Increased In Vivo Regeneration of Cortisol in Adipose Tissue in Human Obesity and Effects of the 11β-Hydroxysteroid Dehydrogenase Type 1 Inhibitor Carbenoxolone

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11β-Hydroxysteroid dehydrogenase type 1 (11HSD1) regenerates cortisol from cortisone within adipose tissue and liver. 11HSD1 inhibitors may enhance insulin sensitivity in type 2 diabetes and be most efficacious in obesity when 11HSD1 is increased in subcutaneous adipose biopsies. We examined the regeneration of cortisol in vivo in obesity, and the effects of the 11HSD1 inhibitor carbenoxolone. We compared six lean and six obese men and performed a randomized, placebo-controlled crossover study of carbenoxolone in obese men. The obese men had no difference in their whole-body rate of regenerating cortisol (measured with 9,11,12,12-[$^2H_4$]cortisol tracer), but had more rapid conversion of [^{3}H]cortisone to [^{3}H]cortisol in abdominal subcutaneous adipose tissue (measured with microdialysis). During insulin infusion, adipose 11HSD1 activity fell markedly in lean but not in obese men. Carbenoxolone inhibited whole-body cortisol regeneration, but did not significantly inhibit adipose 11HSD1 and had no effects on insulin sensitivity (measured by [$^2H_2$]glucose infusion with or without hyperinsulinemia). Thus, in vivo cortisol generation is increased selectively within adipose tissue in obesity, perhaps reflecting resistance to insulin-mediated downregulation of 11HSD1. However, obese men are less susceptible than lean men to the insulin-sensitizing effects of carbenoxolone. To be useful in obese patients, 11HSD1 inhibitors will need to inhibit the enzyme more effectively in adipose tissue.
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GCMS, gas chromatography−mass spectrometry; 11HSD1, 11β-hydroxysteroid dehydrogenase type 1.

11β-Hydroxysteroid dehydrogenase type 1 (11HSD1) is a microsomal enzyme expressed in many tissues, including liver and adipose tissue (1). It catalyzes the regeneration of the active glucocorticoid cortisol from its inactive 11-keto metabolite cortisone. This intracellular generation of cortisol plays a key role in amplifying glucocorticoid receptor activation independently of the level of cortisol in the circulating plasma. Its potential importance is illustrated in animal models. Transgenic mice that overexpress 11HSD1 by approximately threefold selectively in adipocytes under the AP2 promoter/enhancer (2,3) develop about a twofold increase in intra-adipose glucocorticoid levels despite no change in plasma levels, which are controlled by a compensatory fall in ACTH secretion. This results in central obesity together with hyperinsulinemia, hyperglycemia, hyperlipidemia, and hypertension. Mice with similar overexpression of 11HSD1 in liver under the ApoE promoter develop insulin resistance, dyslipidemia, and hypertension without obesity (4). Conversely, 11HSD1 knockout mice on a high-fat diet are protected from obesity, hyperglycemia, and dyslipidemia and redistribute fat to peripheral rather than central fat depots (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 liver but increased in adipose tissue. These findings substantiate the hypothesis that increased intra-adipose glucocorticoid regeneration by 11HSD1 contributes to obesity and its metabolic complications.

Whether increased 11HSD1 levels have a similar importance in human obesity and associated type 2 diabetes is controversial (10). The conventional measure of 11HSD1 is the ratio of urinary cortisol to cortisone metabolites, which is inconsistently altered in obesity (11–16) and diabetes (17–19). However, this ratio may be confounded by the activity of other enzymes (e.g., 11HSD type 2, 5α- and 5β-reductase) that differ in obesity, and is insensitive to the tissue-specific changes in 11HSD1 that have been observed in animals. Hepatic 11HSD1 has been assessed in humans by measuring the conversion of an oral dose of cortisone into cortisol in peripheral plasma after "first pass" metabolism, and is consistently reduced in obesity

(13–15) more than in type 2 diabetes (18). Adipose 11HSD1 has been assessed in vitro in biopsies. Since our original report (14), many investigators have confirmed substantial increases in subcutaneous adipose 11HSD1 activity and mRNA in obese men and women (15,20–24) of a similar magnitude as the increase in the adipose-overexpressing transgenic mouse (2). One study did not confirm this finding in obese subjects (25), perhaps because the adipose biopsies were obtained during surgery. In lean type 2 diabetic patients, subcutaneous adipose 11HSD1 is not altered (18). It appears, therefore, that the tissue-specific dysregulation of 11HSD1 observed in obese animals also occurs in humans, but that diabetes per se is not associated with dysregulation of 11HSD1.

However, in vitro findings in biopsies cannot establish the magnitude of the effect of altered 11HSD1 levels in obesity. In biopsies, it has not been possible to correlate 11HSD1 activity or mRNA with cortisol levels or mRNA levels for glucocorticoid-dependent genes (21,22). Previous attempts to measure adipose 11HSD1 in vivo using arteriovenous sampling across abdominal subcutaneous adipose tissue have been inconclusive, perhaps because of variability in measurement (26). Recently, we (27) and others (28) have validated a new approach using deuterated cortisol tracer infusion to measure the turnover of 11HSD activities in vivo. Here, we use this technique to quantify systemic regeneration of cortisol by 11HSD1. Recent studies using arteriovenous sampling have suggested that the splanchnic circulation (liver and intra-abdominal adipose tissue) accounts for most of the systemic regeneration of cortisol (28). To assess cortisol regeneration specifically in subcutaneous adipose tissue, we have developed a new technique using in vivo microdialysis.

