Tissue Specific Regulation of Glucocorticoids in Severe Obesity and the Response to Significant Weight Loss Following Bariatric Surgery (BARICORT)

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Context: Tissue cortisol exposure is under the control of the isozymes of 11β-hydroxysteroid dehydrogenase (11β-HSD). 11β-HSD1 in vivo, acts as an oxoreductase converting inactive cortisone to active cortisol. We hypothesized that 11β-HSD1 activity is dysregulated in obesity and alters following bariatric surgery induced weight loss in different tissues.

Methods: We recruited 21 patients prior to undergoing bariatric surgery and performed cortisol generation profiles (following oral cortisone administration), urinary corticosteroid metabolite analysis, adipose tissue microdialysis, and tissue gene expression before and after weight loss, following bariatric surgery. Archived tissue samples from 20 previous bariatric surgery patients were also used for tissue gene expression studies.

Results: Gene expression showed a positive correlation with 11β-HSD1 and BMI in omental adipose tissue (OM) ($r = +0.52$, $P = .0001$) but not sc adipose tissue ($r = +0.28$, $P = .17$). 11β-HSD1 expression in liver negatively correlated with body mass index (BMI) ($r = -0.37$, $P = .04$). 11β-HSD1 expression in sc adipose tissue was significantly reduced after weight loss (0.41 ± 0.28 vs 0.17 ± 0.1 arbitrary units, $P = .02$). Following weight loss, serum cortisol generation increased during a cortisol generation profile (area under the curve $26\,768 \pm 16\,880$ vs $47\,579 \pm 16\,086$ nmol/L/minute, $P \leq .0001$.) Urinary corticosteroid metabolites demonstrated a significant reduction in total cortisol metabolites after bariatric surgery ($15\,224 \pm 6595$ vs 8814 ± 4824 μg/24 h, $P = .01$). Microdialysis of sc adipose tissue showed a threefold reduction in cortisol/cortisone ratio after weight loss.

Conclusions: This study highlights the differences in tissue specific regulation of cortisol metabolism in obesity and after weight loss. Following bariatric surgery hepatic 11β-HSD1 activity increases, sc adipose tissue 11β-HSD1 activity is reduced and total urinary cortisol metabolites are reduced indicating a possible reduction in hypothalamic pituitary adrenal axis drive. 11β-HSD1 expression correlates positively with BMI in omental adipose tissue and negatively within hepatic tissue. 11β-HSD1 expression is reduced in sc adipose tissue after weight loss. (***J Clin Endocrinol Metab*** **100: 1434–1444, 2015)**

Obesity has reached epidemic proportions worldwide, where severe obesity (body mass index [BMI] > 40) is commonplace (1–4). Obesity increases the risk of developing diabetes, cancer, and cardiovascular disease and is associated with an increased mortality (5–9). Patients with glucocorticoid (GC) excess (Cushing's syndrome)

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Abbreviations: AU, arbitrary units; AUC, area under the curve; BMI, body mass index; BP, blood pressure; bp, base pairs; DHEA, dehydroepiandrosterone; F/E, cortisol to cortisone ratio; GC, glucocorticoid; GC/MS, gas chromatograph mass spectrometry; GR, glucocorticoid receptor; HDL, high-density lipoprotein; HPA, hypothalamic pituitary adrenal; 11β-HSD, 11β-hydroxysteroid dehydrogenase; IQR, interquartile range; LDL, low-density lipoprotein; OM, omental adipose tissue; RYGB, Roux En Y Gastric Bypass; TAG, trigaclycerol; THE, tetrahydrocortisone; THF, tetrahydrocortisol; UFE, urinary free cortisone; UFF, urinary free cortisol; VLCD, very low calorie diet.

bear a strikingly similar phenotype to patients with obesity. In obesity however, circulating serum GC concentrations are in the normal range or lower than controls (10). Circulating serum GC concentrations are regulated by the hypothalamic pituitary adrenal (HPA) axis. Tissue cortisol metabolism is under the control of the isozymes of the 11β-hydroxysteroid dehydrogenase (11β-HSD) system. In vivo, 11β-HSD1 acts primarily as an oxoreductase converting inactive cortisone to active cortisol. Dysregulated 11β-HSD1 activity has been implicated in obesity, metabolic, and inflammatory diseases (11, 12).

