

The Contribution of Visceral Adipose Tissue to Splanchnic Cortisol Production in Healthy Humans

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Cortisol is regenerated from cortisone by 11 β -hydroxysteroid dehydrogenase type 1 (11HSD1), amplifying glucocorticoid action in adipose tissue and liver. 11HSD1 inhibitors are being developed for type 2 diabetes and may be most effective in obesity, where adipose 11HSD1 is increased. However, the magnitude of regeneration of cortisol in different tissues in humans is unknown, hindering understanding of the pathophysiological and therapeutic importance of 11HSD1. In eight healthy men, we infused 9,11,12,12-²H₄-cortisol and measured tracer enrichment in the hepatic vein as an indicator of total splanchnic cortisol generation. Oral cortisone (25 mg) was then given to measure first-pass hepatic cortisol generation. In steady state, splanchnic cortisol production was 45 \pm 11 nmol/min when arterial plasma cortisone concentration was 92 \pm 7 nmol/l. Extrapolation from hepatic cortisol generation after oral cortisone suggested that, at steady state, the liver contributes 15.2 nmol/min and extrahepatic splanchnic tissue contributes 29.8 nmol/min to the total splanchnic cortisol production. We conclude that tissues draining into the portal vein, including visceral adipose tissue, contribute substantially to the regeneration of cortisol. Thus, in addition to free fatty acids and adipokines, the portal vein delivers cortisol to the liver, and inhibition of 11HSD1 in visceral adipose tissue may indeed be valuable in ameliorating insulin resistance in obesity. *Diabetes* 54:1364–1370, 2005

The enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11HSD1) is widely expressed, including in the liver and adipose (1). It catalyzes regeneration of the glucocorticoid cortisol from its inactive 11-keto metabolite cortisone, thus amplifying glucocorticoid receptor activation independently of the level of cortisol in the blood. Its potential importance as a tissue-specific regulator of metabolism is illustrated in

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11HSD1, 11 β -hydroxysteroid dehydrogenase type 1; ICG, indocyanin green; TTR, tracer-to-tracee ratio.

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animals. Transgenic mice overexpressing 11HSD1 in adipocytes (2,3) develop central obesity with hyperinsulinemia, hyperglycemia, hyperlipidemia, and hypertension. Mice overexpressing 11HSD1 in the liver develop insulin resistance, dyslipidemia, and hypertension without obesity (4). Conversely, 11HSD1 knockout mice are protected from obesity, hyperglycemia, and dyslipidemia on high-fat feeding (5–7). Moreover, inbred rodent models of obesity and diabetes show tissue-specific dysregulation of 11HSD1 (2,8,9). Most commonly, 11HSD1 is reduced in the liver but increased in adipose tissue.

Similar tissue-specific dysregulation of 11HSD1 has been inferred in human obesity from indirect measurements. The rate of conversion of an oral dose of cortisone into cortisol in peripheral plasma is impaired (10–12), suggesting downregulation of hepatic 11HSD1. In subcutaneous adipose tissue, 11HSD1 activity and mRNA are increased in biopsies from obese subjects in most studies (11–17), and in vivo microdialysis confirms increased cortisone-to-cortisol conversion in obesity (18). Furthermore, the nonselective 11HSD1 inhibitor carbenoxolone enhances insulin sensitivity in healthy men and in patients with type 2 diabetes (19,20). Against this background, development of selective 11HSD1 inhibitors has become a highly competitive goal for the pharmaceutical industry, with some evidence of success (21,22).

However, fundamental questions remain about the role of 11HSD1 in humans. Crucially, the magnitude of regeneration of cortisol within individual tissues has not been quantified; therefore, consequences of dysregulation or enzyme inhibition remain uncertain. A suspicion persists that 11HSD1 catalyzes dehydrogenase conversion of cortisol to cortisone under some circumstances (23); therefore, enzyme expression or activity in vitro cannot be extrapolated in vivo. Studies measuring endogenous cortisol and cortisone (24) or dilution of stable isotope cortisol tracer (25) in the hepatic vein suggest that there is substantial splanchnic regeneration of cortisol but do not distinguish activity in the liver from the contribution of visceral adipose tissue, which in vitro studies suggest is substantial (26). Unfortunately, measurement of cortisol in the portal vein has only been achieved during surgery, when high stress levels probably obscure any influence of local regeneration (27). In subcutaneous adipose tissue, venous sampling has indicated local regeneration of cortisol, but the errors of measurement are wide (28), while in vivo microdialysis provides relative rather than absolute quantification (18).

