Reduced Glucocorticoid Production Rate, Decreased 5α-Reductase Activity, and Adipose Tissue Insulin Sensitization After Weight Loss

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OBJECTIVE—The epidemics of obesity, insulin resistance, and type 2 diabetes have heightened the need to understand mechanisms that contribute to their pathogenesis. Increased endogenous glucocorticoid production has been implicated based on parallels with Cushing's syndrome. We have assessed the impact of weight loss on glucocorticoid secretion and metabolism (notably 11β-hydroxysteroid dehydrogenase type 1 and 5α-reductase [5αR] activity) and insulin sensitivity.

RESEARCH DESIGN AND METHODS—Twenty obese volunteers were investigated before and after weight loss. Patients underwent hyperinsulinemic-euglycemic clamps with simultaneous adipose microdialysis and oral cortisone acetate administration. Changes in glucocorticoid secretion and metabolism were assessed using 24-h urine collections.

RESULTS—Before weight loss, fat mass correlated with glucocorticoid secretion rate (total fat, $r = 0.46$, $P < 0.05$; trunk fat, $r = 0.52$, $P < 0.05$); however, glucocorticoid secretion rate was inversely related to insulin sensitivity ($r = -0.51$, $P < 0.05$). Hyperinsulinemia failed to suppress adipose tissue interstitial fluid glycerol release (180 ± 50 μmol [basal] vs. 153 ± 10 μmol [steady state], NS). After oral cortisone (25 mg), cortisol concentrations within adipose interstitial fluid increased (4.3 ± 1.1 vs. 14.2 ± 2.6 nmol/l, $P < 0.01$), but glycerol concentrations did not change. After weight loss, insulin sensitivity increased. Consistent with insulin sensitization, adipose tissue interstitial fluid glycerol concentrations fell under hyperinsulinemic conditions (186 ± 16 vs. 117 ± 9 μmol, $P < 0.05$). Glucocorticoid secretion decreased (11,751 ± 1,520 vs. 7,464 ± 937 μg/24 h, $P < 0.05$) as did 5αR activity (5α-tetrahydrocortisol–to–tetrahydrocortisol ratio 1.41 ± 0.16 vs. 1.12 ± 0.17, $P < 0.005$).

CONCLUSIONS—Obesity is associated with insulin resistance within adipose tissue and increased cortisol secretion rates; both are reversed with weight loss. Reduced 5αR activity after weight loss may decrease hypothalamo-pituitary-adrenal axis activation and reduce glucocorticoid metabolite production. ***Diabetes* 57: 1536–1543, 2008**

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5αR, 5α-reductase; 5βR, 5β-reductase; 11β-HSD1, 11β-hydroxysteroid dehydrogenase type 1; AUC, area under the curve; DEXA, dual-energy X-ray absorptiometry; HPA, hypothalamo-pituitary-adrenal; RIA, radioimmunoassay; THE, tetrahydrocortisone; THF, tetrahydrocortisol; VLCD, very low–calorie diet.

The global epidemic of obesity continues to progress at an alarming rate in both adults and children (1). The health consequences of obesity are well described along with significant increases in both mortality and morbidity (2). The risks of type 2 diabetes and insulin resistance associated with obesity are perhaps the most alarming; once BMI reaches 35 kg/m^2, the risk of developing type 2 diabetes increases by 42-fold in men and by 92-fold in women (3,4).

Parallels with patients with glucocorticoid excess and Cushing's syndrome have highlighted the potential role that endogenous glucocorticoids may play on insulin sensitivity and obesity. However, obesity and insulin resistance are not states of subclinical glucocorticoid excess; circulating cortisol levels are normal or even slightly reduced (5). Although activation of the hypothalamo-pituitary-adrenal (HPA) axis is well described, this has been attributed to changes in body composition (6,7), and importantly, the relationship with insulin sensitivity per se has not been explored. Cortisol availability to bind and activate the glucocorticoid receptor is modified by a series of enzymes through the concept of prereceptor hormone metabolism. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is highly expressed in liver and adipose tissue (8), and its predominant activity is to convert the inactive glucocorticoid cortisone to active cortisol and thereby locally amplify the action of glucocorticoids. In contrast, the A-ring reductases (5α- and 5β-reductase [5αR and 5βR]) inactivate cortisol (in conjunction with 3α-hydroxysteroid dehydrogenase) to its tetrahydro-metabolites (5α-tetrahydrocortisol [5αTHF] and tetrahydrocortisone [THE]). The activities of both these enzyme systems can limit glucocorticoid availability and also impact on HPA axis activation, such that inhibition of 11β-HSD1 (decreasing local cortisol generation) and activation of A-ring reductases (enhancing cortisol inactivation) may drive the HPA axis.

Although the health consequences of obesity are severe, there is clear evidence as to the benefits of significant weight loss. A reduction of 10 kg in weight is associated with a 20% reduction in total mortality (with a specific 30% reduction in diabetes-related deaths), a 10-mmHg reduction in systolic blood pressure, and a 50% decrease in the absolute risk of development of type 2 diabetes (9). Moreover, recent data have now confirmed the dramatic improvement in morbidity and mortality after bariatric surgery, which achieves sustained weight loss of between 14 and 25% (10,11). Weight loss arises when there is a net calorie deficit and energy expenditure exceeds intake. However, the precise molecular mechanisms that convey the health benefits are largely unknown, and it is possible