Most studies examining 11β-HSD1 activity and expression in adipose tissue have been carried out using sc adipose tissue. In general, they show 11β-HSD1 activity and expression is increased in obesity (13–17). However, some studies have not found an increase in sc tissue 11β-HSD1 expression (18). Conflicting data on changes in 11β-HSD1 expression following dietary weight loss have been published (19, 20), but gastric bypass induced weight loss, resulted in reduced expression in 1 study (21). Data analyzing the omental adipose depot are understandably more sparse, but increased expression of 11β-HSD1 mRNA levels in omental adipose tissue have been reported in obesity (18, 23–25, 30). In hepatic tissue the results are also conflicting; 11β-HSD1 expression has been shown to be elevated in obesity (24), correlating positively with metabolic disease (26), but other studies have reported a reduction in obese patients (13) and increase following short-term dietary weight loss (19). Analyses of urinary corticosteroid metabolites are similarly mixed. Urinary cortisol metabolites correlate positively with increasing waist circumference (27). Urinary cortisol metabolite analysis shows reduced 11β-HSD1 activity in central obesity (28). Following weight loss, urinary glucocorticoids and metabolites have been reported to be reduced (27, 29).

As increased activity of 11β-HSD1 may augment tissue cortisol exposure and contribute to a metabolically unhealthy phenotype (30), the prospect of specific 11β-HSD1 inhibitors raises the possibility of these drugs as potential treatments for obesity and the metabolic syndrome (11, 31, 32). Early trials examining 11β-HSD1 inhibitors in diabetes and metabolic syndrome (33, 34) have been reported, but long-term data are lacking. Of note, all published clinical trials looking at specific 11β-HSD1 inhibitors have shown modest but significant weight loss (33–35).

The interpretation of studies to date is limited by the lack of data from multiple tissue depots in an individual patient. Not all studies utilize a cortisol generation profile to measure hepatic 11β-HSD1 activity, instead of relying on urinary corticosteroid metabolite ratios, which may reflect more global 11β-HSD1 activity. Further studies are required to establish the global and tissue-specific role of 11β-HSD1 in obesity and following weight loss.

We aimed to establish the activity and expression of 11β-HSD1 in multiple tissue depots by several methods of assessment (urinary corticosteroid metabolite analysis, cortisol generation profile, in vivo adipose tissue microdialysis, and PCR) in obese individuals and the effect of weight loss.

Materials and Methods

Patients, ethics, and anthropometric measurements

The study received ethical approval from the Ethics Committee of St Vincent's University Hospital group. Participants were recruited between autumn 2010 and spring 2013. We recruited participants who were awaiting bariatric surgery in St Columcille's Hospital, Loughlinstown, Dublin. Patients awaiting Roux En Y Gastric Bypass (RYGB) had attended a multidisciplinary team for at least 12 months with input from monthly physiotherapy, psychology, and nutritional support and were deemed appropriate candidates for RYGB surgery, having failed conservative measures for weight loss. Participants who were recruited gave informed consent and attended detailed assessment (as outlined below) prior to surgery with repeat testing after surgery (Figure 1). Patients were operated on between April 2011 and April 2013. In addition, archived tissue samples from patients who underwent RYGB surgery between 2005 and 2010 and gave

