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Hepatic 11 β -hydroxysteroid dehydrogenase type 1 activity in obesity and type 2 diabetes using a novel triple tracer cortisol technique

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Abstract

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Contribution statement SD researched the data, managed the data handling and data analyses, and assisted with writing the manuscript. BN assisted with the acquisition of data and conduct of the study. VP assisted with the conduct of the study and the acquisition and analyses of data. REC and RKL assisted with the acquisition of data and the statistical analyses. RJS assisted with the mass spectrometry data acquisition and analyses. AB assisted with the study design, data analyses and manuscript editing. RB assisted with the study design, researched the data, assisted with the conduct of the study, data analyses and writing the manuscript. All authors were involved with editing the manuscript and approving the final version to be published. RB is the guarantor of this manuscript and is responsible for the integrity of this work.

Aims/hypothesis—Dysregulation of 11 β -hydroxysteroid dehydrogenase (11 β -HSD) enzyme activities are implicated in the pathogenesis of obesity and insulin resistance. The aim of the study was to determine whether hepatic 11 β -HSD type 1 (11 β -HSD-1) enzyme activity differs in people with and without obesity and type 2 diabetes.

Methods—We measured hepatic 11 β -HSD-1 activity in the overnight fasted state in 20 lean non-diabetic participants (LND), 21 overweight/obese non-diabetic participants (OND) and 20 overweight/obese participants with type 2 diabetes (ODM) using a non-invasive approach. One mg doses of [9,12,12-²H₃]cortisol (D cortisol) and [4-¹³C]cortisone ([¹³C]cortisone) were ingested, while [1,2,6,7-³H]cortisol ([³H] cortisol) was infused intravenously to enable concurrent measurements of first-pass hepatic extraction of ingested D cortisol and hepatic conversion of ingested [¹³C]cortisone to C13 cortisol derived from the ingested cortisone (a measure of 11 β -HSD-1 activity in the liver) using an isotope dilution technique. One-way ANOVA models and Kruskal–Wallis tests were used to test the hypothesis.

Results—Plasma D cortisol and C13 cortisol concentrations were lower in OND than in LND ($p<0.05$) over 6 h of the study. There was no difference ($p=0.15$) in C13 and D cortisol concentrations between OND and ODM and between LND and ODM for the same study period. Hepatic conversion of [¹³C]cortisone to C13 cortisol was similar between groups.

Conclusions/interpretation—Hepatic conversion of [¹³C]cortisone to C13 cortisol did not differ between the groups studied. We conclude that hepatic 11 β -HSD-1 activity is similar in individuals who are overweight/obese or who have type 2 diabetes.

Keywords

Diabetes; 11 β -Hydroxysteroid dehydrogenase; Obesity

Introduction

Glucocorticoids are potent regulators of glucose, fat and protein metabolism. Tissue-specific conversion of cortisone to cortisol via the 11 β -hydroxysteroid dehydrogenase type 1 enzyme pathway (11 β -HSD-1) results in high local cortisol concentrations [1]. 11 β -HSD-1 is present in multiple tissues, including the liver and adipose tissue, with activity being greater in omental than in subcutaneous fat [2–5]. The 11 β -HSD-1 pathway has attracted considerable attention both as a therapeutic target and a potential contributor to the pathogenesis of diabetes, obesity and the so-called ‘metabolic syndrome’ [1, 6, 7]. Our observation that the splanchnic bed of obese humans produces large amounts of cortisol, all of it occurring within the liver [8], strongly implies that the resultant high local cortisol concentrations within the liver could be important determinants of hepatic insulin action. If so, inhibitors of hepatic 11 β -HSD-1 could have dramatic effects on hepatic insulin action and could become an important new therapy for type 2 diabetes. However, the enthusiasm for the development of inhibitors of hepatic 11 β -HSD-1 has been dampened by reports that hepatic 11 β -HSD-1 activity is reduced in obesity and insulin resistance [9–13]. In those reports, cortisol concentrations were measured following cortisone ingestion based on indirect (i.e. plasma concentrations, not flux) and non-specific measurements of hepatic 11 β -HSD-1 enzyme activity. These papers have indicated that hepatic cortisol production is reduced in obesity

and type 2 diabetes [9–13]. By contrast, a narrative review by Anagnostis et al reported higher cortisol concentrations in obese individuals [14], leading to a metabolic syndrome-type picture. These authors provide evidence of a need to develop tissue-specific inhibitors of 11 β -HSD-1 as targets for obesity and metabolic syndrome. Hence there is considerable controversy in this field which requires resolution. Since we have previously demonstrated that splanchnic cortisol uptake is increased in both obesity and type 2 diabetes [15], we hypothesise that the lower plasma cortisol concentrations observed in obese participants by these investigators following ingestion of cortisone were perhaps due to increased hepatic cortisol clearance/uptake rather than to decreased hepatic cortisol production.