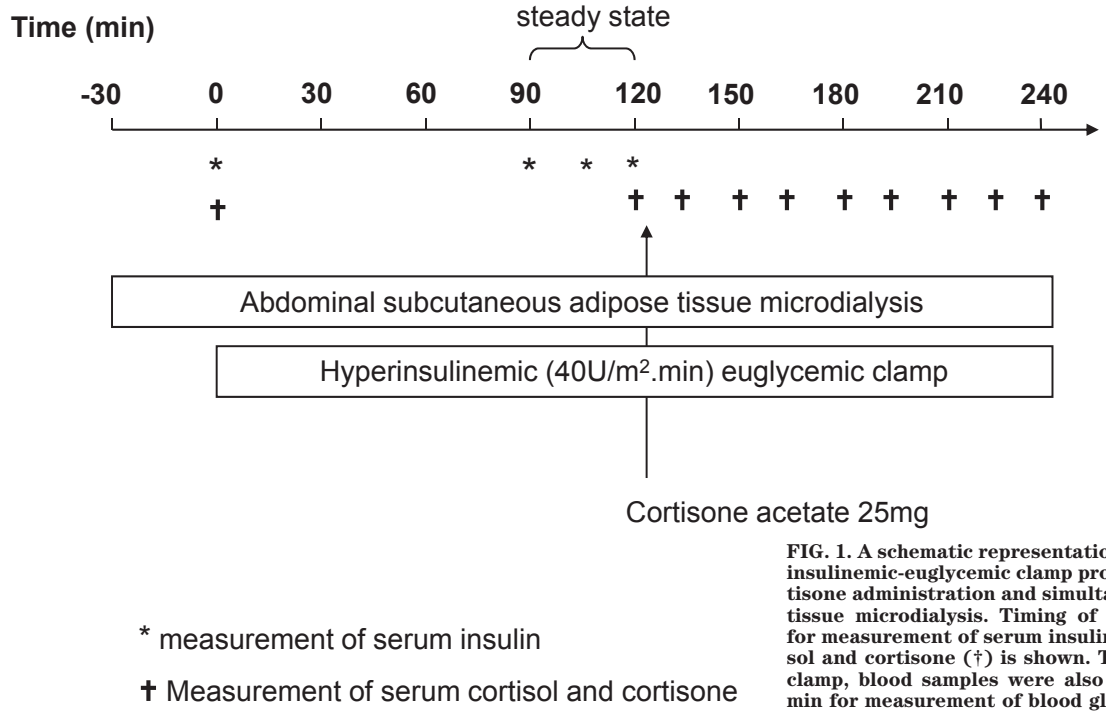

FIG. 1. A schematic representation of the hyperinsulinemic-euglycemic clamp protocol with cortisone administration and simultaneous adipose tissue microdialysis. Timing of samples taken for measurement of serum insulin (*) and cortisol and cortisone (†) is shown. Throughout the clamp, blood samples were also taken every 5 min for measurement of blood glucose.

that changes in glucocorticoid secretion and/or metabolism may play a role. Previously, we have shown that weight loss was associated with adipocyte-specific increases in 11β-HSD1 mRNA expression but no change in global 11β-HSD1 activity (12). In light of recent studies that have highlighted the role of selective 11β-HSD1 inhibitors as agents that cause insulin sensitization (13–16), we have conducted a detailed clinical study that has examined the relationship between insulin sensitivity (both globally and locally within adipose tissue) and glucocorticoid secretion and metabolism before and after significant weight loss. The underlying hypothesis is that changes in glucocorticoid secretion and metabolism may be crucial determinants of global and tissue-specific insulin sensitivity.

RESEARCH DESIGN AND METHODS

The study was approved by South Birmingham Local Research Ethics Committee, and all subjects gave their informed, written consent. Twenty obese volunteers (10 men and 10 women aged 42 ± 3 years, with mean BMI 36.6 ± 1.0 kg/m^2 [mean ± SD]) were recruited after local advertisement and underwent the clinical protocol described below. Patients had no significant past medical history, and none had received glucocorticoid therapy. All patients had normal blood counts, fasting glucose, and renal, liver and thyroid function. Patients with diabetes were excluded from the study.

Clinical protocol

Day 1. Subjects were investigated in the fasting state. Fasting blood samples were drawn at 0900 h for measurement of total cholesterol, triglycerides, cortisol, cortisone, glucose, and insulin. Measurements of BMI, waist circumference (measured supine, at the level of the umbilicus), hip circumference (at the level of the greater trochanter), and blood pressure (average of three readings, measured supine after 10-min rest using Dynamap [Critikon, Tampa, FL]) were also taken. In addition, all patients performed a 24-h urine collection for corticosteroid metabolite analysis using gas chromatography/mass spectrometry as previously described (17).

Body composition analysis was performed using dual-energy X-ray absorptiometry (DEXA) with a total body scanner (QDR 45OO; Hologic, Bedford, MA). Coefficients of variation for multiple scans were <3%. Regional fat mass (trunk and leg) was analyzed as previously described (12).

Day 2. Subjects were again investigated in the fasted state, with water available ad libitum, and underwent a hyperinsulinemic (40 mU · m^{-2} · min^{-1})-euglycemic clamp for 240 min with simultaneous adipose tissue microdialysis.

Subjects were asked to abstain from caffeine, alcohol, and heavy exercise for the preceding 24 h. At 0800 h, an adipose tissue microdialysis catheter (CMA60; CMA Microdialysis, Stockholm) was inserted into subcutaneous adipose tissue under local anesthetic (2 ml 1% lidocaine) 10 cm lateral to the umbilicus. The CMA60 catheters are 0.5 mm in diameter and 50 mm in length, the distal 30 mm consisting of a semipermeable membrane with a 20-kDa cutoff. After a flush sequence (15 μl/min for 5 min), microdialysis was performed at a rate of 0.5 μl/min and continued for 30 min before sample collection as described previously (18). The first sample was collected before the commencement of the hyperinsulinemic-euglycemic clamp, and sample collection continued for its entire duration (240 min).