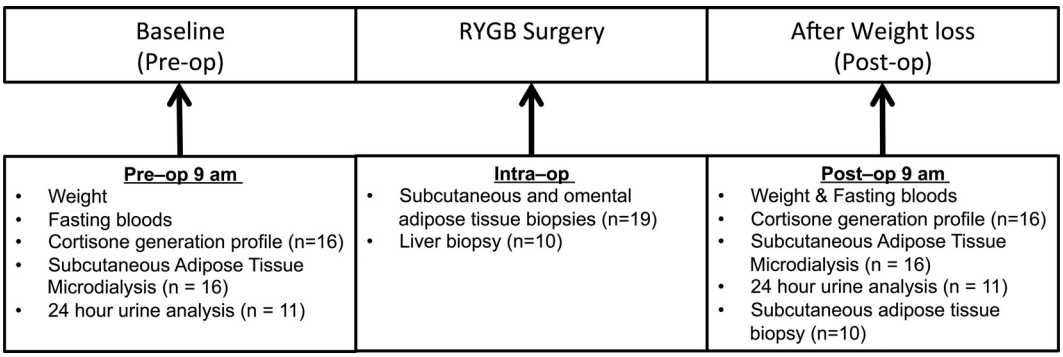

Figure 1. Baricort study protocol involving baseline, surgical, and postoperative testing

informed consent for further testing were used for baseline gene expression analysis.

Exclusion criteria included current or prior steroid use within the previous 6 months or significant weight fluctuations 6 months prior to surgery or recent illness at time of assessment, pregnancy, age > 70 years or < 18 years, and persons unable to give informed consent.

Assays

Presurgical blood tests including cholesterol profile, electrolytes, liver profile, blood counts, thyroid function tests, and glucose indices were all carried out with in-hospital assays. Serum glucose was collected in sodium fluoride tubes and measured using the hexokinase methodology in an automated analyzer (Olympus AU640). Serum total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol, and trigaclycerol (TAG) concentrations were collected in serum tubes and measured using enzymatic reagents (Olympus AU640). Serum insulin was measured using an automated monoclonal antibody-based two-site immunoenzymometric assay (AIA-1800 system, Tosoh Europe NV), and glycosylated hemoglobin (HbA1c) was measured with an automated HPLC instrument-reagent system (model HLC-723 G7; Tosoh Europe NV).

Cortisol generation profile

Participants were studied after overnight fasting. They attended for testing at 9:00 AM after an overnight fast and having ingested 2 mg of dexamethasone the previous night at 11:00 PM to suppress endogenous adrenal cortisol production. The dose of 2 mg of dexamethasone was chosen based on the high BMI of our patient cohort (mean BMI = 52.13 ± 7.5 kg/m^2) given that previous papers documented difficulty in suppressing adrenal cortisol production in obese cohorts (36). An iv cannula was inserted in the antecubital fossa and a sc adipose tissue micro dialysis catheter (CMA60, CMA Microdialysis) was inserted, under local anesthetic, 10 cm lateral to the umbilicus. The use of CMA microdialysis catheters has been described previously by our group (37). After a flush sequence (15 μL over 5 min) microdialysis was performed at a rate of 0.3 μL per minute and continued for 240 minutes. After baseline fasting bloods were taken, participants were given 25 mg of oral cortisone acetate. Following this, serum and microdialysis samples were taken every 30 minutes for 240 minutes. Serum samples were centrifuged and microdialysis vials were stored at −80°C until analysis using liquid chromatography tandem mass spectrometry.

Urinary glucocorticoid metabolite profile

Participants completed a 24-hour urine collection for cortisol, cortisone, and glucocorticoid metabolite analysis (38) before surgery and again after surgery, following weight loss. The urine collections were carried out on a separate occasion from the cortisol generation profile, when no dexamethasone was taken. The volume of the urine was recorded, and 15 mL aliquots were stored at −20°C until analysis using gas chromatograph mass spectrometry (GC/MS) as previously described (38). Total cortisol metabolites include tetrahydrocortisol (THF), 5α-THF, tetrahydrocortisone (THE), cortols and cortolones and free cortisol metabolites. Urinary (THF + 5α-THF)/THE is regarded as an index of global 11β-HSD activity, if the ratio of urinary free cortisol (UFF) to urinary free cortisone (UFE) is unaltered [reflecting specifically the activity of renal 11β-HSD2], any changes in this ratio result from alterations in 11β-HSD1 activity. Thus, a reduction in the urinary (THF + 5α-THF)/THE ratio correlates well with impaired generation of serum cortisol from orally administered cortisone acetate indicative of reduced 11β-HSD1 activity (28).