The present experiments sought to address and resolve a very important biological question regarding hepatic 11 β -HSD-1 activity in obesity and type 2 diabetes. Previously we have used the splanchnic catheterisation method to determine relative contributions of both 11 β -HSD-1 and -2 enzyme activities in the liver and kidney, respectively. In those studies we were unable to directly measure hepatic 11 β -HSD-1 activity, since the tracer was infused into the systemic circulation. This is the first time we have used oral tracers that entered the liver via the portal vein from the gut (i.e. bypassing the viscera). This methodology enables direct measurement of hepatic 11 β -HSD-1 flux without the need for invasive organ catheterisation and could be used in a variety of clinical settings to study this enzyme pathway and its inhibition via specific liver 11 β -HSD inhibitors as a viable target for the modulation of hepatic insulin action in humans.

Methods

After approval from the Radioactive Drug Research Committee, Food and Drug Administration and Mayo Institutional Review Board, 20 lean non-diabetic (LND; BMI 19–24 kg/m²), 21 overweight/obese non-diabetic (OND; BMI 27–40 kg/m²) and 20 overweight/obese type 2 diabetic individuals (ODM; BMI 27–40 kg/m²) provided written consent to take part in the study. All participants were in good health and had a stable weight, and none engaged in unaccustomed physical exercise. First-degree relatives of the non-diabetic participants did not have a history of diabetes mellitus. Individuals with a history of smoking or alcohol intake over and above American Diabetes Association guidelines, i.e. two drinks per day for men and one drink per day for females, were excluded from participation in the study. At screening, two participants with type 2 diabetes were being treated with lifestyle modifications alone, nine with metformin alone, and nine with a combination of a sulfonylurea and metformin. Oral antihyperglycaemic medications were discontinued 10 days prior to the study visit.

Individuals with type 2 diabetes who were taking thiazolidinediones were excluded, since these agents decrease 11 β -HSD-1 activity in vitro [16]. All participants were instructed to follow a weight maintenance diet (55% carbohydrate, 30% fat and 15% protein) for at least 2 weeks prior to the study. Body composition (total fat and lean body mass) was measured in the Mayo Clinic Center for Translational Science Activities (CTSA) using dual-energy x-ray absorptiometry (Lunar iDXA software version 6.10; GE Healthcare Technologies, Madison, WI, USA). Detailed characteristics of the participants are provided in Table 1.

Experimental design

A schematic of the experimental design is shown in Fig. 1. Participants were admitted to the Mayo Clinic Clinical Research Unit at 17:00 hours on the evening before the study. A standard 42 kJ/kg (10 kcal/kg) meal (55% carbohydrate, 30% fat and 15% protein) was eaten between 17:30 and 18:00 hours. Thereafter, participants remained fasting until the end of the study. Sips of water were permitted ad libitum. At 05:00 hours on the morning of study an intravenous catheter was placed in a forearm vein for tracer infusion and another in the dorsum of the opposite hand placed in a heated box (~55°C) to permit sampling of arterialed venous blood. Primed (740,000 Bq) continuous (7,400 Bq/min) infusion of [1,2,6,7-³H]cortisol ([³H]cortisol) was started at -240 min. As part of separate experiments, a subset of participants were infused with tracers of cortisone/cortisol locally via microdialysis catheters in the subcutaneous fat of the abdomen and the leg. These tracers were undetectable in the plasma, i.e. there was no interference with results obtained from this study.

At time 0, participants ingested 1.0 mg [4-¹³C]cortisone ([¹³C]cortisone) and 1.0 mg [9,12,12-²H₃]cortisol (D cortisol) mixed in 38 ml water over 15 min. At time 0, the [³H]cortisol infusion rate was changed (Table 2) in order to mimic the temporal pattern of change in cortisol concentrations previously observed by Stewart et al following ingestion of cortisone [9]. Arterialed venous blood was sampled at -30, -20, -10, 0, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min for measurement of D cortisol, D cortisone, [¹³C]cortisone and C13 cortisol derived from the ingested [¹³C]cortisone enrichment, and [³H]cortisol-specific activity.