Cannulae were inserted into an antecubital vein (glucose/insulin infusion) and into a contra-lateral dorsal hand vein for repeated sampling. The hand was placed in a hot-air box maintained at 50–55°C to arterialize the blood. After resting for 15 min, a baseline blood sample was obtained for measurement of fasting blood glucose, insulin, cortisol, and cortisone. Intravenous infusion of human soluble insulin (Actrapid; Novo Nordisk, Copenhagen, Denmark) was then started initially with a decreasing loading dose (over 10 min) and then continuing for the remainder of the clamp (10–240 min) at a rate of 40 mU · m^{-2} · min^{-1}. Dextrose (20%) was infused (starting at minute 4) at a variable rate to maintain blood glucose concentrations at the fasting level. Blood glucose monitoring was performed every 5 min and measured on a bedside blood glucose analyzer (YSI, Yellow Springs, OH). Steady state was taken as 90–120 min, and during this time period, three serum samples were taken for measurement of insulin concentrations. Glucose utilization during the steady-state period was expressed per kilogram body weight (*M* value) and insulin sensitivity (*M/I* value) defined as the *M* value divided by the mean insulin concentration, as previously described (19). At $t = 120$, further blood samples were taken for measurement of serum cortisol and cortisone. Cortisone acetate (25 mg) was then administered orally, and subsequently, additional serum samples were taken every 15 min for a further 2 h (120–240 min) for measurement of cortisol and cortisone as an index of 11β-HSD1 activity. During the 120- to 240-min period, the insulin infusion continued with 5-min blood sampling for blood glucose concentrations. The dextrose infusion rate continued to be adjusted to maintain euglycemia. A schematic representation of this protocol is shown in Fig. 1.

Very low–calorie diet

After all investigations outlined in the clinical protocol had been completed, patients entered into a weight-loss program using a total meal replacement, very low–calorie diet (VLCD) (Lipotrim; Howard Foundation, Cambridge, U.K.) (12). This diet provides 425 kcal/day for women and 559 kcal/day for men. After significant weight loss (>10% initial body wt), all subjects returned to a normal diet, and once refeeding had been commenced for at least 1 week, all of the investigations described above were repeated. Investigations were not repeated sooner to avoid the confounding effect that the stress of the hypocaloric diet may have had on the HPA axis.

Biochemical assays

Serum. Blood counts, urea, creatinine and electrolytes, cholesterol, triglycerides, liver chemistry, and plasma glucose were measured using standard laboratory methods (Roche Modular System; Roche, Lewes, U.K.). Cortisol was measured using a coat-a-count radioimmunoassay (RIA) (Diagnostic Products, Los Angeles, CA) per the manufacturer's guidelines. Cortisone was assayed after extraction from serum followed by RIA of the extract with ^{125}I-labeled cortisone and Sac-Cel (IDS, Tyne and Weir, U.K.) second antibody separation (20). The coefficients of variation for 10 consecutive assays were <15% for values between 50 and 100 nmol/l and <10% for values >100 nmol/l.

Microdialysate. Microdialysate samples were collected in microvials and exchanged every 30 min. Samples were analyzed using a mobile photometric, enzyme-kinetic analyzer (CMA600; CMA Microdialysis) for glucose, pyruvate, lactate, and glycerol.

Cortisol was measured using a commercially available colorimetric competitive ELISA (R&D Systems, Minneapolis, MN). The minimum detectable dose range for the assay was 30–111 pg/ml with intra-assay coefficients of variation of 6–9%.

Urinary corticosteroid metabolites. Urinary corticosteroid metabolite analysis was performed by gas chromatography/mass spectrometry as described previously (17). The sum of total cortisol metabolites (THF, THE, 5α-THF, α-cortolone, cortisone, cortisol, β-cortolone, β-cortol, and α-cortol) provides a reflection of cortisol secretion rate. The ratio of tetrahydrometabolites of cortisol (THF plus 5αTHF) to those of cortisone (THE) provides a reflection of 11β-HSD1 activity when considered with the ratio of urinary free cortisol to cortisone, which more accurately reflects renal 11β-HSD2 activity (17). In addition, the ratios of cortols to cortolones and of 11β-hydroxy-etiocholanolone plus 11β-hydroxy-androsterone to 11oxo-etiocholanolone also reflect 11β-HSD1 activity (21). The activities of 5αRs and 5βRs can be inferred from measuring the ratios of 5αTHF to THF and androsterone to etiocholanolone.

Statistical analysis

Data are presented as means ± SE unless otherwise stated. Power calculations and cohort size requirements were calculated based on observations from our previous studies using a VLCD (12). They took into account the possibility of a high dropout rate, bearing in mind the intensity of the clinical protocol. Area under the curve (AUC) analysis was performed using the trapezoidal method. For comparison of single variables before and after weight loss, paired *t* tests have been used (or nonparametric equivalents where data were not normally distributed). Where repeated samples were taken (either during an individual investigation or for comparison of the same investigation before and after weight loss), repeated-measures ANOVA on ranks was used incorporating Dunn's test as a post hoc analysis. Regression analyses were performed using Pearson correlations; where more than one variable was considered, multiple linear regression analysis was used. All analysis was performed using the SigmaStat 3.1 software package (Systat Software, Point Richmond, CA).