We aimed to establish the relative contribution of liver and extrahepatic splanchnic tissues (principally visceral adipose tissue) to total splanchnic cortisol production in healthy men. This was achieved using hepatic vein catheter-

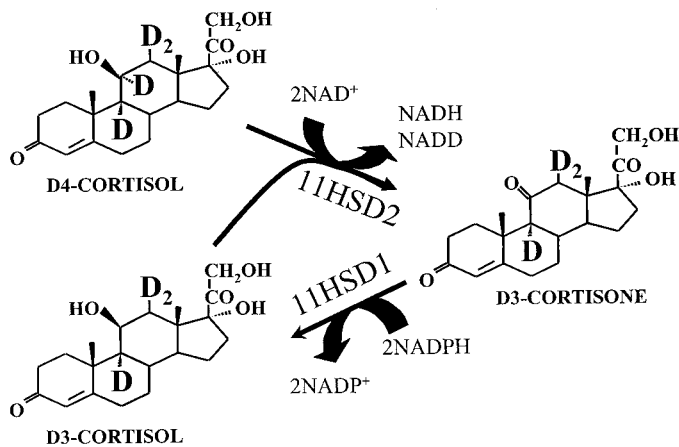


FIG. 1. Metabolism of d4-cortisol to form d3-cortisone and d3-cortisol. D, deuterium = ²H.

terization and systemic infusion of 9,11,12,12-²H₄-cortisol (d4-cortisol) (29) (Fig. 1). d4-Cortisol is converted to 9,12,12-²H₃-cortisone (d3-cortisone) by 11HSD type 2 in the kidney, and d3-cortisone is converted to d3-cortisol by 11HSD1. Unlike cortisol or d3-cortisol, d4-cortisol is not regenerated by 11HSD1; for this reason, dilution of d4-cortisol tracer with either cortisol or d3-cortisol in a selective venous sample reflects 11HSD1 activity in the tissues draining to that vein. Whole splanchnic cortisol generation was measured in steady state, and hepatic production of cortisol was measured after oral administration of cortisone. Subtraction of hepatic production from splanchnic production allowed calculation of cortisol production from extrahepatic visceral tissues.

RESEARCH DESIGN AND METHODS

Eight healthy Caucasian men (age 27.1 ± 1.5 years, BMI 22.7 ± 0.5 kg/m²) were recruited with the following inclusion criteria: aged 18–60 years, no history of acute or chronic disease, physical examination and laboratory tests (blood counts, creatinine, liver enzymes, thyroid-stimulating hormone, electrolytes, and electrocardiogram), no medication, alcohol intake <20 g/day, seronegative for hepatitis B or C and autoimmune hepatitis, and no use of hepatotoxic toxins or drugs. Written informed consent and ethics committee approval (Karolinska Hospital, Stockholm) were obtained.

Protocol. Subjects took 1 mg dexamethasone by mouth at 2300, fasted thereafter, and attended the clinical research center at 0700. Cannulae were

positioned in an antecubital vein for infusions and in a dorsal vein of a hand placed in a hot box for sampling arterialized blood.

Intravenous infusions commenced at 0730 ($t = 0$ h) and continued for 6.5 h (Fig. 2). Dexamethasone was infused at 4 μ g/min to suppress ACTH and endogenous cortisol production. 9,11,12,12-²H₄-Cortisol (Cambridge Isotopes, Andover, MA) (Fig. 1) was infused at 40% enrichment in unlabeled cortisol at 1.74 mg/h after a priming dose of 3.6 mg.

At $t = 2$ h, a no. 7 Courmand catheter was inserted under local anesthesia via the right femoral vein and positioned in the hepatic vein under fluoroscopic control. Indocyanin green (ICG) (ICG-Pulsion; Pulsion Medical Systems, Munich, Germany) was infused at 0.5 mg/min for measuring hepatic blood flow as described (30). Simultaneous samples of arterialized and hepatic vein blood were obtained between $t = 3$ and $t = 3.5$ h for assessment of cortisol metabolism in steady state (Fig. 2). ICG was analyzed in duplicate three times during steady state, and the mean blood flow in each subject was used in subsequent calculations.

At $t = 3.5$ h, cortisone acetate (25 mg) was administered by mouth. Sampling continued until $t = 5$ h when the hepatic vein catheter was removed (Fig. 2).

Laboratory analyses

Steroid analysis by gas chromatography mass spectrometry. Plasma (1.5 ml) containing epicortisol (500 ng) was extracted with chloroform (15 ml) and methoxime-trimethylsilyl derivatives prepared as described (29). Derivatized steroids were quantified using a Polaris Q ion trap electron-impact mass spectrometer with a Trace gas chromatograph (ThermoFinnigan, Winsford, U.K.) with electron energy 70 eV, source temperature 200°C, and interface temperature 280°C. Separation used a DB17MS column (column length 15 m, internal diameter 0.25 mm, film thickness 0.25 μ m; J&W Scientific). Oven temperature was 60°C and was increased after 1 min at 30°C per min to 200°C, then increased at 10°C per min to 300°C, and then maintained for 8 min. Injection temperature was 240°C. Quantitation was against two calibration lines for quantities (50–250 ng) and enrichment (10–50%) of cortisol. Enrichment was corrected for background interference from naturally occurring isotopes.