We used a triple tracer approach (we believe for the first time) to directly measure first-pass hepatic cortisone to cortisol conversion via the 11 β -HSD-1 pathway in obesity and type 2 diabetes. The conversion of ingested [¹³C]cortisone to C13 cortisol directly measures how much cortisol is converted from cortisone, while the ingested D cortisol directly measures fractional hepatic extraction of ingested D cortisol. Infusion of [³H]cortisol is used to measure hepatic C13 cortisol production using an isotope dilution technique. The systemic rates of appearance (Ra) of D cortisol and C13 cortisol were measured using intravenously infused [³H]cortisol, permitting concurrent measurements of hepatic extraction of the ingested D cortisol and conversion of the ingested [¹³C]cortisone to C13 cortisol.

Analytical techniques

Blood samples were immediately placed on ice, centrifuged at 4°C, separated and stored at -80°C until the analyses were carried out. Plasma unlabelled cortisol and cortisone, and labelled cortisol and cortisone, enrichments were measured using liquid chromatography-tandem mass spectrometry as previously described [17, 18], with slight modification and additional monitoring. Briefly, protein was precipitated from 100 μ l plasma samples and standards made in stripped sera with 250 μ l cold acetonitrile after adding 25 μ l internal standard solution (0.02 μ g/ml prednisolone). Fifty microlitres of the supernatant fraction was injected onto an Aria TLX-2 liquid chromatographic system (Thermo Fisher Scientific, San Jose, CA, USA) configured with a 0.5 $\times$ 50 mm C18 HTLC Column (Thermo Fisher Scientific) that served as an extraction column, prior to being transferred directly to an

Alltima C18 4.6 mm×150 mm×5 µmol/l analytical column (Grace, Columbia, MA, USA). Analytes were separated via an analytical column using a gradient buffer system, with buffer A being 10% methanol/0.1 mmol/l ammonium acetate and B being 90% methanol/0.1 mmol/l ammonium acetate, prior to being introduced into an API 5000 triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA, USA) for multiple reaction monitoring acquisition. The transitions monitored were 363.2> 121.0, 364.2 > 122.0, 366.2 > 121.0, 361.1 > 163.0, 362.1>164.0, 364.1>164.0, 361.3>147.0 for cortisol, C13 cortisol, D cortisol, cortisone, [¹³C]cortisone, D cortisone and prednisolone, respectively. Cortisol and cortisone concentrations were calculated against a six-point standard curve using the ratios between cortisol/ prednisolone and cortisone/prednisolone. Mole per cent enrichment (MPE) of C13 cortisol, D cortisol, [¹³C]cortisone and D cortisone was calculated using the following formula for each species:

$$\text{MPE} = \left\{ \text{Area ratio}_{(\text{sample}-\text{baseline})} / \left[\text{Area ratio}_{(\text{sample}-\text{baseline})} \right] + 1 \right\} \times 100$$

For each participant the baseline MPE was subtracted from each of their reported time points and values reported were expressed as the observed enrichment above natural abundance level. Compared with D4 cortisol, which we previously used in our splanchnic catheterisation studies, C13 cortisol is sterically and chromatographically much more similar to cortisol, and the inter-assay and intra-assay CVs obtained were 1.06% and 4.9%, respectively. [³H]Cortisol radioactivity was measured using HPLC followed by liquid scintillation counting [19].

Calculations

Steele steady-state and non-steady-state equations [20] were used to calculate cortisol fluxes analogous to our triple tracer approach to measure postprandial glucose turnover [21]. Although the [³H]cortisol infusion profile was adjusted to minimise the change in plasma [³H]cortisol/C13 cortisol and plasma [³H]cortisol/D cortisol to maintain ratios constant throughout the experiment, minor perturbations did occur immediately following ingestion of oral tracers. Hence we have opted to present both steady- and non-steady-state calculations for fluxes.

In order to calculate the fluxes, the tracer and tracee cortisol concentrations were derived from plasma enrichments.

Definitions

Tracee	Unlabelled cortisol
Tracer I (i.v.)	[³ H]Cortisol
Tracer II (oral)	[¹³ C]Cortisone
Tracer III (oral)	D cortisol
Plasma concentration of tracee (unlabelled cortisol)	Cortisol (nmol/l)
Plasma concentration of tracer I ([³ H]cortisol)	[³ H]Cortisol (dpm/l)

Plasma concentration of C13 cortisol	C13 cortisol per cent molar ratio [MR%]/100×unlabelled cortisol (nmol/l)
Plasma concentration of tracer III (D cortisol)	D cortisol MR%/100× unlabelled cortisol (nmol/l)
$F[{}^3\text{H}]\text{cortisol}$	Rate of intravenous infusion of $[{}^3\text{H}]\text{cortisol}$ (dpm/min)

For the purposes of calculation, concentrations at any given time point are averaged between that time point and the one prior to it.