TABLE 1
Baseline analysis of 20 obese individuals

Anthropometric measurements	
Weight (kg)	106.3 ± 3.7
BMI (kg/m^2)	36.6 ± 1.0
Waist circumference (cm)	102 ± 6
Waist-to-hip ratio	0.92 ± 0.02
Body composition (DEXA)	
Total fat mass (kg)	41.6 ± 2.8
Total lean mass (kg)	59.2 ± 1.8
Fat (%)	39.8 ± 1.5
Trunk fat mass (kg)	19.8 ± 1.2
Trunk-to-limb fat ratio	1.07 ± 0.05
Serum biochemistry and hyperinsulinemic-euglycemic clamp	
Glucose (mmol/l)	5.1 ± 0.2
Insulin (mU/l)	13.9 ± 1.4
HOMA-B	151.6 ± 10.1
HOMA-S	62.3 ± 9.9
HOMA-IR	2.0 ± 0.2
M value (mg · kg^{-1} · min^{-1})	3.2 ± 0.3
M/I value (mg · kg^{-1} · min^{-1} · mU^{-1} · l^{-1})	3.5 ± 0.4
Cholesterol (mmol/l)	5.0 ± 0.2
Triglycerides (mmol/l)	1.32 ± 0.17
Cortisol (180–550 nmol/l)	318 ± 40
Cortisone (nmol/l)	61 ± 4
Urine corticosteroid metabolites	
Total glucocorticoid metabolites (μg/24 h)	10,525 ± 1,285
Urinary free cortisol/urinary free cortisone	0.75 ± 0.06
(THF plus 5αTHF)/THE	0.92 ± 0.05
Cortols/cortolones	0.42 ± 0.03
(11OH-androst plus 11OH-etio)/11oxo-etio	2.21 ± 0.20
5αTHF/THF	1.13 ± 0.15
Androsterone/etiocholanolone	1.40 ± 0.2

Data are means ± SE. HOMA-B, homeostasis model assessment of β-cell function; HOMA-IR, HOMA of insulin resistance; HOMA-S, HOMA of insulin sensitivity.

RESULTS

Baseline analysis. Baseline characteristics of the patients are presented in Table 1. Before weight loss, total fat mass and trunk fat mass correlated with total glucocorticoid secretion rate (total fat, $r = 0.46$, $P < 0.05$; trunk fat, $r = 0.52$, $P < 0.05$; Fig. 2*A*). There was no significant correlation between fat mass (total or trunk) and insulin sensitivity as measured by the *M/I* value (total fat, $r = -0.17$, $P = 0.52$; trunk fat, $r = -0.11$, $P = 0.68$). However, the *M/I* value was inversely related to glucocorticoid secretion rate ($r = -0.51$, $P < 0.05$), even after correction for fat mass ($P < 0.05$).

Within this cohort, insulin sensitivity and fat mass (total or regional) did not correlate significantly with 11β-HSD1 activity as measured by either the urinary THF plus 5αTHF–to–THE ratio or the generation of cortisol following oral cortisone.

Adipose tissue microdialysis. During the steady-state period (90–120 min), adipose tissue interstitial fluid glucose concentration did not differ significantly from basal glucose values (2.2 ± 0.2 vs. 2.7 ± 0.2 mmol/l, NS), confirming that in addition to successful clamping of blood glucose (4.9 ± 0.2 [fasting] vs. 4.8 ± 0.1 mmol [steady state], NS), adipose tissue interstitial fluid glucose concentrations had also been clamped. Consistent with insulin promoting glucose utilization, interstitial fluid pyruvate (38.1 ± 5.8 [basal] vs. 77.5 ± 5.2 μmol/l [steady state], $P < 0.05$) and lactate (0.68 ± 0.1 [basal] vs. 2.1 ± 0.2 mmol/l [steady state], $P < 0.05$) increased significantly. Basal glycerol release did not correlate with insulin sensitivity, and consistent with underlying insulin resistance in this obese cohort, hyperinsulinemia failed to suppress glycerol release (180 ± 50 [basal] vs. 153 ± 10 μmol/l [steady state], NS).

Under hyperinsulinemic conditions, oral cortisone acetate administration did not have any impact on interstitial fluid glucose, lactate, pyruvate, or glycerol concentrations (data not shown).

Consistent with previous observations (18), cortisol was detectable in adipose tissue interstitial fluid, and concentrations were not altered by hyperinsulinemia (4.3 ± 1.1 [basal] vs. 5.4 ± 1.0 nmol/l [steady state, 90–120 min], NS). After oral cortisone acetate administration, interstitial fluid cortisone concentrations increased significantly (14.2 ± 2.6 nmol/l, 210–240 min, $P < 0.005$ vs. 90–120 min).

Impact of weight loss. Of the 20 patients who were recruited and enrolled into the study, 14 (7 men and 7 women) completed the full investigative protocol using the VLCD for 10 (7–15) weeks (median [range]). Six volunteers were unable to tolerate the VLCD and were not reinvestigated.