Development of selective 11HSD1 inhibitors for the treatment of type 2 diabetes is a highly competitive goal for the pharmaceutical industry (29). The first such agents, arylsulfonamidothiazoles, are effective in enhancing hepatic insulin sensitivity and lowering blood glucose levels in diabetic mice (30,31). In humans, the nonselective 11HSD1-inhibitor carbenoxolone has been shown to enhance hepatic insulin sensitivity in healthy men and in nonobese type 2 diabetic patients (32,33). However, in one study, carbenoxolone did not improve insulin sensitivity in Zucker obese rats (34); in these animals, hepatic 11HSD1 was downregulated so that additional inhibition by carbenoxolone may have been less effective, and carbenoxolone had no measurable effect on 11HSD1 in adipose tissue. It is widely believed that 11HSD1 inhibitors will be most efficacious in patients in whom hyperglycemia is associated with obesity. However, the efficacy of such agents in human obesity has yet to be reported. Here, we examine the effect of carbenoxolone on 11HSD1 activity and insulin sensitivity in obese men.

RESEARCH DESIGN AND METHODS

Healthy men age 20–50 years were recruited by advertisement. They were classified as lean (BMI $< 26 kg/m^2$) or obese (BMI $>30 kg/m^2$). Exclusion criteria included abnormal liver, renal, and thyroid function tests on biochemical screening; alcohol consumption >21 units per week; glucocorticoid therapy by any route in the previous 3 months; a history of depression in the previous 3 months; or the taking of regular medication. The study was approved by the local ethics committee, and written informed consent was obtained.

We recruited six obese men for this randomized double-blind crossover study comparing carbenoxolone (Biorex, Enfield, UK; 100 mg every 8 h orally for 7 days) with placebo. Phases were separated by a 2-week wash-out period. At the end of each phase, subjects collected urine for 24 h and then attended the clinic on two successive mornings after overnight fasts for measurements. Lean men attended only once, unmedicated, for the same measurements.

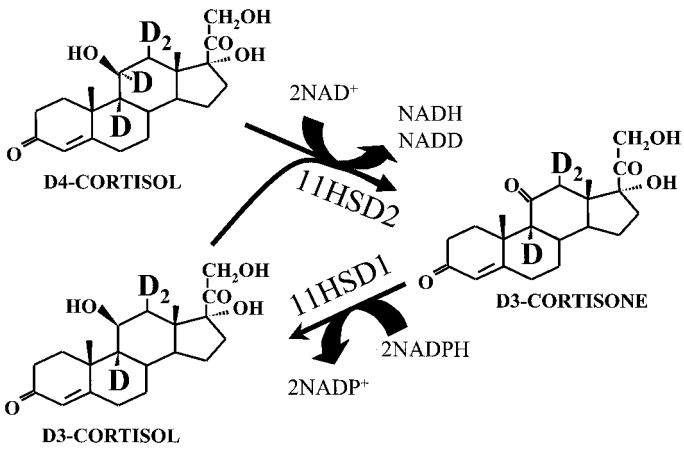

FIG. 1. Metabolism of [$^2H_4$]cortisol (D4-cortisol) by 11HSDs. Note that D3-cortisol is subject to reversible interconversion, whereas D4-cortisol is not regenerated by 11HSD1.

On one morning, systemic 11HSD activities were measured. Weight, height, waist and hip circumferences, and blood pressure after 5 min sitting were measured. Two antecubital venous cannulas were sited, one for infusions and the other for sampling. 9,11,12,12-[$^2H_4$]Cortisol (Cambridge Isotope, Andover, MA) was infused for 5 h at 20% enrichment in unlabeled cortisol at 1.74 mg/h after a priming dose of 3.6 mg. Samples were withdrawn at the intervals indicated on Fig. 1. Drinks of 100 ml water were given to encourage urine flow, and timed urine aliquots were collected close to hourly intervals throughout the study.

On the second morning, insulin sensitivity was measured in a euglycemic clamp with and without hyperinsulinemia, and 11HSD1 activity was measured in subcutaneous adipose tissue by in vivo microdialysis. A microdialysis cannula (CMA Microdialysis, Solna, Sweden) was inserted in paraumbilical subcutaneous abdominal adipose tissue and infused with Ringer lactate solution (0.3 μl/min) containing 1,2,6,7-[^{3}H]cortisone (50 nmol/l) from $t = 0$ to 5 h. Effluent was collected from the cannula each hour. Two venous cannulas were sited, one in an antecubital vein for infusions and the other in the retrograde direction in a contralateral hand vein for sampling; the hand was placed in a hot box to arterialize the blood. 6,6-[$^2H_2$]Glucose (Cambridge Isotope) was infused at $3 mg \cdot kg^{-1} \cdot h^{-1}$ from $t = 0$ to 5 h after a priming dose of 1.7 mg. Samples were taken during steady-state conditions at $t = 2.5$–3 h. Insulin was then infused at $t = 3$–5 h at $0.8 mU \cdot kg^{-1} \cdot min^{-1}$, and 20% dextrose (with [$^2H_2$]glucose added at 1.4 mg/ml) was infused at a variable rate to maintain arterialized blood glucose concentrations at 5 mmol/l. Samples were taken again at $t = 4.5$–5 h (i.e., 1.5–2 h after introducing hyperinsulinemia).