Steroid extraction and analysis from urine, serum, and microdialysis fluid

Corticosteroid extraction and analysis from urine was performed using GC/MS as described in detail previously by our group (38–40). For corticosteroid extraction and analysis from serum samples, liquid chromatography tandem mass spectrometry was used as previously described by our group (41). Due to low volumes of dialysate, microvial samples were pooled into 2 groups (pre- and postoperatively) according to the various time points. The semipermeable membrane in the microdialysis catheter (20 kilodalton limit) only allows collection of free unbound cortisol from the sc adipose tissue. Serum measurements represent unbound cortisol. Urine measurements represent total corticosteroid metabolites (steroids are deconjugated from sulfated and glucuronidated to produce a total steroid profile by GC/MS).

Intra operative tissue sampling

Tissue samples were collected during surgery. Omental, sc adipose tissue, and hepatic tissue biopsies were placed immediately into RNA later (Life Technologies) and stored at 4°C overnight. The following day, the samples were frozen and stored at −80°C until analysis. Repeat sc adipose tissue biopsies were obtained more than 6 months following surgery (mean time of biopsy was 14 mo). Under an aseptic technique and using 2% lignocaine as analgesia, a 1-cm incision was made 10 cm lateral to the umbilicus and a small sample of adipose tissue was removed. These sc adipose samples were immediately placed into RNA later and stored as above.

RNA extraction

Total RNA was extracted from tissue using the Tri-Reagent (Trizol Life Technologies) single step system. Concentration was determined spectrophotometrically at OD_{260}.

Reverse transcription

In a 20-μL volume, 500 ng of total RNA was incubated with 0.8 μL random hexamers, 2 μL deoxyribonucleotide triphosphates (10×), 1 μL RNase inhibitor, 1 μL Multiscribe reverse transcriptase, 5.5 mM MgCl, and 1 μL reaction buffer. The reverse transcription reaction was carried out at 25°C for 10 minutes and 37°C for 120 minutes before the reaction was terminated by heating to 85°C for 5 minutes. Reagents were purchased from Applied Biosystems.

Pre-PCR amplification

For pre-PCR amplification of cDNA, Taqman Pre-Amp master mix and pooled Taqman assays were used. Pre-PCR amplification reaction was done at 5 μL containing 2.5 μL Taqman Pre-Amp Master Mix (2×), 1.25 μL of pooled Taqman assay mix, and 1.25 μL cDNA. Preamplification PCR was carried out at 1 cycle of 95°C for 10 minutes followed by 14 cycles of 95°C for 15 seconds and then 60°C for 4 minutes. After preamplification, the product was diluted 1:5 and stored at −20°C.

Real time PCR

Real time PCR (RT-PCR) was carried out using the Fluidigm platform as described previously (42). The sequence for 11ß-HSD1 was 5′-AGGAAAGCTCATGGGAGGACTAG-3′ (antisense, 23 bp), 5′-ATGGTGAATATCATCATGAAAAAGATTC-3′ (sense, 28 base pairs [bp]), and 11ß-HSD1 TaqMan probe 5′-CATGCTCATTCTCAACCACATCACCAACA-3′ (29 bp). Reactions were performed in singleplex in 10-μL volumes on 96-well plates in reaction buffer containing 2 × TaqMan Universal PCR Master Mix (Applied Biosystems). Primers and probes were supplied by Applied Biosystems as premade "assay on demand." All reactions were normalized against the housekeeping gene 18S rRNA, provided as a preoptimized control probe. All target genes were labeled with FAM, and the reference gene with VIC. The reaction conditions were as follows: 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Data were obtained as Ct values (Ct = cycle number at which logarithmic PCR plots cross a calculated threshold line) and used to determine ΔCt values [ΔCt = (Ct of the target gene) − (Ct of the reference gene)]. Data were expressed as arbitrary units [arbitrary units (AU) = $1000\ x(2^{-\Delta ct})$].