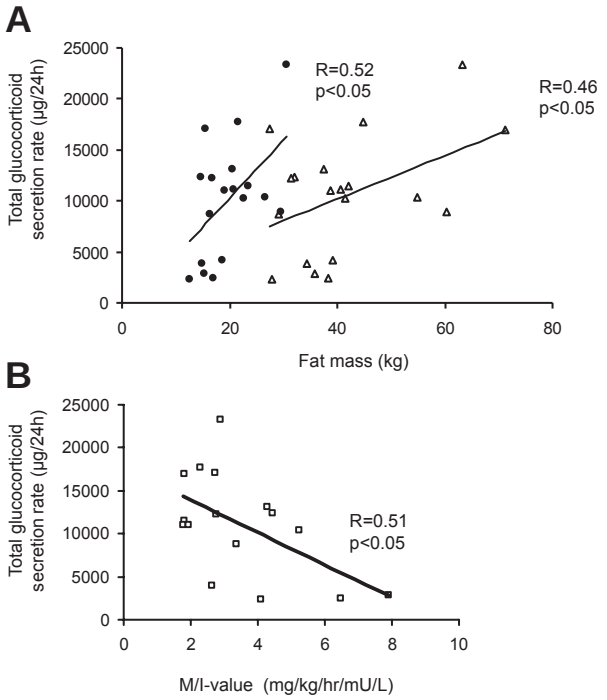

FIG. 2. Total glucocorticoid secretion rate correlates positively with trunk (●) and total fat mass (△) in 20 healthy obese individuals before weight loss (*A*) and inversely correlates with insulin sensitivity as measured by hyperinsulinemic-euglycemic clamp (*B*).

Anthropometry, fasting biochemistry, and blood pressure. After the VLCD, mean BMI fell from 36.6 ± 1.3 to 31.4 ± 1.0 kg/m^2 (n = 14, $P < 0.0001$). We observed significant reductions in waist circumference, total and trunk fat mass, lean mass, and percentage fat (Table 2). However, there was little change in fat distribution, as waist-to-hip ratio and trunk-to-limb fat mass ratio on DEXA scanning remained unchanged (Table 2).

Consistent with a decrease in muscle mass, serum creatinine decreased significantly (97 ± 2 vs. 91 ± 2 μmol/l, $P < 0.05$). Total cholesterol decreased with no significant change in triglyceride concentrations. Complete data are presented in Table 3. In addition, systolic blood pressure fell significantly (129 ± 3 vs. 119 ± 3 mmHg, $P < 0.005$) without an alteration in diastolic blood pressure (74 ± 3 vs. 70 ± 2 mmHg, $P = 0.26$).

Insulin sensitivity. Although fasting plasma glucose remained unchanged (5.2 ± 0.2 vs. 4.8 ± 0.1 mmol/l, $P = 0.16$), fasting insulin fell significantly (15.0 ± 1.9 vs. 8.3 ± 1.9 mU/l, $P < 0.01$). Glucose disposal as reflected by the M value increased (3.2 ± 0.3 vs. 5.1 ± 0.7 mg · kg^{-1} · min^{-1}, $P < 0.005$) as did insulin sensitivity (M/I value) (3.7 ± 0.5 vs. 7.0 ± 1.1 mg · kg^{-1} · min^{-1} · mU^{-1} · l^{-1}, $P < 0.005$). Calculated measures of insulin sensitivity (homeostasis model assessment) also increased (Table 3).

Cortisol secretion and metabolism. After weight loss, total glucocorticoid secretion decreased (11,751 ± 1,520 vs. 7,464 ± 937 μg/24 h, $P < 0.05$). Decreases in absolute levels of 5α-reduced metabolites were more marked compared with 5β-reduced metabolites (Table 4), consistent with decreased 5αR activity (androsterone/etiocholanolone, 1.65 ± 0.23 vs. 1.2 ± 0.19, $P = 0.002$; 5αTHF/THF, 1.41 ± 0.16 vs. 1.12 ± 0.17, $P = 0.004$). Urinary steroid metabolite ratios that reflect 11β-HSD1 activity (THF plus 5αTHF–to–THE and cortols-to-cortolones ratios) did not change significantly, with the exception of the 11OH-androsterone plus 11OH-etiocholanolone–to–11oxo-etiocholanolone ratio, which decreased (2.20 ± 0.23 vs. 1.65 ± 0.22, $P = 0.01$) (Table 4).

Consistent with our previous observations (12), cortisol generation from oral cortisone within serum did not change after weight loss (AUC 938 ± 179 [before] vs. 854 ± 97 nmol · l^{-1} · h^{-1} [after], $P = 0.44$) (Fig. 4*A*).

Adipose tissue microdialysis. After weight loss, hyperinsulinemia increased pyruvate and lactate concentrations in adipose tissue interstitial fluid (as observed before weight loss), although values before and after weight loss were not different (Fig. 3*A* and *B*). Similarly, there were no significant differences in glucose concentrations (Fig. 3*C*).

In contrast to the results before weight loss, glycerol concentrations fell under hyperinsulinemic conditions, consistent with repression of lipolysis (186 ± 16 [basal] vs. 117 ± 9 μmol/l [180–210 min], $P < 0.05$). Furthermore, glycerol levels were significantly reduced compared with those before weight loss, suggesting insulin sensitization (162 ± 16 vs. 117 ± 10 μmol/l, $P < 0.05$; and 171 ± 18 vs. 117 ± 9 μmol/l, $P < 0.05$) (Fig. 3*D*).

Cortisol concentrations in adipose tissue interstitial fluid at the 180–210 min time point were higher after weight loss (8.6 ± 1.4 [before] vs. 12.3 ± 1.9 nmol/l [after], $P = 0.03$) (Fig. 4*B*), however, total cortisol production as measured by AUC analysis did not differ (AUC 12.8 ± 1.9 [before] vs. 13.8 ± 2.3 nmol · l^{-1} · h^{-1} [after], $P = 0.23$). Before and after weight loss, immediately after the administration of oral cortisone acetate, the ratio of interstitial fluid to serum cortisol decreased (Fig. 4*C*), suggesting a more rapid appearance of cortisol in serum rather than interstitial fluid. After weight loss, there was a significant

TABLE 2
Anthropometric measurements and body composition data as measured by DEXA in 14 individuals before and after weight loss associated with a VLCD