Laboratory measurements. Enzyme immunoassays (Eurogenetics Tasah, Hampton, U.K.) were used to measure plasma insulin and C-peptide levels. Electrolytes were measured on a Vitras 950 (Ortho Clinical Diagnostics, Amersham, U.K.) and glucose on a Cabas Mira Plus (Roche Diagnostics, East Sussex, U.K.). Free fatty acids were measured by a colorimetric technique (Wako, Neuss, Germany). Triglycerides, total cholesterol, and HDL cholesterol were measured using enzyme-linked immunosorbent assay kits (Roche Diagnostics). HbA_{1c} was measured by ion-exchange high-performance liquid chromatography (Variant 11; Bio-Rad, Hemel Hempstead, U.K.). Carbenoxolone was measured by high-pressure liquid chromatography with ultraviolet detection (at 254 nm), using 18α-glycyrrhetinic acid as the internal standard (33). Gas chromatography–mass spectrometry (GCMS) was used to measure 24-h urine excretion of endogenous cortisol metabolites, as previously described (35).

The isotopomers of [$^2H_4$]cortisol (D4-cortisol), [$^2H_3$]cortisone (D3-cortisone), and [$^2H_3$]cortisol (D3-cortisol) were measured as previously described (27) in plasma and urine by GCMS after hydrolysis and formation of methoximetrimethylsilyl derivatives. Epicortisol was added to plasma and epicortisol and epi-5β-tetrahydrocortisol were added to urine as internal standards. Enrichments were calculated from peak areas. Results for D4-cortisol were corrected for isotopic interference from endogenous mass + 4 cortisol and mass + 1 D3-cortisol. Results for D3-cortisol were corrected for isotopic interference from endogenous mass + 3 cortisol.

[$^2H_2$]Glucose enrichment in plasma was measured by GCMS, as previously described, after the formation of di-*O*-isopropylidene derivatives (36).

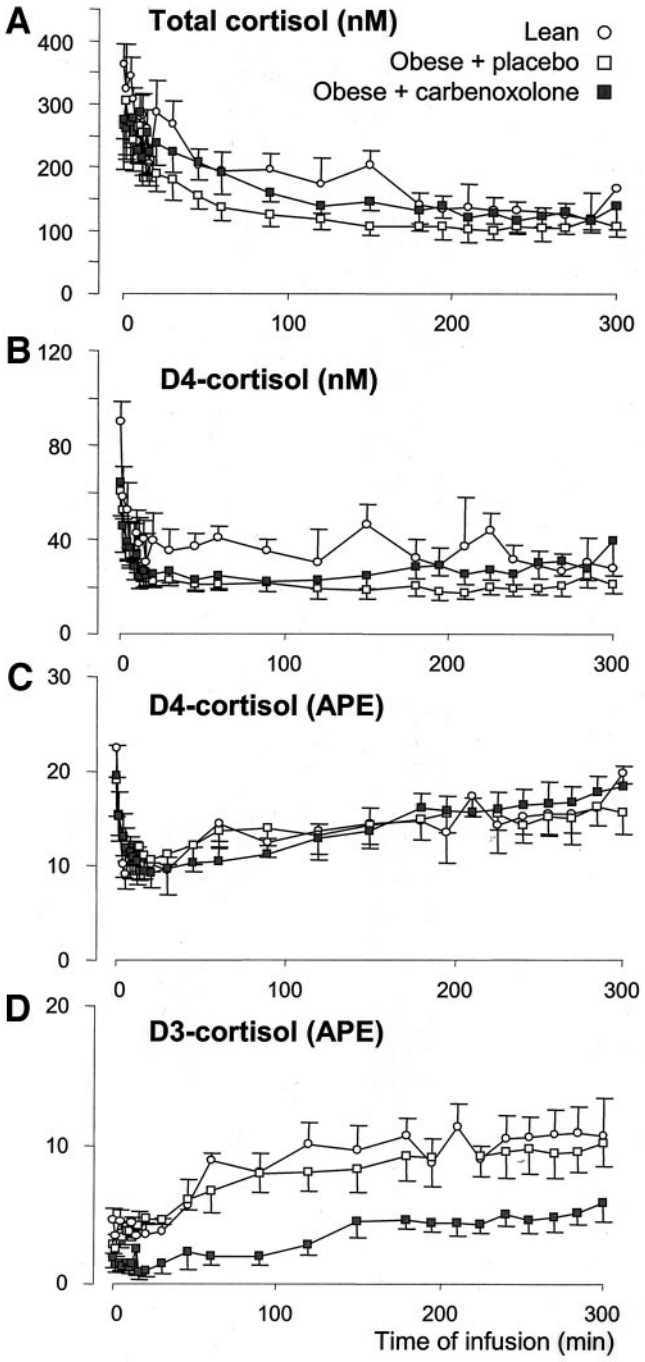

FIG. 2. D4-Cortisol infusion to measure systemic 11HSD activities. Data are means ± SE for six subjects per group after priming dose at $t = 0$ and infusion for 300 min. APE, atoms percent enrichment. Statistical comparisons of calculated kinetic data are shown in Table 3 and are based on steady-state conditions at 240–300 min of infusion.