Equations— $[{}^3\text{H}]\text{Cortisol}$ was used to trace the systemic Ra of both C13 cortisol and D cortisol. Thus the systemic Ra ($\mu\text{g}/\text{min}$) of C13 cortisol derived from the ingested $[{}^{13}\text{C}]\text{cortisone}$ was calculated by using Steele's non-steady-state equation:

$$\text{Ra}_{\text{C13 cortisol}} = \frac{F[{}^3\text{H}]\text{cortisol} - V \times \text{C13 cortisol} \times (\delta[{}^3\text{H}]\text{cortisol}/\text{C13 cortisol})/\delta t}{[{}^3\text{H}]\text{cortisol}/\text{C13 cortisol}} \quad (1)$$

where t denotes time (min); V (ml) is the volume of distribution of cortisol, according to Andrew et al [10], which is a qualitative estimation, since V_{of} glucose is used in substitution of true V_{of} cortisol in their calculations, and δt is the time derivative.

The systemic Ra ($\mu\text{g}/\text{min}$) of D cortisol derived from the ingested D cortisol tracer was calculated as:

$$\text{Ra}_{\text{D cortisol}} = \frac{F[{}^3\text{H}]\text{cortisol} - V \times \text{D cortisol} \times (\delta[{}^3\text{H}]\text{cortisol}/\text{D cortisol})/\delta t}{[{}^3\text{H}]\text{cortisol}/\text{D cortisol}} \quad (2)$$

Since $[{}^3\text{H}]\text{cortisol}$ was infused intravenously in a pattern designed to minimise the change in the plasma tracer-to-tracee ratio, the second term, i.e. $\delta([{}^3\text{H}]\text{cortisol}/\text{C13 cortisol})$ and $([{}^3\text{H}]\text{cortisol}/\text{D cortisol})/\delta t$ in Eqs 1 and 2 approached zero, thereby minimising any non-steady-state error that otherwise would occur if Steele's equation were used to calculate turnover in the presence of large changes in the plasma tracer-to-tracee ratio. This creates in essence a model-independent calculation of appearance, since the first term merely represents dilution of tracer by tracee.

After the first sampling time point (~10 min) following ingestion of oral tracers, we found that the tracer-to-tracee ratios (specific activity) were constant; hence we opted to use steady-state equations to calculate cortisol fluxes.

$$\text{Ra}_{\text{C13 cortisol}} = \frac{F[{}^3\text{H}]\text{cortisol}}{[{}^3\text{H}]\text{cortisol}/\text{C13 cortisol}} \quad (3)$$

$$Ra_{D \text{ cortisol}} = \frac{F[{}^3\text{H}]\text{cortisol}}{[{}^3\text{H}]\text{cortisol}/D \text{ cortisol}} \quad (4)$$

Hepatic extraction (HE) of D cortisol was calculated as:

$$HE_{D \text{ cortisol}} = \frac{\text{Dose D cortisol} - \int_0^{360} Ra_{D \text{ cortisol}}(t) \delta t}{\text{Dose D cortisol}} \quad (5)$$

where dose D cortisol denotes the amount of D cortisol ingested and $\int_0^{360} Ra_{D \text{ cortisol}}(t) \delta t$ is the AUC of D cortisol (Eq. 4) that reaches the systemic circulation over 360 min of the study.

Hepatic production (HP) of C13 cortisol was calculated as the AUC for the Ra of C13 cortisol (Eq. 3) divided by 1 minus the hepatic extraction (HE) of D cortisol (Eq. 5).

$$HP_{C13 \text{ cortisol}} = \frac{\int_0^{360} Ra_{C13 \text{ cortisol}}}{1 - HE_{D \text{ cortisol}}} \quad (6)$$

Total body cortisol production was calculated using Steele's equation:

$$Ra_{\text{total cortisol}} = \frac{F[{}^3\text{H}]\text{cortisol}}{[{}^3\text{H}]\text{cortisol}/\text{cortisol}_{\text{total}}} \quad (7)$$

where $\text{cortisol}_{\text{total}}$ denotes the total plasma cortisol (i.e. sum of unlabelled cortisol plus C13 cortisol plus D cortisol) concentration. $[{}^3\text{H}]\text{Cortisol}$ clearance was calculated using established methodology by Hughes et al [22].