ICG analysis. ICG concentrations were measured using high-performance liquid chromatography as described (30).

Data interpretation

Steady-state calculations. Concentrations of cortisol, ²H₄-cortisol (d4-cortisol), and ²H₃-cortisol (d3-cortisol) were calculated. Enrichment of cortisol with d4-cortisol was calculated as peak area of d4-cortisol/[peak areas of (d4-cortisol + cortisol)]. Enrichment with d3-cortisol was calculated as peak area of d3-cortisol/[peak areas of (d3-cortisol + d4-cortisol)]. The tracer-to-tracee ratios (TTRs) (TTRs of d4-cortisol to cortisol and d4-cortisol to d3-cortisol) were calculated from the peak areas. Steady-state (ss) concentrations, enrichments, and blood flows were calculated as the means for each subject between $t = 3$ and $t = 3.5$ h.

Clearances (l/min) of cortisol and d4-cortisol were calculated as the rate of infusion of cortisol or d4-cortisol divided by the steady-state concentrations of cortisol or d4-cortisol, respectively.

The rate of appearance (R_a) of endogenous cortisol (Eq. 1) and d3-cortisol (Eq. 2) were calculated in arterialized samples.

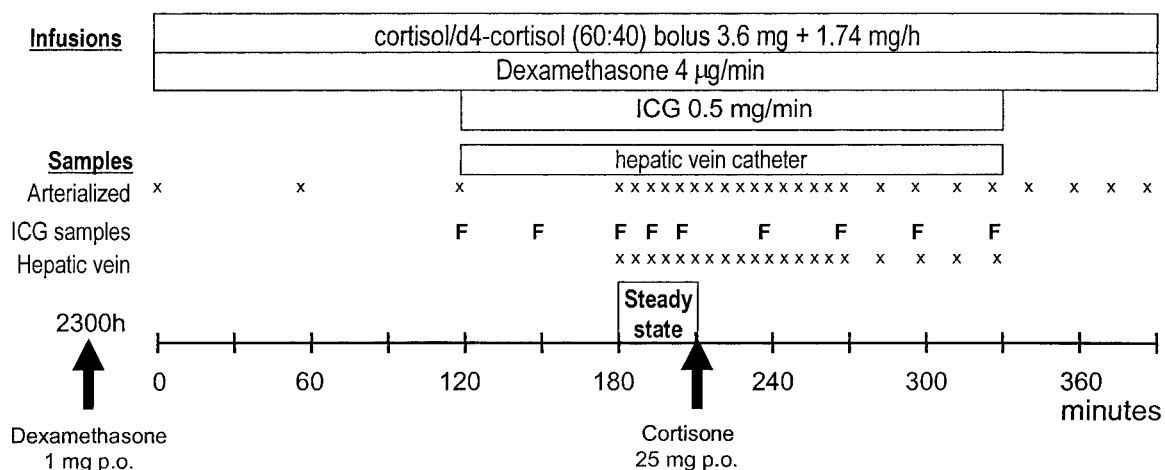


FIG. 2. Protocol for sampling and hepatic blood flow measurements. x, blood sample; F, measurement of blood flow.

$$R_a \text{ cortisol} = \left(\frac{\text{infusion rate of d4-cortisol}}{\text{d4-cortisol enrichment}} \right) - (\text{infusion rate of cortisol}) - (\text{infusion rate of d4-cortisol}) \quad (1)$$

$$R_a \text{ d3-cortisol} = \frac{\text{infusion rate of d4-cortisol}}{\text{TTR d4-cortisol/d3-cortisol}} \quad (2)$$

Tissue production of cortisol (Eq. 3) and d3-cortisol (Eq. 4) across the splanchnic bed were calculated as follows.

$$\text{Tissue cortisol production} = (\text{blood flow}_{ss} \times [\text{cortisol}_{\text{arterial}}]_{ss}) - \left((\text{blood flow}_{ss} \times [\text{cortisol}_{\text{arterial}}]_{ss}) \times \frac{\text{TTR d4-cortisol/cortisol}_{\text{arterial ss}}}{\text{TTR d4-cortisol/cortisol}_{\text{venous ss}}} \right) \quad (3)$$

$$\text{Tissue d3-cortisol production} = (\text{blood flow}_{ss} \times [\text{d3-cortisol}_{\text{arterial}}]_{ss}) - \left((\text{blood flow}_{ss} \times [\text{d3-cortisol}_{\text{arterial}}]_{ss}) \times \frac{\text{TTR d4-cortisol/d3-cortisol}_{\text{arterial ss}}}{\text{TTR d4-cortisol/d3-cortisol}_{\text{venous ss}}} \right) \quad (4)$$