Microdialysis measurement of conversion of cortisone to cortisol. 1,2-[$^3H_2$]Cortisone and 1,2-[$^3H_2$]cortisol were measured in microdialysis buffer after extraction in ethyl acetate, the addition of internal standard unlabeled cortisol and cortisone, and separation by thin-layer chromatography; cortisol and cortisone fractions were identified by ultraviolet detection, as previously described (37). ^3H in each fraction was measured by liquid scintillation counting to an error of <2%.

To assess the efficiency of the transfer of [^3H]cortisol and [^3H]cortisone across the microdialysis membrane, in vitro experiments were conducted in which the probe was perfused with Ringer lactate and immersed in saline containing [^3H]steroid. Recovery of cortisol was 56.0 ± 2.9% ($n = 4$) over 5 h for cortisol and did not differ between cortisol and cortisone.

Kinetic analysis

D4-cortisol. The initial rate of D3-cortisone appearance after priming has been used as an index of 11HSD type 2 conversion of cortisol to cortisone (27). However, curves for D3-cortisone levels could not be accurately fitted in the current study because of the high variance in the measurements at low concentration and because of a co-eluting interfering analyte present only in samples from obese subjects.

Cortisol kinetics were calculated as previously described (27) from mean D3- and D4-cortisol measurements in steady state between 4th and 5th h of infusion (Figs. 1 and 2). Briefly, the rate of appearance of endogenous cortisol was calculated as [(infusion rate of D4-cortisol)/(D4-cortisol–to–cortisol ratio)] − (infusion rate of cortisol) − (infusion rate of D4-cortisol). Clearance of cortisol and D4-cortisol was calculated as (infusion rate)/(steady-state concentration). The rate of appearance of D3-cortisol was calculated as (infusion rate of D4-cortisol)/(D4-cortisol–to–D3-cortisol ratio).

Urine deuterated steroid excretion was calculated from the urine sample supplied by each subject at the end of the 5-h infusion; subjects had last voided after 3.5–4 h of infusion. After adjusting for the exact timing of voiding in each subject, results were expressed as excretion rates per hour.

D2-glucose. Rates of glucose disposal, appearance, and hepatic glucose production were calculated from [$^2H_2$]glucose enrichment and infusion rates in steady state in the final 30 min of normoinsulinemia and hyperinsulinemia.

Statistical analysis. Groups were compared by unpaired Student's t tests or repeated-measures ANOVA, as appropriate. The effects of carbenoxolone were tested by paired Student's t tests or, for time courses, by subtracting the value for placebo from the value for carbenoxolone at each time point and performing repeated-measures ANOVA to test differences from zero.

RESULTS

Comparison obese placebo-treated subjects and lean subjects. Characteristics of lean and obese participants are shown in Table 1. Obese subjects had higher fasting triglycerides and tended to have higher systolic blood pressure, but were otherwise well matched to control subjects.

The excretion of cortisol metabolites in 24-h urine is shown in Table 2. Obese subjects had higher total metabolite excretion, relatively higher excretion of cortisone than cortisol metabolites, and relatively higher excretion of 5β- rather than 5α-reduced metabolites.

Results of systemic D4-cortisol infusion are shown in Fig. 2 and the kinetic analyses in Table 3. Steady-state conditions were achieved in all groups. The clearance rates of unlabeled and labeled cortisol and the endogenous cortisol production rates tended to be greater in obese than in lean men, but none of these differences reached statistical significance. The rate of appearance of D3-cortisol at steady state was not different in the obese group. Of the 348 μg of D4-cortisol infused each hour, 272 ± 70 μg were recovered in the urinary metabolites shown in Table 3. Obese subjects excreted less tracer as free cortisol, but substantially more as 5β-tetrahydrocortisone. Turnover

TABLE 1
Characteristics of study participants

	Lean	Obese
n	6	6
Age (years)	37.3 ± 2.2	41.8 ± 2.6
BMI (kg/m^2)	24.6 ± 0.7	36.6 ± 1.6*
Waist-to-hip ratio	0.89 ± 0.01	1.02 ± 0.02*
Systolic blood pressure (mmHg)	109 ± 7	123 ± 7
Diastolic blood pressure (mmHg)	68 ± 3	68 ± 3
Fasting plasma glucose (mmol/l)	5.1 ± 0.2	5.0 ± 0.2
Fasting plasma triglycerides (mmol/l)	1.6 ± 0.2	2.9 ± 0.2†
Total plasma cholesterol (mmol/l)	5.6 ± 0.5	5.4 ± 0.2
HDL cholesterol (mmol/l)	1.4 ± 0.1	1.2 ± 0.1

Data are means ± SE. *$P < 0.001$, †$P < 0.01$ by Student's t test.

