</td>
<td>17 ± 2</td>
<td>14 ± 2</td>
</tr>
<tr>
<td>d3-cortisone (nmol/l)</td>
<td>8 ± 1</td>
<td>6 ± 2</td>
</tr>
<tr>
<td>d4-cortisol/ cortisol ratio</td>
<td>0.40 ± 0.03</td>
<td>0.38 ± 0.03 *</td>
</tr>
<tr>
<td>d4-cortisol/ d3-cortisol ratio</td>
<td>1.13 ± 0.04</td>
<td>1.02 ± 0.04 **</td>
</tr>
</tbody>
</table>
Figure 1. Quantifying cortisol production using deuterated cortisol.

9,11,12,12^-2H_4-cortisol (d4-cortisol) is converted mainly in the kidney to d3-cortisone, with the loss of the deuterium on C11. The d3-cortisone is then reduced by 11β-HSD1, predominantly in the liver and adipose tissue, with the addition of an unlabelled hydrogen to form d3-cortisol. Differences between d3-cortisol and d4-cortisol metabolism therefore reflect 11β-HSD1 reductase activity.

Figure 2. Plasma measurements during deuterated cortisol infusion.

Data are mean ± SEM for n=6 (subcutaneous measurements) and n=4 (visceral measurements) during deuterated cortisol infusion, with plasma samples from arterialised (filled squares), portal or subcutaneous (open diamonds) and hepatic (filled triangles) cannulae. a) Plasma cortisol concentrations, b) Plasma d4-cortisol enrichment, and c) d4-cortisol/ d3-cortisol ratio for subcutaneous study. d) Plasma cortisol concentrations, e) Plasma d4-cortisol enrichment, f) d4-cortisol/ d3-cortisol ratio for visceral study. Statistical comparison of mean values in steady state (180-210 minutes) is shown in Table 2.
Quantification of adipose 11β-HSD1 in humans

Subcutaneous sampling

a) Plasma cortisol

b) d4-Cortisol Enrichment

c) d4-Cortisol/ d3-Cortisol

Visceral sampling

d) Plasma cortisol

e) d4-Cortisol Enrichment

f) d4-Cortisol/ d3-Cortisol

Duration of infusion (min)