Postoperative cortisol generation profile

Participants in this study had normal surgical follow-up with regular clinic attendances at 6 weeks and 12 weeks postoperatively and then at 3-month intervals. Participants returned for repeat testing no earlier than 6 months postoperatively. The mean length of time for postoperative reassessment following bariatric surgery was 9.3 ± 2.2 months.

Postoperative sc adipose tissue biopsy

On a separate visit to the cortisol generation profile, with no dexamethasone suppression, the participant attended a sc adipose tissue biopsy. Under local anesthetic (5 mL 2% lignocaine) adipose tissue was biopsied from under the skin of the abdomen, 10 cm lateral and 5 cm inferior to the umbilicus. Adipose tissue was stored initially in RNA later and then at −80°C until analysis.

Statistical analysis

Results are expressed as means ± SD for parametric data and medians and interquartile ranges for non-Gaussian data. Statistical analyses comparing groups were done using Student's *t*-test and Mann Whitney U-test for non-Gaussian datasets. Spearman and Pearson correlation tests were used to analyze associations between datasets. Figures and statistics were performed using Graph Pad Prism version 6.

Patients

All participants were Caucasian. A total of 21 patients (11 women) were recruited and 20 of them underwent RYGB surgery with 1 participant undergoing sleeve gastrectomy. Preoperatively, the mean weight of patients was 152.5 ± 26.4 kg and mean BMI was 52.13 ± 7.5 kg/m^2. The mean age of participants was 44.3 ± 6.5 years. Weight was measured to the nearest 0.1 kg, using a Seca 665 (Seca Ltd). Height was measured to the nearest 0.1 cm using a Seca 242 stadiometer (Seca Ltd). BMI was calculated using kilograms per meter squared. Blood pressure was recorded as the mean of 3 recordings using a mercury sphygmomanometer with the participant sitting.

For gene expression assays, archived tissue samples were also used from patients who had undergone bariatric surgery in St Columcille's Hospital previously (43, 44). These adipose tissue samples were snap frozen in liquid nitrogen and stored at −80° C. Omental and sc tissue samples from 24 nonobese patients undergoing elective abdominal surgery in the Queen Elizabeth hospital in Birmingham were used as a control group for gene expression (15 women, mean age 63.8 ± 11.5y and median BMI was 24 kg/m^2, interquartile range [IQR] 23.3–24.3 kg/m^2).

Results

Patient metabolic changes following bariatric surgery

There were improvements in several measures of metabolic health following bariatric surgery. The mean preoperative BMI was 52.1 ± 7.5 kg/m^2 and this decreased to 34.9 ± 5.5 kg/m^2 ($P \leq .0001$) following bariatric surgery. The mean preoperative weight was 152.5 ± 26.4 kg, and 104.8 ± 20.7 kg ($P \leq .0001$) at repeat testing following surgery. The mean systolic blood pressure (BP) preoperatively was 139 ± 14.75 mmHg and this decreased to 123.2 ± 12.32 mmHg ($P = .0002$). Diastolic BP improved significantly from 86.7 ± 10.5 mmHg to 74.1 ± 8.8 mmHg postoperatively ($P = .014$). The cholesterol profile did not improve significantly after RYGB surgery. Total cholesterol was 4.5 ± 0.9 mmol/L preoperatively and 4.2 ± 0.8 postoperatively ($P = .4$). LDL was 2.8 ± 0.6 mmol/L preoperatively and to 2.4 ± 0.6 mmol/L postoperatively ($P = .08$). HDL remained unchanged before and after RYGB surgery at 1.3 ± 0.3 mmol/L. Triglycerides were 1.1 ± 0.4 mmol/L preoperatively and 0.9 ± 0.3 postoperatively ($P = .3$). Despite a lack of significant changes in lipid profiles, only 3 patients were on statin therapy after weight loss, whereas 9 patients were on statins prior to surgery. Fasting plasma glucose was 6.3 mmol/L (IQR 5.7–7.3) preoperatively and was significantly lower following RYGB surgery at 4.9 mmol/L (IQR 4.8–5.9), ($P = .001$). HbA1C decreased significantly from 43 (IQR 37.5–52.5) to 37.5 mmol/mol (IQR 33–39), ($P = .005$).