TABLE 2
Urine cortisol metabolite excretion (24 h)

	Lean subjects	Obese subjects with placebo	Obese subjects with carbenoxolone
5αTHF	5.91 ± 0.63	3.62 ± 0.49*	8.17 ± 2.59
5βTHF	1.80 ± 0.42	2.70 ± 0.99	2.27 ± 0.70
5βTHE	4.50 ± 0.58	11.89 ± 1.26†	10.82 ± 4.43
Cortisol	0.12 ± 0.02	0.17 ± 0.02	0.19 ± 0.05
Cortisone	0.16 ± 0.03	0.26 ± 0.03	0.27 ± 0.10
(5αTHF + 5βTHF)-to-5βTHE	1.79 ± 0.20	0.57 ± 0.11†	1.56 ± 0.65
Cortisol-to-cortisone ratio	0.91 ± 0.29	0.74 ± 0.13	0.84 ± 0.08
5βTHF-to-5αTHF ratio	0.33 ± 0.08	0.96 ± 0.40	0.74 ± 0.37‡
Total cortisol metabolites	14.04 ± 1.24	21.16 ± 1.90§	24.47 ± 7.28

Data are means ± SE and are expressed as milligrams per 24 h. Total cortisol metabolites are the sum of 5α-tetrahydrocortisol (5αTHF) + 5β-tetrahydrocortisol (5βTHF) + 5β-tetrahydrocortisone (5βTHE) + cortols + cortolones. *$P < 0.05$, †$P < 0.001$, §$P < 0.01$ for unpaired Student's t test between lean and obese subjects; ‡$P < 0.05$ for paired Student's t test for effect of carbenoxolone in obese subjects.

of D4-cortisol to D3-cortisol metabolites in urine tended to be lower, rather than higher, in the obese subjects.

Measurements of intra-adipose 11HSD1 by in vivo microdialysis are shown in Fig. 3. Obese subjects showed more rapid initial conversion of [$^3H_2$]cortisone to [$^3H_2$]cortisol. Conversion in both groups plateaued at a similar level after 3 h. However, after the introduction of hyperinsulinemia, there was a rapid, temporary fall in cortisol generation in lean subjects, but no change in obese subjects.

Measurements of glucose turnover are shown in Table 4. Glucose concentrations were similar in the two groups. [$^2H_2$]glucose enrichment was in steady state at the time of kinetic measurements (data not shown). Obese subjects had higher plasma insulin in basal conditions than lean subjects and were markedly peripherally insulin resistant during hyperinsulinemia, as judged by a lower glucose infusion rate and impaired induction of glucose disposal. They also showed impaired insulin clearance, with higher insulin levels during infusion. However, hepatic insulin sensitivity, as judged by the glucose production rate, was not measurably impaired in the obese group.

Effects of carbenoxolone in obese men. Carbenoxolone levels were undetectable after placebo treatment and were 5.7 ± 1.0 μg/ml after carbenoxolone treatment. Carbenoxolone increased the ratio of cortisol to cortisone metabolites in 24-h urine (Table 2) and reduced the rate of appearance of D3-cortisol (Table 3). However, carbenoxolone did not significantly inhibit adipose 11HSD1 activity (Fig. 3), as measured by in vivo microdialysis.

The effects of carbenoxolone on glucose kinetics in obese subjects are shown in Table 4. Carbenoxolone did not significantly alter glucose or insulin concentrations, the glucose infusion rate in steady state, or glucose disposal and production rates.

DISCUSSION

Our results show for the first time that generation of cortisol from cortisone by 11HSD1 is increased in vivo

TABLE 3
[$^2H_4$]Cortisol kinetics

	Lean	Obese subjects with placebo	Obese subjects with carbenoxolone
Steady-state plasma calculations			
Total cortisol (nmol/l)	165 ± 15	152 ± 57	230 ± 112
Cortisol clearance (ml/min)	399 ± 41	604 ± 111	504 ± 125
D4-cortisol clearance (ml/min)	732 ± 194	774 ± 172	620 ± 220
Rate of appearance of endogenous cortisol (nmol/min)	23.1 ± 12.5	26.0 ± 13	17.2 ± 11.5
Rate of appearance of D3-cortisol (nmol/min)	13.9 ± 0.6	14.1 ± 0.6	9.2 ± 0.4*
Urinary labeled steroid excretion in steady state			
D4-cortisol (mg/h)	3.1 ± 1.0	0.5 ± 0.1†	3.0 ± 0.7
D4–5αTHF (mg/h)	49 ± 27	27 ± 8	130 ± 60
D4–5βTHF (mg/h)	26 ± 5	38 ± 3	37 ± 10
D3-cortisone	5.6 ± 1.1	5.0 ± 0.7	3.3 ± 0.5
D3–5βTHE (mg/h)	34 ± 5	80 ± 5‡	61 ± 12
D3-cortisol (mg/h)	4.5 ± 1.2	2.3 ± 0.7	3.2 ± 0.4
D3–5αTHF (mg/h)	45 ± 26	27 ± 9	44 ± 16
D3–5βTHF (mg/h)	22 ± 4	32 ± 2	123 ± 110
D3-THFs/D4-THFs	0.89 ± 0.06	0.89 ± 0.03	0.62 ± 0.22
D3–5βTHE/D4-THFs	0.67 ± 0.15	1.26 ± 0.08§	0.52 ± 0.15†
D3-THFs/D3–5βTHE	2.18 ± 0.71	0.81 ± 0.05	3.01 ± 0.77