Regional cortisol production was also calculated by the alternative approach used by Basu et al. (25), as in Eq. 5.

$$\begin{aligned} \text{Tissue cortisol production} &= \left(\frac{\text{blood flow}_{ss} \times ([\text{d4-cortisol}_{\text{arterial}}]_{ss} - [\text{d4-cortisol}_{\text{venous}}]_{ss})}{\text{TTR d4-cortisol/cortisol}_{\text{arterial ss}}} \right) \\ &- \left(\text{blood flow}_{ss} \times ([\text{cortisol}_{\text{arterial}}]_{ss} - [\text{cortisol}_{\text{venous}}]_{ss}) \right) \end{aligned} \quad (5)$$

Non-steady-state calculations. Two approaches were applied to calculate rates of appearance of cortisol, using the mean data for all eight subjects: Steele equation calculations at individual time points and curve fitting of change in enrichment of d4-cortisol with time.

The Steele equation was applied using Eq. 6, where t = time, V = volume of distribution, $C(t)$ = total cortisol concentration at time = t , and $E(t)$ = TTR d4-cortisol/cortisol_{venous} at time = t . The volume of distribution was assumed to be 12 l, as has been widely used for glucose (31). However, V for cortisol could not be measured in the current studies, so the rates estimated from the Steele equation were used only to estimate the time course of appearance of cortisol, not to quantify absolute cortisol production rates.

$$R_a \text{ cortisol} (t) = \left(\frac{\text{infusion rate of d4-cortisol}}{E(t)} \right) - \left(\frac{V \times \frac{C(t)}{1 + E(t)} \times \frac{dE(t)}{dt}}{E(t)} \right) \quad (6)$$

Following the cortisone bolus, cortisol production resulted in a reduction in d4-cortisol enrichment in the hepatic vein (Fig. 3). This was fitted to a curve using a one-compartment model with Kinetica software (Innaphase, Philadelphia, PA). The area under the curve for enrichment was used to calculate the total production of cortisol per unit time as follows.

$$\text{At steady state, d4-cortisol enrichment}_{ss} = \frac{R_a \text{ d4-cortisol}}{R_a \text{ d4-cortisol} + R_a \text{ cortisol}}$$

where “ R_a cortisol” represents the rate of infusion plus endogenous generation (from adrenal secretion and from regeneration by 11HSD1).

At a given time (t) after administration of cortisone, “extra” cortisol is produced (R_a extra), resulting in a change in d4-cortisol enrichment (E).

$$E(t) = \frac{R_a \text{ d4-cortisol}}{R_a \text{ d4-cortisol} + R_a \text{ cortisol} + R_a \text{ extra}}$$

$$\Delta E_{(SS-t)} = E_{SS} - E(t)$$

$$E(t) = E_{SS} - \Delta E_{(SS-t)}$$

$$E_{SS} - \Delta E_{(SS-t)} = \frac{R_a \text{ d4-cortisol}}{R_a \text{ d4-cortisol} + R_a \text{ cortisol} + R_a \text{ extra}}$$

Thus, “ R_a extra” could be calculated as an integrated parameter across a time period of t using Eq. 7, where AUC ΔE is the area under the curve for change in d4-cortisol enrichment.

$$R_a \text{ extra} = \left(\frac{R_a \text{ d4-cortisol}_{ss}}{\text{AUC } \Delta E} \right) - (R_a \text{ d4-cortisol}_{ss}) - (R_a \text{ cortisol}_{ss}) \quad (7)$$