Hepatic 11ß-HSD1 activity (cortisol generation profile)

All participants suppressed their morning fasting cortisol levels to < 50 nmol/L following 2 mg dexamethasone ingestion the previous night, before and after bariatric surgery. The mean fasting cortisol level after dexamethasone suppression was 13.7 ± 11.1 nmol/L preoperatively and 12.1 ± 5.2 nmol/L after weight loss. The peak cortisol generated following 25 mg oral cortisone acetate was 160.4 ± 105.6 nmol/L preoperatively and this rose to 248.3 ± 75.3 nmol/L after weight loss. Comparing results before and after weight loss, there was an increase in cor-

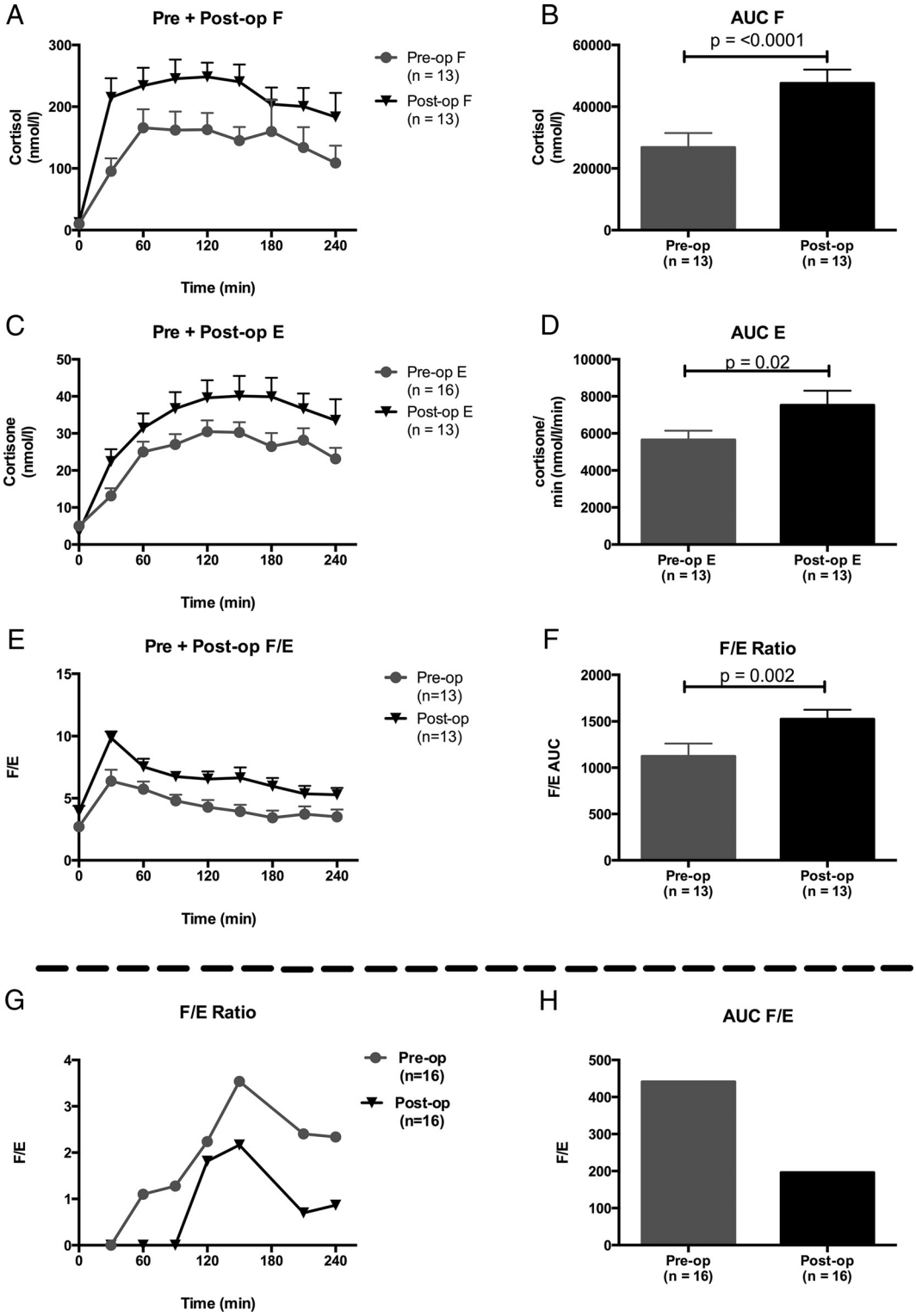

Figure 2. Analysis of serum cortisol, cortisone, and cortisol/cortisone (F/E) ratio before and after weight loss following bariatric surgery. Analysis of pooled microdialysis subcutaneous adipose tissue samples of 16 patients undergoing bariatric surgery before and after weight loss (G + H). All samples taken after ingestion of oral 25 mg cortisone acetate. A, Serum cortisol generation time curve. B, AUC analysis of serum cortisol with significant increase in AUC serum cortisol after weight loss. C, Serum cortisone time curve. D, AUC analysis of serum cortisone shows a significant increase after bariatric surgery. E, Time curve of ratio of F/E. F, AUC of F/E analysis with a significant increase in postop F/E ratio. G, Microdialysis sc tissue cortisol generation of cortisol after ingestion of 25 mg oral cortisone acetate. H, AUC shows a threefold reduction in F/E ratio after weight loss following bariatric surgery. Data expressed as means ± SEM. Pre-op, black filled-in circe; Post-op, grey filled-in inverted triangle.