Data are means ± SE. 5αTFH, 5α-tetrahydrocortisol; 5βTHF, 5β-tetrahydrocortisol; 5βTHE, 5β-tetrahydrocortisone. †$P < 0.05$, §$P < 0.01$, ‡$P < 0.001$, for unpaired Student's t test for difference between lean and obese subjects; *$P < 0.001$ for paired Student's t test for effect of carbenoxolone in obese subjects.

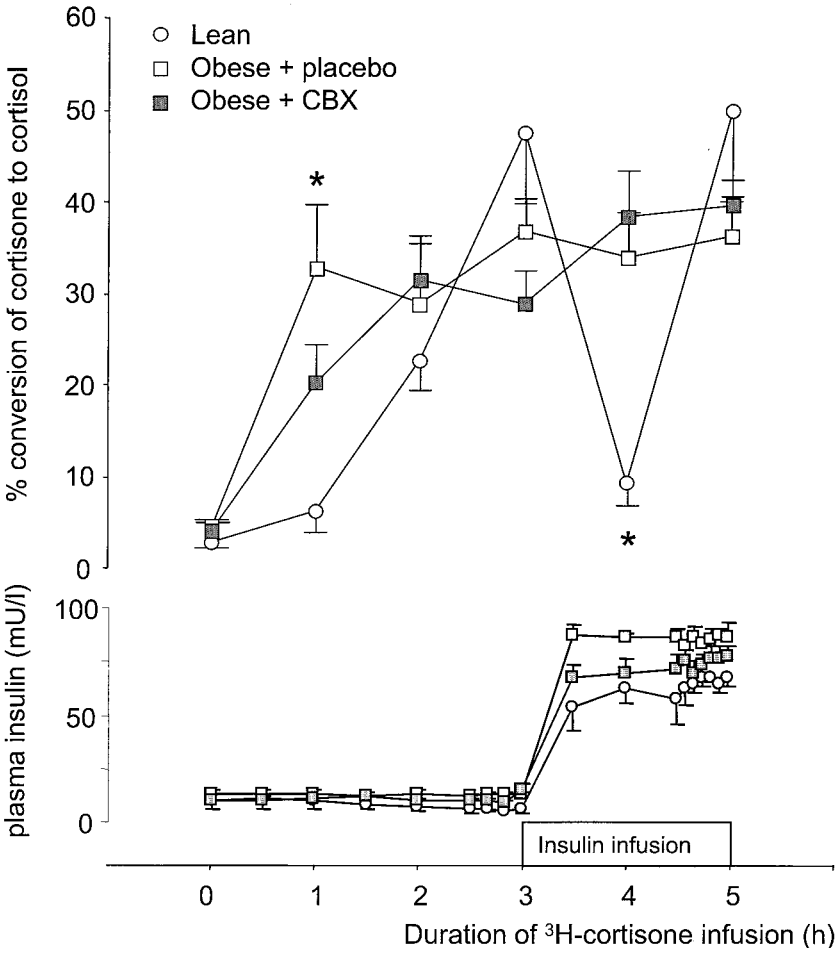

FIG. 3. In vivo microdialysis measurement of subcutaneous adipose 11HSD1 activity with and without hyperinsulinemia. Data are means ± SE for six subjects per group for the conversion of [^{3}H]cortisone to [^{3}H]cortisol (microdialysis; *upper panel*) and plasma insulin levels (*lower panel*). Comparisons of insulin levels are shown in Table 4. For microdialysis, comparison of lean and obese subjects was performed by repeated-measures ANOVA. The changes with time of perfusion ($P < 0.001$) showed a highly significant interaction by group ($P < 0.002$ lean vs. obese). Post hoc testing by Student's t tests showed significant differences in the 1st h after cannula insertion (*$P < 0.02$) and in the 1st h after introducing hyperinsulinemia (*$P < 0.002$). For microdialysis, the effect of carbenoxolone (CBX) was determined by repeated-measures ANOVA of the difference between placebo and carbenoxolone; analysis at each time point revealed no effect of carbenoxolone ($P = 0.64$).

within subcutaneous adipose tissue in obese men, as has been inferred from findings in adipose biopsies (14,15,20–24). In contrast, systemic whole-body regeneration of cortisol from cortisone, measured here for the first time in a pathological state with 9,11,12,12-[$^2H_4$]cortisol tracer infusion (27), was not increased in the obese subjects. This suggests that the increase in cortisol regeneration in adipose tissue is offset by the previously documented decrease in 11HSD1 activity in liver (13–15). The nonselective 11HSD1 inhibitor carbenoxolone enhances insulin sensitivity in liver in lean healthy (32) and diabetic (33) men, but had no effect on insulin sensitivity in the obese