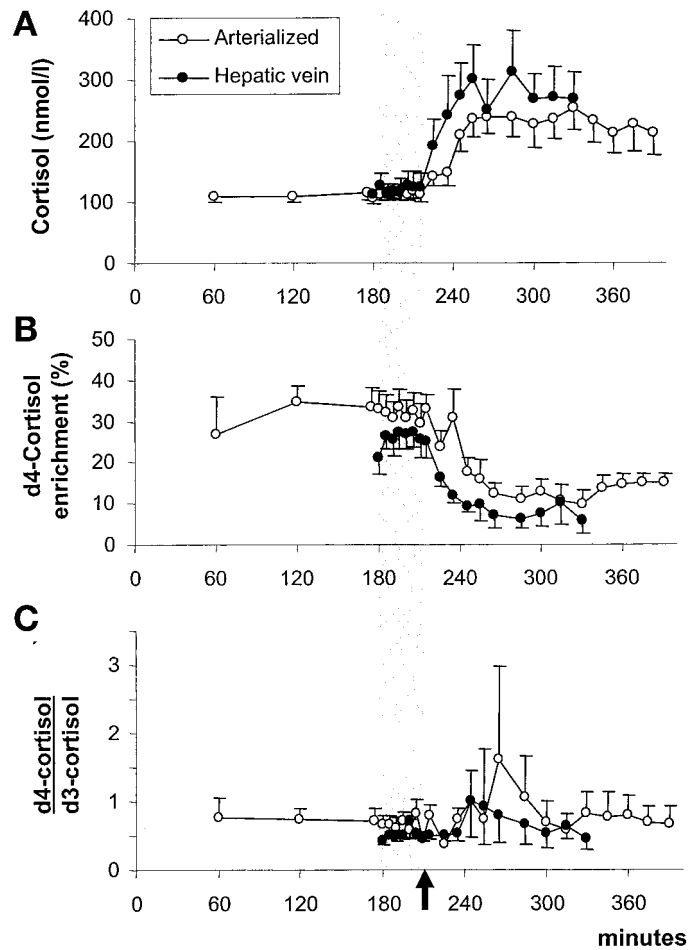


FIG. 3. Steady-state d4-cortisol infusion followed by administration of oral cortisone. Data are means \pm SE for concentration of cortisol (A), enrichment of d4-cortisol (B), and TTR of d4-cortisol to d3-cortisol (C). \circ , samples from the arterialized hand vein; \bullet , samples from hepatic vein. The shaded box indicates the period of steady-state calculations and the arrow indicates the time of administration of cortisone 25 mg orally.

Estimating extrahepatic (visceral) versus hepatic contribution to splanchnic cortisol generation. To estimate the extent to which hepatic metabolism of cortisone to cortisol accounts for cortisol generation in the whole of the splanchnic circulation, and thereby to deduce the likely contribution of visceral adipose tissue, the following applies, where R_a is the net rate of appearance.

$$R_a \text{ cortisol}_{\text{splanchnic ss}} = R_a \text{ cortisol}_{\text{visceral ss}} + R_a \text{ cortisol}_{\text{hepatic ss}}$$

$$R_a \text{ cortisol}_{\text{visceral ss}} = R_a \text{ cortisol}_{\text{splanchnic ss}} - R_a \text{ cortisol}_{\text{hepatic ss}}$$

This can be reexpressed in Eq. 8, where [cortisone_{liver}] is the concentration of cortisone reaching the liver in steady state or at time = t .

$$R_a \text{ cortisol}_{\text{visceral ss}} = R_a \text{ cortisol}_{\text{splanchnic ss}} - \left(\frac{[\text{cortisone}_{\text{liver ss}}]}{[\text{cortisone}_{\text{liver } t}]} \times R_a \text{ cortisol}_{\text{hepatic } t} \right) \quad (8)$$

$R_a \text{ cortisol}_{\text{splanchnic ss}}$ is known (Eq. 3) and $R_a \text{ cortisol}_{\text{hepatic}}$ in non-steady state at time = t is known (Eq. 7). Cortisone_{liver} t was estimated as follows. Since very little cortisone was detected in hepatic vein, bioavailability of cortisone was calculated as a percentage of the administered dose from the area under the total curve of cortisol appearance (fitted as above). The time-course of absorption was assumed to be distributed as a symmetrical bell-shaped curve with a peak at the time of maximum rate of production of cortisol, calculated in Eq. 6. Thus, the amount of cortisone reaching the liver between time 0 and the time of the peak rate of appearance of cortisol was estimated as half the administered dose \times bioavailability %/100. The mean concentration of cortisone reaching the liver between administration and the peak rate of appearance of cortisol was then calculated as the amount of cortisone reaching the

TABLE 1
Steady-state concentrations, enrichments, and TTRs of steroids in plasma from arterialized venous and hepatic vein samples

	Arterialized hand vein	Hepatic vein
Cortisol (nmol/l)	118.1 ± 10.1	124.1 ± 16.3
d4-cortisol (% APE)	31.7 ± 4.5	25.6 ± 4.0*
Tracer-to-tracee ratio _{D4F/D3F}	0.66 ± 0.14	0.46 ± 0.04

Data are means ± SE. * $P \leq 0.005$. APE, atom percent excess; D4F, d4-cortisol; D3F, d3-cortisol.

liver × hepatic blood flow, assuming equal mixing between blood from portal vein and hepatic artery.

The two unknown variables in Eq. 8 (cortisone concentration reaching the liver in steady state and rate of visceral appearance of cortisol) were then modeled to identify combinations that fit the observed data. At each estimated cortisone concentration reaching the liver in steady state, the rate of extraction of cortisone by visceral tissues was calculated using Eq. 9.

$$\text{Cortisone extraction rate}_{ss} = \frac{([\text{cortisone}]_{\text{arterial ss}} - [\text{cortisone}]_{\text{liver ss}}) \times \text{blood flow}}{\text{blood flow}} \quad (9)$$

Equation 8 was solved by finding an estimated cortisone concentration reaching the liver in steady state at which the visceral cortisone extraction rate was equal to the visceral cortisol production rate.

Statistical comparisons. Data were compared using paired Student's *t* tests and are presented as means ± SE.