Anthropometric measurements	Before weight loss	After weight loss	P value
Weight (kg)	106.0 ± 4.4	91.0 ± 3.5	<0.0001
BMI (kg/m^2)	36.6 ± 1.3	31.4 ± 1.0	<0.0001
Waist circumference (cm)	112 ± 3	100 ± 2	<0.0001
Waist-to-hip ratio	0.91 ± 0.02	0.89 ± 0.02	0.25
Body composition (DEXA)			
Total fat mass (kg)	44.0 ± 3.4	33.4 ± 2.6	<0.0001
Total lean mass (kg)	59.0 ± 2.0	55.0 ± 2.0	<0.0001
Fat (%)	41.1 ± 1.8	36.3 ± 1.8	<0.0001
Trunk fat mass (kg)	20.6 ± 1.4	15.1 ± 1.1	<0.0001
Trunk-to-limb fat ratio	1.04 ± 0.05	1.00 ± 0.06	0.44

Data are means ± SE.

TABLE 3
Biochemical characterization of 14 individuals before and after weight loss associated with a VLCD

	Local reference range (where applicable)	Before weight loss	After weight loss	*P* value
Na (mmol/l)	134–146	141 ± 1	141 ± 1	0.39
K (mmol/l)	3.4–5.2	4.1 ± 0.1	4.2 ± 0.1	0.44
Urea (mmol/l)	3.2–7.6	4.7 ± 0.3	4.3 ± 0.3	0.44
Creatinine (μmol/l)	60–126	97 ± 2	91 ± 2	0.02
Glucose (mmol/l)		5.2 ± 0.2	4.8 ± 0.1	0.16
Insulin (mU/l)		15.0 ± 1.9	8.3 ± 1.9	0.008
HOMA-B		156.9 ± 13.8	111.3 ± 11.4	0.005
HOMA-S		59.7 ± 13.5	119.7 ± 18.6	0.002
HOMA-IR		2.2 ± 0.3	1.2 ± 0.3	0.009
Cholesterol (mmol/l)		5.1 ± 0.3	4.5 ± 0.3	0.04
Triglycerides (mmol/l)		1.4 ± 0.2	1.2 ± 0.2	0.09
Cortisol (nmol/l)		349 ± 52	302 ± 29	0.08
Cortisone (nmol/l)		58 ± 4	66 ± 5	0.33

Data are means ± SE. HOMA-B, homeostasis model assessment of β-cell function; HOMA-IR, HOMA of insulin resistance; HOMA-S, HOMA of insulin sensitivity.

increase in interstitial fluid cortisol availability compared with serum ($P < 0.05$ vs. before weight loss) (Fig. 4*C*), consistent with enhanced local generation of cortisol.

DISCUSSION

We have characterized glucocorticoid secretion and metabolism and insulin sensitivity in a cohort of obese individuals before and after weight loss. Total glucocorticoid secretion rate was related to both regional and total fat mass and inversely correlated with insulin sensitivity. Interestingly, in this cohort, there was no relationship between fat mass (or distribution) and insulin sensitivity. Before weight loss, adipose tissue was insulin resistant, with failure of insulin to suppress lipolysis. After weight loss, we observed marked increases in insulin sensitivity globally and also within adipose tissue, as evidenced by insulin-mediated suppression of glycerol release. Furthermore, we observed a decrease in total glucocorticoid secretion rate and a specific reduction in 5αR activity.

The role of endogenous glucocorticoid production and metabolism has been implicated in the pathogenesis of obesity and insulin resistance. Much of this work has cited phenotypic similarities between patients with Cushing's syndrome and those with simple obesity. Activation of the HPA axis and hypersensitivity of the axis to stimulation and suppression have been described previously in obesity (6,7). However, the independent relationship between glucocorticoid secretion and insulin sensitivity is a novel and interesting finding; we suggest that the increased adrenal glucocorticoid output could have an impact on insulin sensitivity and glucose utilization in liver, muscle, and perhaps adipose tissue.

The majority of glucocorticoid metabolism occurs within the liver. Over recent years, there has been a focus

TABLE 4
Urinary corticosteroid metabolite analysis performed by gas chromatography/mass spectrometry on 24-h urine samples from 14 obese volunteers before and after weight loss associated with a VLCD

Corticosteroid metabolites (μg/24 h)	Before weight loss	After weight loss	*P* value
Total glucocorticoid metabolites	11751 ± 1520	7464 ± 937	0.02
Total cortisol metabolites (Fm)	5194 ± 640	3278 ± 439	0.02
Total cortisone metabolites (Em)	6661 ± 912	4288 ± 537	0.02
THE	4456 ± 682	2877 ± 384	0.03
5αTHF	2275 ± 285	1250 ± 214	0.002
THF	1878 ± 371	1304 ± 209	0.14
UFE	120 ± 17	103 ± 18	0.45
UFF	78 ± 12	70 ± 11	0.56
α-Cortolone	1405 ± 181	861 ± 110	0.009
β-Cortolone	680 ± 82	447 ± 61	0.02
α-Cortol	327 ± 41	209 ± 32	0.03
β-Cortol	533 ± 70	341 ± 65	0.06
Corticosteroid metabolite ratios			
Urinary free cortisol/urinary free cortisone	0.66 ± 0.04	0.70 ± 0.05	0.25
(THF plus 5αTHF)/THE	0.99 ± 0.05	0.91 ± 0.06	0.13
Cortols/cortolones	0.43 ± 0.03	0.41 ± 0.03	0.40
(11OH-androst plus 11OH-etio)/11oxo-etio	2.20 ± 0.23	1.65 ± 0.22	0.01
5αTHF/THF	1.41 ± 0.16	1.12 ± 0.17	0.004
Androsterone/etiocholanolone	1.65 ± 0.23	1.20 ± 0.19	0.002

Data are means ± SE. 11OH-androst, 11hydroxyandrosterone; 11OH-etio, 11hydroxyetiocholanolone; 11oxo-etio, 11oxo-etiocholanolone; Em, cortisone plus THE plus α-cortolone plus β-cortolone; Fm, cortisol plus THF plus 5αTHF plus α-cortol plus β-cortol.