Statistical analysis

Data in the text and figures are expressed as mean $\pm$ SD. Concentrations are expressed as nmol/l and Ra as $\mu\text{g}/\text{min}$. One-way ANOVA models and Kruskal–Wallis tests were used to test the hypothesis that hepatic cortisol production differs among LND, OND and ODM. The primary outcome measures to quantify hepatic cortisol production were AUC, which is conceptually a weighted average of the serial measurements of each tracer; the Ra of the tracers; and hepatic extraction and production. A p value <0.05 was considered statistically significant for each outcome measure in the overall (omnibus) test for differences among the three groups. There were two planned post hoc comparisons: testing the effect of diabetes (i.e. OND vs ODM) and testing the effect of obesity (i.e. LND vs OND) using Wilcoxon's rank sum test at a Bonferroni-corrected level of significance of 0.025 ($=0.05/2$). This lower threshold was used to determine statistical significance; hence p values reported in the paper have not been inflated for multiple testing. When the study was designed, the sample size

($n=20$ for each group) was estimated to provide a power greater than 80% to detect a 25% reduction in hepatic C13 cortisol production in OND and ODM. Statistical analysis was conducted using SAS software version 9.3 (SAS Institute, Cary, NC, USA).

Results

Cortisol, C13 cortisol, D cortisol and [^3H]cortisol concentrations

Unlabelled cortisol concentrations were not statistically different ($p=0.67$) among the three groups; however, following [^{13}C]cortisone and D cortisol ingestion, C13 cortisol concentrations and D cortisol concentrations differed ($p<0.05$) among groups. Both C13 cortisol ($p<0.02$) and D cortisol ($p<0.05$) concentrations were lower in OND compared with LND over 6 h of the study period. There was no difference ($p=0.15$) in C13 and D cortisol concentrations between OND and ODM. [^3H]Cortisol concentrations were no different ($p=0.40$) between the groups (Table 3, Fig. 2a–d). Clearance of [^3H]cortisol (Table 2) calculated at baseline (steady state) was numerically lower in LND than in OND and ODM; however, statistically there was no difference ($p=0.36$).

Ratios of plasma [^3H]cortisol to C13 cortisol and [^3H]cortisol to D cortisol

Ratios of plasma [^3H]cortisol to C13 cortisol and [^3H]cortisol to D cortisol remained constant in all three groups throughout the study except for minor perturbations during approximately the first 10–15 min (Fig. 3), thereby permitting accurate calculations of cortisol fluxes using both steady-state and non-steady-state equations.

Ra of C13 cortisol and D cortisol

The Ra of C13 cortisol from ingested [^{13}C]cortisone provides an index of hepatic 11β -HSD-1 activity. The Ra of C13 cortisol was not different in all three groups whether calculated using steady-state or non-steady-state equations (Fig. 4 and Table 4). Similarly, the Ra of D cortisol also did not differ in OND compared with LND or between OND and ODM calculated over 20 min, 30 min, 1 h, 2 h, 4 h or the total 6 h of the study using steady-state or non-steady-state equations (Fig. 4a–d and Table 4).

Hepatic C13 cortisol production

Fractional hepatic cortisol extraction calculated using steady-state or non-steady-state equations was not statistically different among the study groups (Table 4). First-pass hepatic conversion of the ingested [^{13}C]cortisone to C13 cortisol calculated over 1, 2, 4 and 6 h of the study using steady-state or non-steady-state equations did not differ in all three groups (Fig. 5 and Table 4). This implies that hepatic C13 cortisol production is similar in lean vs obese vs type 2 diabetic individuals.

Discussion

The data indicate that hepatic C13 cortisol production following ingestion of [^{13}C]cortisone was not different in OND and ODM compared with LND. Plasma C13 and D cortisol concentrations observed following ingestion of tracers were consistently lower in OND and ODM compared with LND. However, fractional hepatic extraction of D cortisol as well as

clearance of [^3H]cortisol estimated at steady state (basal), though numerically lower in LND, was not statistically different across the three study groups. In the absence of differences in hepatic C13 cortisol production between LND and OND, our data suggest that the previously reported lower plasma cortisol concentrations observed after cortisone ingestion in obesity [9, 10] were perhaps due to increased hepatic cortisol clearance/uptake rather than to decreased hepatic cortisone to cortisol conversion (i.e. 11 β -HSD-1 activity). We also show no differences in hepatic cortisol production in ODM compared with OND and LND. This has previously not been reported. In addition, these observations are consistent with previous studies conducted by us using the splanchnic catheterisation technique in which we observed that splanchnic cortisol uptake was higher with no difference in splanchnic cortisol production rate in people who were obese or who had diabetes [15]. This is also consistent with a prior report by Stimson et al [23] that splanchnic cortisol production was not different in lean vs obese patients with type 2 diabetes. We conclude that, regardless of degree of obesity or diabetes, hepatic 11 β -HSD-1 activity is unchanged. Therefore modulation of hepatic cortisol by selective 11 β -HSD-1 inhibitors may be a therapeutic option for type 2 diabetes.