RESULTS

Splanchnic cortisol production in steady state. Concentrations of cortisol, d4-cortisol, and d3-cortisol were in steady state between $t = 3$ and $t = 3.5$ h (Fig. 3 and Table 1). In arterialized blood, clearance of cortisol was slower than that of d4-cortisol (0.43 ± 0.07 vs. 0.73 ± 0.04 l/min; $P < 0.001$), reflecting the contribution of reappearance of cortisol but not d4-cortisol. Cortisone was readily detected in arterialized blood (92.0 ± 7.0 nmol/l) but rarely detected in hepatic vein samples, making kinetic calculations based on cortisone or d3-cortisone impossible.

The whole-body cortisol production rate was 37 ± 22 nmol/min (Eq. 1), and d3-cortisol production rate was 60 ± 10 nmol/min (Eq. 2). d4-Cortisol enrichment was lower in hepatic vein than arterialized blood, indicating splanchnic cortisol production, which was calculated (Eq. 3) as 45 ± 11 nmol/min ($P = 0.007$ vs. 0, $P = 0.81$ vs. whole-body cortisol production). The rate of splanchnic d3-cortisol production (Eq. 4) in seven of the eight subjects was 20 ± 9 nmol/min ($P = 0.06$ vs. 0, $P = 0.08$ vs. whole-body d3-cortisol production). However, there was one outlier in whom d3-cortisol extraction appeared to occur.

Applying Eq. 5, as suggested by Basu et al. (25), gave similar results. Cortisol production was 34 ± 17 nmol/min ($P = 0.65$ vs. result from Eq. 3 above), and d3-cortisol "production" was -0.9 ± 3.2 nmol/min (not different from zero or from result from Eq. 4 above). Equations 3 and 4 are preferred, however, because they are based on peak area ratios and do not require extrapolation from calibration lines to calculate steroid concentrations and are, therefore, less prone to error. Splanchnic cortisol uptake was 23 ± 12 nmol/min.

Hepatic "first-pass" metabolism of cortisone in non-steady state. Following administration of cortisone (69 μmol) by mouth, cortisol concentrations rose in hepatic vein earlier than in arterialized blood (time to peak 76 ± 16 vs. 118 ± 11 min; $P = 0.01$). d4-Cortisol enrichment fell in

TABLE 2
Hepatic cortisol production after oral administration of cortisone calculated using the Steele equation

Time after oral cortisone (min)	Rate of cortisol production (nmol/min)
0 (steady state)	45*
5	218
15	800
25	966
35	1037
45	949

*From steady-state Eq. 3; the other rates of cortisol production are using mean data for all subjects in Eq. 6.

both hepatic vein and arterialized blood (Fig. 3), but this occurred within 5 min of dose administration in hepatic vein and after 35 min in arterialized blood, and tended to be more pronounced in hepatic vein ($\Delta 24.5 \pm 3.0$ vs. $22.0 \pm 2.2\%$; $P = 0.07$).

The peak rate of appearance of cortisol (Eq. 6) occurred 35 min after the dose (Table 2). This calculation assumes mixing of the cortisol generated only within the "immediate" pool with an estimated size of 12 l (31). Therefore, the accuracy of time points beyond 45 min is questionable, given that distribution will be occurring into the adipose and extracellular fluid compartments. Over the first 35 min, 29 μmol of cortisol was generated, equivalent to 42% of the administered cortisone dose. Assuming a symmetrical bell-shaped curve for cortisol generation (as supported by the calculated rates in Table 2) and complete conversion of "available" cortisone to cortisol, this indicates a bioavailability of 84% and complete absorption of the dose over 70 min. Curve fitting of change in d4-cortisol enrichment revealed a total area under the curve of $5,150 \pm 730\% \cdot \text{min}$ (hepatic vein) or $5,093 \pm 1,234\% \cdot \text{min}$ (arterialized blood), which equates with a very similar bioavailability of cortisone of 85%. The remainder of the cortisone dose was either not absorbed or metabolized by other enzymes in liver, since only a trivial rise in cortisone was detected in the hepatic vein (data not shown).

The mean hepatic rate of cortisol appearance between 0 and 35 min after the cortisone dose, calculated from the area under the curve of change in d4-cortisol enrichment (Eq. 7), was 156 nmol/min. The mean cortisone concentration reaching the liver between 0 and 35 min after the dose was estimated as 337 nmol/l.