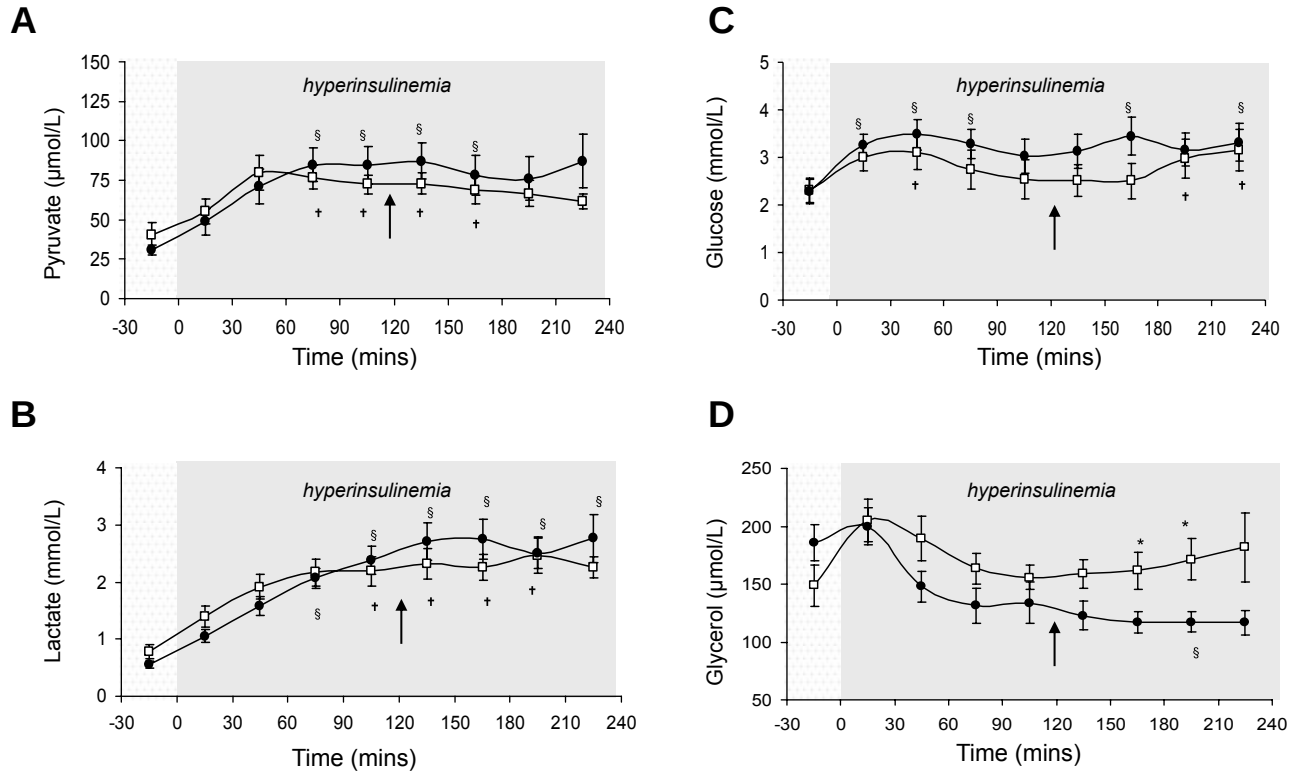

FIG. 3. Adipose tissue microdialysate analysis in 14 obese individuals before (□) and after weight loss (●) under basal and hyperinsulinemic conditions. Hyperinsulinemia increased interstitial fluid pyruvate (*A*) and lactate (*B*) concentrations equally before and after weight loss. Glucose concentrations were unaltered, consistent with successful clamping of blood glucose levels, and were also similar before and after weight loss. Glycerol concentrations failed to suppress before weight loss, in keeping with adipose tissue insulin resistance, but fell significantly after weight loss (†$P < 0.05$ vs. baseline before weight loss; §$P < 0.05$ vs. baseline after weight loss; *$P < 0.05$ before vs. after weight loss. Arrow denotes administration of 25 mg cortisone acetate orally).

on the role of 11β-HSD1 as a local amplifier of cortisol action in the pathogenesis of obesity and insulin resistance (22). Moreover, inhibitors of 11β-HSD1 have shown considerable potential in rodents (13–16) and primates (23) as insulin sensitizers and as agents that may aid weight loss. We were unable to show significant changes in 11β-HSD1 activity in either serum or urine in agreement with our previous observations (12). However, our data do suggest increased cortisol availability within adipose tissue interstitial fluid after weight loss. Previously, we have shown increased adipocyte (but not whole adipose tissue) expression of 11β-HSD1 after weight loss (12), and this may be responsible for the modest changes that we observed, although we have not evaluated adipocyte-specific gene expression in this study. Insulin resistance in obesity specifically within adipose tissue has long been postulated but not demonstrated. Insulin is a potent suppressor of adipose tissue lipolysis, and the lack of effect of hyperinsulinemia on adipose tissue interstitial glycerol levels before weight loss is suggestive of insulin resistance. These observations contrast with those after weight loss when hyperinsulinemia decreased glycerol concentrations consistent with adipose tissue insulin sensitization. The modest increase in cortisol following cortisone acetate suggestive of increased (and certainly not decreased) cortisol availability may be important here, bearing in mind the recent description that cortisol can sensitize insulin action in subcutaneous adipose tissue (24).