The proposed experiments are based on certain assumptions that are unlikely to confound data interpretation. First, we assumed that the intestinal conversion of cortisone to cortisol is negligible [8, 24, 25]. Second, we assumed that metabolic fates of [^3H]cortisol, D cortisol and C13 cortisol are similar (i.e. there is no isotopic discrimination). Therefore, once the tracers (i.e. ingested D cortisol and C13 cortisol) reach the systemic circulation, both are metabolised in parallel to the tracer (i.e. intravenously infused [^3H]cortisol), including conversion to cortisone then back to cortisol, without losing their respective labels. Therefore, since no dilution occurs relative to one another, this cycling is 'invisible' to the infused [^3H]cortisol which only traces the systemic Ra of cortisol derived from the ingested tracers. Third, there is no reason to suspect that hepatic clearance of the ingested D cortisol will be any different from that of C13 cortisol derived from hepatic conversion of [^{13}C]cortisone. Compartmentalisation of 11 β -HSD-1 across the liver is still under evaluation, with studies demonstrating centripetal rather than periportal expression [26]. However, since cortisol readily diffuses across plasma membranes, we doubt this will be the case. Clearly future studies will be required to evaluate this further. Fourth, based on previous studies [9, 11], we assumed that both [^{13}C]cortisone and D cortisol would be completely absorbed over the 6 h of the proposed study. Upon inspection of the data from the first few studies we found this to be true for 4 h as well as for 6 h of the study duration, but we opted to continue for the entire 6 h planned duration of the experiment. Furthermore we also noted that the Ra of C13 cortisol and D cortisol calculated as the AUC was no different between the three groups in the first 60 min of the study when the orally ingested tracers were being rapidly absorbed by the gut and transported by the portal vein to the liver (i.e. during first-pass hepatic extraction). Fifth, we assumed that we could achieve relatively stable plasma tracer-to-tracee ratios. We varied the infusion rate of [^3H]cortisol in order to minimise the change in the plasma ratios of [^3H]cortisol/D cortisol and [^3H]cortisol/C13 cortisol. As anticipated, we had no difficulty in achieving this goal based on our prior experience [21, 27]. D cortisol and [^{13}C]cortisone were administered in equimolar amounts. Assuming near complete conversion of cortisone to cortisol, appropriate adjustment of the

[³H]cortisol infusion rate resulted in stable ratios for [³H]cortisol/D cortisol and [³H]cortisol/C13 cortisol, as shown in Fig. 3, after the initial 10–15 min when the concentrations were rapidly changing in the plasma.

Previously we used the splanchnic catheterisation method for measurement of splanchnic (i.e. viscera and liver) cortisol production which also requires a series of assumptions regarding rates of flux between the cortisone and cortisol pools within the viscera, kidney and the liver (i.e. four compartments), as well as rates of irreversible loss of both cortisone and cortisol from these pools [8, 15, 17]. The validity of the resultant flux calculations depends on the accuracy of the assumed four-compartment model as well as accurate measurements of splanchnic blood flow. Hence we proposed the triple tracer approach, since, if changes in the plasma tracer-to-tracee ratios can be minimised during the experiment, this (rather than the splanchnic catheterisation method) could become the gold standard to measure cortisol turnover because this method is essentially model-independent. As we had anticipated, we were able to minimise changes in plasma tracer-to-tracee ratios and use steady-state equations without the need to calculate volume of distribution of cortisol, thereby achieving a simpler yet sophisticated method for measurement of hepatic 11 β -HSD-1 activity in humans. To our knowledge, the volume of distribution of cortisol has previously been reported by Andrew et al assuming it to be similar to that observed for glucose [10], which is a qualitative estimation.

As expected, though numerically different, the conclusions in our study are virtually identical whether one uses steady-state or non-steady-state calculations for cortisol turnover. Additionally, splanchnic catheterisation techniques are invasive and can only be performed under very controlled circumstances in centres that have the necessary infrastructure and expertise to conduct such studies, thus limiting their wide applicability.