tisol generated at each time point postoperatively (Figure 2A), and area under the curve (AUC) analysis showed a statistically significant increase in total cortisol generated (26 768 ± 16 880 vs 47 579 ± 16 086 nmol/L/min, $P \leq .0001$), (Figure 2B). Fasting serum cortisone preoperatively was 5.0 ± 4.5 nmol/L compared to 3.4 ± 1.2 nmol/L postoperatively, ($P = .9$, Figure 2C). Peak serum cortisone was 30.5 ± 12.1 nmol/L preoperatively and 40.1 ± 18 nmol/L postoperatively ($P = .1$, Figure 2C). At each time point, during the 240-minute cortisol generation profile, cortisone was higher in the postoperative setting (Figure 2C). AUC analysis revealed a statistically significant difference after bariatric surgery, 5739 ± 2048 vs 7523 ± 2815 nmol/min ($P = .01$, Figure 2D).

Finally, we looked at the ratio of cortisol to cortisone (F/E). Serum F/E AUC was significantly raised after bariatric surgery showing as increase in hepatic 11β-HSD1 activity (Figure 2E). The mean AUC for the F/E ratio was 943.8 (729.4–1097) preoperatively vs 1486 U (1067–1547) postoperatively ($P = .02$, Figure 2F).

Subcutaneous adipose tissue microdialysis

Analysis of microdialysis samples from sc adipose tissue demonstrated a reduction in 11β-HSD1 activity after weight loss in sc adipose tissue as seen by a threefold reduction in the F/E ratio between pooled pre and post-RYGB surgery samples (Figure 2, G and H).