Estimating extrahepatic (visceral adipose tissue) cortisol generation. Table 3 shows models for the steady-state conditions that would satisfy the observed steady-state splanchnic rate of cortisol generation of 45 nmol/min with an observed steady-state arterialized cortisone concentration of 92 nmol/l, given a rate of conversion of oral cortisone to cortisol by the liver of 156 nmol/min at a cortisone concentration of 337 nmol/l. The predicted steady-state concentration of cortisone being delivered to the liver at which rates of visceral adipose production of cortisol and extraction of cortisone are identical is 67 nmol/l. Under these conditions, the predicted hepatic rate of cortisol generation was 15.2 nmol/min, and the calculated visceral cortisol production and visceral cortisone extraction were both 29.8 nmol/min. Other solutions to the model are unfeasible because they demand a mismatch

TABLE 3
Modeling of extrahepatic (visceral) and hepatic contributions to splanchnic cortisol generation at steady state

Arterial cortisolone _{ss} (nmol/l)*	Hepatic cortisolone _{ss} (nmol/l)†	R _a cortisol splanchnic _{ss} (nmol/min)*	R _a cortisol hepatic _{ss} (nmol/min)‡	R _a cortisol visceral _{ss} (nmol/min)‡	Visceral cortisone extraction rate (nmol/min)§
92	100	45	22.6	22.4	-9.7
92	92	45	20.8	24.2	0
92	80	45	18.1	26.9	14.5
92	67	45	15.2	29.8	29.8
92	60	45	13.6	31.4	38.6
92	40	45	9.1	35.9	62.8
92	20	45	4.5	40.5	86.9
92	0	45	0	45	111.0

Models were calculated using Eq. 8 in which *denotes measured known variable; †denotes unmeasured estimated variable; ‡denotes calculated variable. §In addition, visceral cortisone extraction was calculated from Eq. 9.

between production of cortisol and extraction of cortisone by visceral adipose tissue.

This model can be extrapolated to estimate cortisol and cortisone concentrations in the portal vein. Cortisone is “delivered” to liver at 67 nmol/l in a mixture of portal vein and hepatic artery blood. The hepatic artery cortisone concentration at steady state is 92 nmol/l. So, if portal blood flow is two-thirds of total splanchnic blood flow, then the portal vein cortisone concentration at steady state would be 55 nmol/l (in order to deliver cortisone at $[55 \times 0.67] + [92 \times 0.33] = 67$ nmol/l to the liver). The corresponding incremental rise in plasma cortisol concentration across the visceral adipose tissue in steady state would be the same as the incremental fall in cortisone concentration (i.e., $92 - 55 = 37$ nmol/l). Thus, cortisol would rise from 118 nmol/l in arterialized blood to 155 nmol/l in portal vein, and mixing with hepatic artery blood would deliver cortisol to the liver at 143 nmol/l. However, since relative portal vein and hepatic artery blood flow was not measured here, these figures can only be approximate.

DISCUSSION

In 1997, Bujalska et al. (26) showed that cultured cells from human omental adipose tissue converted cortisone to cortisol and hypothesized that cortisol generation in visceral adipose tissue may be sufficient to generate “Cushing’s disease of the omentum.” It has been difficult to establish the in vivo significance of these in vitro observations. Here, we confirm that the splanchnic circulation makes a significant contribution to systemic cortisol production (25). We show that the whole splanchnic circulation is substantially more effective at generating cortisol from cortisone than first-pass metabolism in the liver alone. This strongly suggests that 11HSD1 in visceral adipose tissue regenerates cortisol, contributing approximately two-thirds, and the liver one-third, to splanchnic cortisol production in healthy men. This allows us for the first time to predict the magnitude of cortisol regeneration in the human liver and visceral adipose tissue and the extent to which the visceral adipose tissue “delivers” cortisol to the liver.

The absolute rate of splanchnic cortisol production depends on cortisone concentrations, which are typically lower in healthy men (at 50–70 nmol/l [24]) than those achieved during prolonged cortisol infusion here. The relative rate of splanchnic and systemic cortisol production

depends upon adrenal secretion, which varies widely according to ACTH levels. Here, ACTH was suppressed with dexamethasone; therefore, the similarity between splanchnic and whole-body cortisol production suggests that extrasplanchnic tissues do not make a major contribution to non-ACTH-dependent cortisol production. Dexamethasone is converted by 11HSD2 to 11-dehydrodexamethasone, which might compete with cortisone for metabolism by 11HSD1 (32); however, given the low concentrations of this metabolite in plasma (which are predicted to be ~10 nmol/l at this infusion rate), we think this is unlikely to have influenced the results.

The only enzyme known to produce cortisol that is expressed in splanchnic tissues (i.e., in liver and visceral adipose tissue) is 11HSD1. In principle, 11HSD1 can be measured selectively by differential production of d3-cortisol rather than d4-cortisol (Fig. 1). However, by contrast with our previous experience in systemic measurements (18,29), we found measurements of d3-cortisol and, in particular, d3-cortisone to be less reliable than d4-cortisol, cortisol, or cortisone in detecting relatively small arteriovenous differences. As a result, the estimation of substrate d3-cortisone concentration is subject to error, and rates of d3-cortisol generation cannot be compared directly with those of cortisol. d3-Cortisol generation in the whole body tended to be higher than in the splanchnic circulation, consistent with extraadrenal nonsplanchnic 11HSD1 activity. Production of cortisol in other extraadrenal organs has rarely been assessed using tracers in humans. Basu et al. (25) found no cortisol production into the femoral vein, although this is not surprising given that most of leg circulation is in skeletal muscle where 11HSD1 expression is trivial. It will be important to extend the current approach to measurements in subcutaneous adipose tissue.