We measured total body cortisol production as a hypothesis-generating exercise to determine whether the statistically non-significant increase that we have previously observed in obese non-diabetic and type 2 diabetic individuals [15] is more evident in the larger number of participants studied in the current experiments. We found no difference in total body cortisol production between LND and OND. This is in contrast to a prior report that concluded that obesity was associated with excess total body cortisol production [28]. We also found no correlation between 11 β -HSD-1 activity and fasting glucose concentrations in the three groups studied. There were no sex differences noted between groups.

The sample size of the study was based on the power to detect a 25% difference in hepatic cortisol production between the three groups. The observed mean hepatic cortisol production using non-steady-state calculations ranged from 61% to 63%, which was only a 5% difference in means. Thus, while hepatic cortisol production was not statistically significant in this sample, the observed differences were not of the magnitude to be considered clinically relevant. Fractional hepatic extraction, on the other hand, had means that ranged from 10% to 30%, which met the a priori expectation of a 25% change, but the observed variation in extraction yielded less than the planned power. The medians ranged from 15% to 19%, which was approximately a 21% difference.

We conclude that hepatic conversion of ingested [^{13}C]cortisone to C13 cortisol (i.e. an index of hepatic $11\beta\text{-HSD-1}$ activity) is the same in lean as in overweight/obese non-diabetic individuals as well as in those who are overweight/ obese and have type 2 diabetes. Furthermore, the lower cortisol concentrations observed by us and others in obese individuals is likely due to increased first-pass hepatic extraction and/or clearance. Therapeutic modulation by selective $11\beta\text{-HSD-1}$ inhibitors may therefore be useful in targeting hepatic cortisol production in type 2 diabetes.

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Abbreviations

[^{13}C]Cortisone	[4- ^{13}C]Cortisone
[^3H]Cortisol	[1,2,6,7- ^3H]Cortisol
C13 Cortisol	Cortisol derived from ingested [^{13}C]cortisone
CTSA	Mayo Clinic Center for Translational Science Activities
D Cortisol	[9,12,12- $^2\text{H}_3$]Cortisol
D Cortisone	[9,12,12- $^2\text{H}_3$]Cortisone
dpm	Disintegrations per minute
$11\beta\text{-HSD}$	$11\beta\text{-Hydroxysteroid dehydrogenase}$
$11\beta\text{-HSD-1}$	$11\beta\text{-Hydroxysteroid dehydrogenase type 1}$
LND	Lean non-diabetic participants
MPE	Mole per cent enrichment
MR	Molar ratio
OND	Overweight/obese non-diabetic participants
ODM	Overweight/obese participants with type 2 diabetes
Ra	Rate of appearance

References

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Fig. 1.

Schematic outline of the experimental design. A primed continuous infusion of [³H] cortisol was started at -240 min and then varied from time 0 onward to mimic the anticipated Ra of D cortisol and C13 cortisol (derived from ingested [¹³C]cortisone); 1.0 mg of [¹³C]cortisone and 1.0 mg of D cortisol were ingested at time 0

Fig. 2.

Concentrations of unlabelled cortisol (a), C13 cortisol (b), [³H]cortisol (c) and D cortisol (d) observed in LND (black squares), OND (black circles) and ODM (white triangles); 1.0 mg [¹³C]cortisone and 1.0 mg D cortisol were ingested at time 0

Fig. 3. Ratios of plasma $[^3\text{H}]$ cortisol/C13 cortisol and plasma $[^3\text{H}]$ cortisol/D cortisol observed with the triple tracer cortisol technique in LND (black squares), OND (black circles) and ODM (white triangles); 1.0 mg $[^{13}\text{C}]$ cortisone and 1.0 mg D cortisol were ingested at time 0

Fig. 4.

Systemic Ra of C13 cortisol (**a, b**) and D cortisol (**c, d**) observed in LND (black squares), OND (black circles) and ODM (open triangles). Data for steady state are shown on the left panels and those for non-steady state on the right panels; 1.0 mg [^{13}C]cortisone and 1.0 mg D cortisol were ingested at time 0

Fig. 5.