TABLE 4
[$^2H_2$]Glucose kinetics and euglycemic-hyperinsulinemic clamp

	Lean	Obese subjects with placebo	Obese subjects with carbenoxolone
Plasma glucose (mmol/l)			
At baseline	4.9 ± 0.07	4.8 ± 0.16	4.9 ± 0.15
During hyperinsulinemia	5.0 ± 0.03	4.9 ± 0.03	5.0 ± 0.03
Plasma insulin (mU/l)			
At baseline	5.5 ± 1.3	12.6 ± 1.0*	10.7 ± 1.2
During hyperinsulinemia	65.1 ± 4.2	85.1 ± 3.6*	73.9 ± 5.0†
Plasma C-peptide (nmol/l)			
At baseline	0.62 ± 0.12	1.00 ± 0.10‡	0.94 ± 0.17
During hyperinsulinemia	0.62 ± 0.14	1.00 ± 0.11‡	0.82 ± 0.17
Glucose infusion rate during hyperinsulinemia (mg/min)	40.5 ± 5.18	13.4 ± 1.51§	13.3 ± 1.83
Glucose production rate (mg · kg^{-1} · min^{-1})			
At baseline	2.96 ± 0.21	2.57 ± 0.21	2.41 ± 0.11
During hyperinsulinemia	1.93 ± 0.58	1.43 ± 0.10	1.33 ± 0.08
Glucose disposal rate (mg · kg^{-1} · min^{-1})			
At baseline	2.99 ± 0.21	2.59 ± 0.21	2.43 ± 0.11
During hyperinsulinemia	9.16 ± 1.38	3.97 ± 0.21*	3.85 ± 0.36

Data are means ± SE for the mean results at 150–180 min (baseline) and 270–300 min (hyperinsulinemia) of the tracer infusion. ‡$P < 0.05$, *$P < 0.01$, §$P < 0.001$ for unpaired Student's t test for difference between lean and obese subjects; †$P < 0.05$ for paired Student's t test for effect of carbenoxolone in obese subjects.

subjects studied here. Despite its highly effective inhibition of whole-body 11HSD turnover, carbenoxolone did not inhibit conversion of cortisone to cortisol in adipose tissue. This finding is consistent with the results of animal studies (34) and suggests that carbenoxolone inhibits 11HSD1 effectively (and enhances insulin sensitivity) in liver but not in adipose tissue, and that it loses this effect in obese men in whom liver 11HSD1 is already downregulated. This supports the concept that downregulation of hepatic 11HSD1 is advantageous in obesity, and suggests that novel 11HSD1 inhibitors (30,31) will need to inhibit 11HSD1 in adipose tissue if they are to be useful in treating obese patients with type 2 diabetes.

In the microdialysis measurements (Fig. 3), the difference in obese subjects was in the initial rate rather than in the plateau of cortisol generation. In preliminary experiments with microdialysis, we were unable to reliably detect endogenous steroid levels due to small volumes of samples and interferents in immunoassays (R.C.A., R.A., B.R.W., unpublished observations). In the current study, we overcame these problems by using radioactive steroids. However, the infusion of substantial concentrations of [^{3}H]cortisone (50 nmol/l; similar to normal blood cortisone levels) was required to ensure reliable detection. Under these conditions, the [^{3}H]cortisone infused is not a "tracer" because it contributes substantially to the local substrate concentration, the plateau may reflect product inhibition of 11keto-reductase activity, and, as in analogous experiments measuring enzyme kinetics in test tubes, it is the initial rate that is proportional to the amount of enzyme. The observed increased initial rate is consistent with increased mRNA and V_{max} in adipose biopsies in vitro (14,15,20–24). Further, insulin has been previously shown to downregulate 11HSD1 expression in human fibroblasts (38) and liver (39), but to increase 11HSD1 expression in adipocytes in association with stimulating cell differentiation (40). In the current studies, insulin induced a very rapid effect to reduce cortisol generation in lean but not in obese subjects (Fig. 3). It is notable that this effect is unlikely to be due to changes in blood flow, as it is the relative proportion, not the absolute recovery rate, of [^{3}H]cortisol and [^{3}H]cortisone that is measured. This rapid time course suggests that there may be altered kinetics of the enzyme over and above changes in protein levels. One mechanism might involve hexose-6-phosphate dehydrogenase, an enzyme co-localized with 11HSD1 in the lumen of the endoplasmic reticulum that may modulate the supply of NADPH cofactor for cortisone reduction (41–43). Further dissection of the mechanisms of acute regulation of 11HSD1 activity by insulin in adipose tissue may be particularly valuable, as the current data suggest that obese men resist this effect. This is analogous to the relatively poor downregulation of adipose 11HSD1 with high-fat feeding that has been observed in obesity-prone mouse strains (44). Failure to reduce cortisol generation in adipose tissue during hyperinsulinemia (e.g., after food) may be a key mechanism determining susceptibility to obesity.