Glucocorticoids have potent action on insulin signaling. Globally, they induce whole-body insulin resistance (25), perhaps through induction of lipolysis through hormone-sensitive lipase activation (26) and subsequent free fatty acid generation. Tissue specificity of the interaction between glucocorticoids and the insulin signaling cascade is controversial, and in rodents, in the majority of published studies, glucocorticoids appear to cause insulin resistance in adipose tissue (27,28). We have recently extended these findings to humans, demonstrating insulin resistance in muscle with insulin sensitization in subcutaneous adipose tissue (24). Data from this study endorse these observations with global decreased glucocorticoid secretion associated with increased insulin sensitivity (largely reflecting glucocorticoid action on skeletal muscle) and adipose tissue insulin sensitization with increased local glucocorticoid availability.

The changes in 5αR activity are striking. 5αR has two isoforms, which only share ~47% sequence homology (29); the type 1 isoform (5αR1) is located on the short arm of chromosome 5, whereas 5αR2 is located on the short arm of chromosome 2. 5αR1 is expressed in skin and adipose tissue (29,30) and 5αR2 in prostate, epididymis, and seminal vesicles; both isozymes are expressed in the liver (29). For cortisol metabolism, studies suggest that the most significant contributor to 5α-reduction of glucocorticoid metabolites within the liver is 5αR2.

In our study, 5αR activity decreased significantly after weight loss. The role of 5αR in the control of body composition and insulin sensitivity has not been investigated in detail. Studies have shown enhanced 5αR activity to be associated with obesity (31) and type 2 diabetes (32) and sexual dimorphism of expression with increased activity in men (31,33). Dietary macronutrient composition

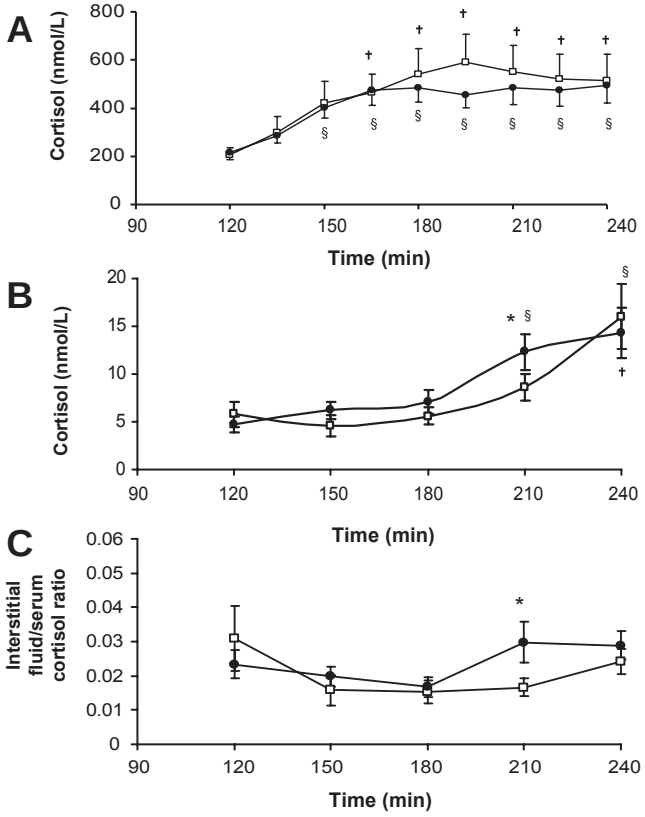

FIG. 4. Serum (*A*) and adipose tissue interstitial fluid (*B*) cortisol generation and the ratio of interstitial fluid cortisol to serum cortisol (*C*) following oral administration of 25 mg cortisone acetate in 14 obese individuals before (□) and after weight loss (●) (†$P < 0.05$ vs. baseline before weight loss; §$P < 0.05$ vs. baseline after weight loss; *$P < 0.05$ before vs. after weight loss).

has also been implicated as a regulator of 5αR and 5βR activity; high-fat, low-carbohydrate and moderate-fat, moderate-carbohydrate diets decrease 5αR and 5βR activity (34). The effect was most pronounced in the high-fat, low-carbohydrate diet, but both diets were associated with significant weight loss (34). In rodents, treatment of obese Zücker rats with insulin sensitizers decreases 5αR1 expression in the liver (35). In patients with polycystic cystic ovary syndrome, 5αR activity correlates positively with markers of insulin resistance (36). Specifically with regard to glucocorticoid metabolism, reduced 5αR activity decreases the inactivation of cortisol to its tetra-hydrometabolites, and as a consequence, this may relax the drive to the HPA axis and be responsible for the reduction in total glucocorticoid secretion that we observed after weight loss. Interestingly, the converse is probably also true in polycystic ovary syndrome, where enhanced 5αR activity may drive the HPA axis to maintain circulating cortisol levels at the expense of adrenal androgen excess (37).

This study adds further evidence as to the role of the HPA axis and glucocorticoid metabolism in the pathogenesis of obesity, insulin resistance, and the metabolic syndrome and has identified a potentially novel role for 5αR. In addition, we have shown adipose tissue–specific changes in metabolism and insulin sensitization after weight loss. Although we are unable to say whether the observed changes are cause or consequence of the improved beneficial phenotype after weight loss, this study raises the possibility that manipulation of 5αR activity, perhaps in a tissue-specific manner, may have potential as a therapeutic strategy.

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