First-pass hepatic conversion of ingested [¹³C]cortisone to C13 cortisol (hepatic 11β-HSD-1 activity) in LND (black bar), OND (grey bar) and ODM (white bar) calculated using non-steady-state equation; 1.0 mg [¹³C]cortisone and 1.0 mg D cortisol were ingested at time 0

Table 1

Participant characteristics

Participant characteristics	LND	OND	ODM
Age, years	52±13	54±11	58±11
Sex, male/female	9/11	11/10	12/8
Weight, kg	78.4±27.1	90.5±13.3	94.2±17.9
BMI, kg/m ²	22.8±1.1 *	31.3±3.9	32.2±4.8
Lean body mass, kg	40.0±12.3 *	51.7±11.5	53.9±10.5
Total fat, %	29.7±5.1 *	37.6±9.8	38.2±5.2
Fasting plasma glucose, mmol/l	4.86±0.35	4.92±0.27	7.69±1.98 ***
HbA _{1c} , % (mmol/mol)	5.3±0.4 (34±5)	5.5±0.3 (37±3)	7.1±0.8 *** (54±9)
Serum creatinine, µmol/l	79.56±3.54	79.56±2.65	70.72±4.42

Data are mean ± SD

*
 $p < 0.05$ vs OND and ODM***
 $p < 0.001$ vs LND and OND

Table 2

Rate of infusion of Tracer I

Time (min)	Infusion rate of [^3H]cortisol (ml/min)
-240 to 10	0.10
11 to 20	0.45
21 to 40	0.40
41 to 75	0.35
76 to 120	0.20
121 to 150	0.10
151 to 360	0.05

Table 3

Concentrations of unlabelled and labelled cortisol

Concentration	LND (n=20)		OND (n=21)		ODM (n=20)		ANOVA <i>p</i> value	Kruskal–Wallis <i>p</i> value	Post hoc comparisons ^a	
	Mean	SD	Mean	SD	Mean	SD			Lean vs obese <i>p</i> value	Obese vs type 2 diabetes <i>p</i> value
AUC unlabelled cortisol, nmol/l×360 min	67,231.96	27,588.00	66,735.37	36,719.63	74,542.78	28,360.46	0.677	0.255	—	—
AUC C13 cortisol, nmol/l×360 min	5,710.72	3,448.50	3,503.68	1,351.81	4,800.31	2,951.92	0.040	0.021	0.006	0.151
AUC D cortisol, nmol/l×360 min	9,407.51	5,296.90	6,262.48	2,096.69	8,359.16	4,083.02	0.045	0.049	0.011	0.175
AUC [³ H]cortisol, dpm/l×360 min	729,652,000	308,447,000	593,979,000	238,354,000	755,779,000	379,208,000	0.212	0.402	—	—
Clearance [³ H]cortisol, l/min	0.25	0.81	0.40	0.12	0.41	0.99	0.362	0.552	—	—

^aComparisons are Wilcoxon's rank sum test if the overall (omnibus) test for group difference was statistically significant ($p < 0.05$ for ANOVA or Kruskal–Wallis test); in the event of post hoc comparisons, $p < 0.025$ should be used to determine statistical significance after adjusting for multiple comparisons (Bonferroni correction)

Table 4

Rate of appearance of C13 and D cortisol, fractional hepatic extraction and hepatic C13 cortisol production

Ra	LND (n=20)		OND (n=21)		ODM (n=20)		ANOVA <i>p</i> value	Kruskal–Wallis <i>p</i> value	Post hoc comparisons ^d	
	Mean	SD	Mean	SD	Mean	SD			Lean vs obese <i>p</i> value	Obese vs type 2 diabetes <i>p</i> value
AUC C13 cortisol, µg/360 min										
Steady state	597.13	465.98	444.19	129.96	503.16	198.30	0.267	0.701	—	—
Non-steady state	562.20	381.02	409.04	181.11	426.86	95.77	0.113	0.185	—	—
AUC D cortisol, µg/360 min										
Steady state	981.34	702.56	818.69	291.67	877.99	268.00	0.529	0.770	—	—
Non-steady state	901.67	566.26	695.98	260.74	754.84	232.04	0.218	0.388	—	—
Fractional hepatic extraction, µg/360 min										
Steady state	0.02	0.70	0.18	0.29	0.12	0.27	0.529	0.770	—	—
Non-steady state	0.10	0.57	0.30	0.26	0.25	0.23	0.218	0.388	—	—
Hepatic C13 cortisol production, µg/360 min										
Steady state	0.61	0.15	0.59	0.22	0.58	0.16	0.862	0.896	—	—
Non-steady state	0.63	0.18	0.61	0.20	0.63	0.29	0.934	0.786	—	—

^aComparisons are not reported, since no omnibus tests reached statistical significance ($p < 0.05$)