The conventional measurement of 11HSD activity is the ratio of cortisol to cortisone metabolites in a 24-h urine sample. This ratio was reduced in obese men in this study, as in some (13–15), but not all (11,12), previous studies. However, this did not correspond with reduced whole-body turnover of 11HSDs. It may be that the urine ratio reflects changes in the liver, where the cortisol and cortisone metabolites are conjugated, more than in the whole body. However, we have previously found a poor association between hepatic 11HSD1 activity, measured by the conversion of oral cortisone to plasma cortisol, and the urinary cortisol-to-cortisone metabolite ratio (16). It is more likely that the ratio is confounded by changes in A-ring reductase enzymes that convert cortisol and cortisone to their most prevalent metabolites (i.e., those that make up the ratio of [5α-tetrahydrocortisol + 5β-tetrahydrocortisol] to 5β-tetrahydrocortisone). Animal models suggest that both 5α-reduction of cortisol and 5β-reduction of cortisol and cortisone are enhanced in obesity (8). The current data allow interpretation of the fractional excretion of cortisol tracer in urine during continuous infusion in the steady state, and show that the most striking increase was in 5β-reduced cortisone metabolites (Table 3). This may be the key mechanism underlying increased metabolic clearance rate for cortisol in obesity (45,46).

Carbenoxolone levels in the obese participants in this study were somewhat lower than in our previous study of lean diabetic patients (33), but nevertheless there was substantial inhibition of whole-body regeneration of D3-cortisol, as measured in both plasma and urine (Table 3). The rate of tracee D3-cortisol generation was calculated with reference to D4-cortisol tracer, so that it reflects 11HSD1 activity (which influences only D3-cortisol), but not 11HSD type 2 activity (which influences both D3-cortisol and D4-cortisol). In principle, the reduced D3-cortisol generation rate could result from the reduced D3-cortisone availability as a result of inhibition of 11HSD type 2 in kidney by carbenoxolone. Unfortunately, we were unable to reliably quantify D3-cortisone in plasma samples from these obese men. However, in our previous study, carbenoxolone inhibited D3-cortisol generation more than was accounted for by lower D3-cortisone (27). Moreover, carbenoxolone inhibits the appearance of cortisol in plasma after oral administration of cortisone (47) (a measure of hepatic 11HSD1 that is independent of endogenous cortisone concentrations, as the exogenous cortisone provides much higher cortisone delivery to the liver), and carbenoxolone administration to rats inhibits 11HSD1 measured directly in excised liver (34). We conclude that carbenoxolone is likely to have effectively inhibited liver 11HSD1 in the obese patients. However, although there was a trend for a lower initial rate of conversion of [^{3}H]cortisone to [^{3}H]cortisol after carbenoxolone in the microdialysis measurements (Fig. 3), this was not statistically significant and carbenoxolone did not reduce adipose 11HSD1 activity in obese men to anything approaching the levels in the lean men. Inhibition of hepatic but not adipose 11HSD1 has been shown in obese Zucker rats (34), and may reflect enterohepatic circulation enhancing intrahepatic drug levels; the volume of distribution of carbenoxolone of ~0.1 l/kg body wt also suggests that the drug does not accumulate in adipose tissue (48). The lack of effect of carbenoxolone on the resistance to insulin-stimulated glucose disposal (Table 4) does not, therefore, indicate that an increase in 11HSD1 does not contribute to peripheral insulin resistance in

obesity. Moreover, it does not follow that all 11HSD1 inhibitors will fail to enhance insulin sensitivity in obese patients. However, it is likely that, to be maximally efficacious in the most prevalent group of patients with type 2 diabetes who are obese, 11HSD1 inhibitors will have to achieve enzyme inhibition in adipose tissue.

In the nondiabetic obese patients in the current studies, although they had marked insulin resistance as measured by peripheral glucose uptake, they did not exhibit an elevated endogenous glucose production rate. This observation is consistent with the results of previous studies that have suggested that elevated glucose production is not a feature of obesity alone (49), although among diabetic subjects, the increased glucose production rate is exaggerated by obesity (50). A recent study, albeit relying on urinary cortisol-to-cortisone metabolite ratios, has suggested that the downregulation of 11HSD1 in the liver with increasing obesity is less pronounced in type 2 diabetic patients (19). The incremental effect on hepatic insulin sensitivity with carbenoxolone was greater in lean healthy subjects (32) and diabetic patients (33) than we found here in obese patients. We suggest that the downregulation of 11HSD1 in liver of obese subjects protects the liver to some extent from insulin resistance, and that this may fail in patients with inadequate insulin secretion. Thus, the amount to be gained by inhibition of 11HSD1 in liver might be greater in diabetic obese patients than it is in nondiabetic obese patients. This possibility requires further experimentation with novel selective 11HSD1 inhibitors.

In summary, these studies support the concept of tissue-specific dysregulation of 11HSD1 in obese men and illustrate the potential impact in liver. Understanding the importance of increased 11HSD1 in adipose tissue will, however, require more potent inhibitors of the enzyme in adipose tissue.

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