We have not established whether 11β-dehydrogenase conversion of cortisol to cortisone, catalyzed by either 11HSD isozyme, occurs in the splanchnic circulation. The low levels of d3-cortisone and cortisone in hepatic vein precluded the calculation of tracer dilution for cortisone.

Portal vein cannulations cannot be performed ethically in healthy humans, so we relied on indirect calculation of extrahepatic splanchnic cortisol generation. Extrapolation of the hepatic cortisol production rate following oral cortisone to deduce the relative contribution of liver and extrahepatic splanchnic tissues in steady state required

some assumptions. Crucially, it is assumed that the rate of appearance of cortisol changes in linear proportion to cortisone concentration in the range of 0 to ~ 700 nmol/l (the maximum concentration estimated after oral cortisone administration). This has not been tested directly but is consistent with the K_m of human 11HSD1 for cortisone concentrations of ~ 1 μ mol/l (33), which suggests that it is unlikely to reach V_{max} in physiological conditions and with studies of 11HSD1 in isolated perfused liver in animals that demonstrate linear increases in product generation at substrate concentrations in excess of 1 μ mol/l (34,35). A second assumption is that the bioavailability of cortisone can be inferred from the proportion that appears as cortisol. However, this is likely to underestimate rather than overestimate bioavailability. The model in Table 3 is remarkably robust to increases in cortisone bioavailability; for example, if bioavailability is assumed to be 100% rather than 84%, then the estimated cortisone concentration reaching the liver at steady state is 65 rather than 67 nmol/l, scarcely affecting the estimates of relative extrahepatic visceral and hepatic cortisol production. A third assumption is that oral administration of cortisone results in generation of cortisol exclusively in the liver. However, any additional conversion (e.g., in blood vessels or mesenteric adipose tissue) would result in overestimation of the hepatic rate of cortisol generation and hence underestimation of the rate in visceral adipose tissue; therefore, the major conclusion that extrahepatic tissues contribute to splanchnic cortisol generation would not be undermined. Finally, the model assumes that there is equimolar exchange between cortisone extraction and cortisol production in extrahepatic splanchnic tissues; therefore, rates of disappearance of cortisone and production of cortisol are identical. From what is known of adipose steroid metabolism, it is a reasonable assumption that there is no additional cortisone extraction other than by 11HSD1, since the other enzyme that metabolizes cortisone in liver, 5 β -reductase, has not been reported in adipose tissue.

Measurement of peripheral venous plasma cortisol after an oral dose of cortisone has been used extensively to measure hepatic 11HSD1 (10–12,36,37). Our findings validate this, since there was a close relationship between results in hepatic vein and in peripheral plasma, albeit the peripheral changes occurred later. This emphasizes that hepatic first-pass metabolism is highly efficient at converting cortisone to cortisol, allowing little “leak” of cortisone into the systemic circulation (38) and hence little opportunity for 11HSD1 in other tissues to contribute. In contrast, in evaluating whole-body 11HSD1, our data illustrate that there are substantial contributions from both liver and visceral adipose tissue; thus, the tissue-specific dysregulation proposed in obesity, with decreased 11HSD1 in liver and increased 11HSD1 in adipose (11), may not alter either total splanchnic or whole-body regeneration of cortisol (18).

We have estimated the likely impact of 11HSD1 on cortisol concentrations in the portal vein. The incremental increase in cortisol concentrations between arterial blood and portal vein was estimated at 37 nmol/l. In conditions where cortisol levels are elevated and variable, it may not be possible to detect such a small increment (27). However, the impact on intracellular cortisol concentrations in

cells expressing 11HSD1 is much greater and has not been estimated here. In mice, transgenic overexpression of 11HSD1 in adipose results in a 2.7-fold increase in enzyme activity and a twofold increase in visceral fat mass, which is associated with an ~ 500 nmol/l increase in portal vein corticosterone concentrations (2). This suggests a similar order of magnitude of the influence of visceral 11HSD1 on portal vein glucocorticoid levels in mice (~ 90 nmol/l) as in men (~ 37 nmol/l).

In summary, these results confirm the substantial magnitude of cortisol regeneration from cortisone within the splanchnic circulation and suggest that an important component is from nonhepatic tissue, probably visceral adipose tissue. This is a key finding in interpreting the likely impact of altered 11HSD1 expression and activity in obesity and other diseases and in predicting the likely benefits of 11HSD1 inhibition.

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