Urinary glucocorticoids and metabolites

Total cortisol excretion was not statistically different before and after bariatric surgery. Similarly, there was no difference in total cortisone excretion following weight loss (Table 1). (THF+5αTHF)/THE, a marker of global 11β-HSD1 activity was not significantly different following weight loss. The ratio of cortisol to cortisone (F/E), which represents total body 11β-HSD2 activity, remained unchanged following weight loss (0.73 ± 0.14 vs 0.75 ± 0.18, $P = .7$). There were significant reductions in the corticosteroid metabolites THF, 5αTHF, and THE after weight loss (Table 1). There was a statistically significant reduction in total F metabolites (Figure 3A) following weight loss (15 224 ± 6595 vs 8814 ± 4824 μg/24 h, $P = .009$) and a significant reduction in urinary dehydroepiandrosterone (dehydroepiandrosterone [DHEA], Figure 3B) after weight loss (622.5 ± 696 vs 106.8 ± 131.6 μg/24 h, $P = .02$).

Tissue mRNA expression

Expression of 11β-HSD1 was significantly higher in adipose tissue depots in our obese cohort compared to nonobese controls (Figure 4A). In omental adipose tissue, 11β-HSD1 (Figure 4A) and glucocorticoid receptor (GR) (Figure 4C) were significantly higher in severely obese preoperative patients compared to nonobese controls, 0.29 ± 0.21 vs 0.17 ± 0.12 AUs ($P = .02$) and 0.52 ± 0.3 vs 0.32 ± 0.19 AUs ($P = .03$), respectively. Subcutaneous adipose tissue in preoperative severely obese patients had significantly higher expression of 11β-HSD1 (Figure 4B) compared to nonobese controls (0.41 ± 0.28 vs 0.23 ± 0.17 AUs, $P = .03$). Following surgery and weight loss, there was a statistically significant drop in 11β-HSD1 ex-

Table 1. Patient Clinical, Biochemical, and Anthropometric Characteristics.

	Before Bariatric Surgery	After Bariatric Surgery	
	n = 21 (male = 10)	n = 18 (male = 10)	*P* Value
Weight (kg)	152.5 ± 26.4	104.8 ± 20.7	<.0001
BMI (kg/m^2)	52.13 ± 7.5	34.9 ± 5.5	<.0001
HbA1C (mmol/mol)	43 (37.5–52.5)	37.5 (33–39)	.005
Corticosteroid Metabolite (μg/24 h)	(n = 11)	(n = 16)	
Total Cortisol (F)	81 ± 40.1	65.1 ± 31.2	.7
Total Cortisone (E)	116.7 ± 63.8	88.2 ± 34.6	.3
F/E	0.73 ± 0.14	0.74 ± 0.16	.7
(THF+5αTHF)/THE	0.86 ± 0.15	0.94 ± 0.36	.9
Total F metabolites	15 224 ± 6595	8814 ± 4824	.009
Androsterone	2970 ± 1533	2706 ± 1935	.7
Etiochoanolone	2070 ± 1082	1387 ± 1082	.14
17 OH Progesterone	129.3 ± 76	131 ± 109.4	.9
DHEA	622.5 ± 696	106.8 ± 131.6	.02
THF	2453 ± 1140	1480 ± 885.3	.02
5αTHF	2096 ± 858.4	1212 ± 866.9	.02
THE	5628 ± 2986	3213 ± 2157	.02

Abbreviation: 5αTHF, allo-tetrahydrocortisol. Data expresssed as means ± SD for parametric data and medians and interquartile ranges for non-Gaussian data. Urinary corticosteroid metabolites analyzed on gas chromatography mass spectrometry, data expressed as means ± SD. After weight loss there is a significant reduction in total F metabolites (Cortisol+THF+5αTHF+αCortol+βCortol+Cortisone+αCortolone+βCortolone+THE) and DHEA. Total body 11β-HSD1 activity {(THF+5αTHF)/THE} and total body 11β-HSD2 activity (UFF/UFE), remains unchanged following bariatric surgery.

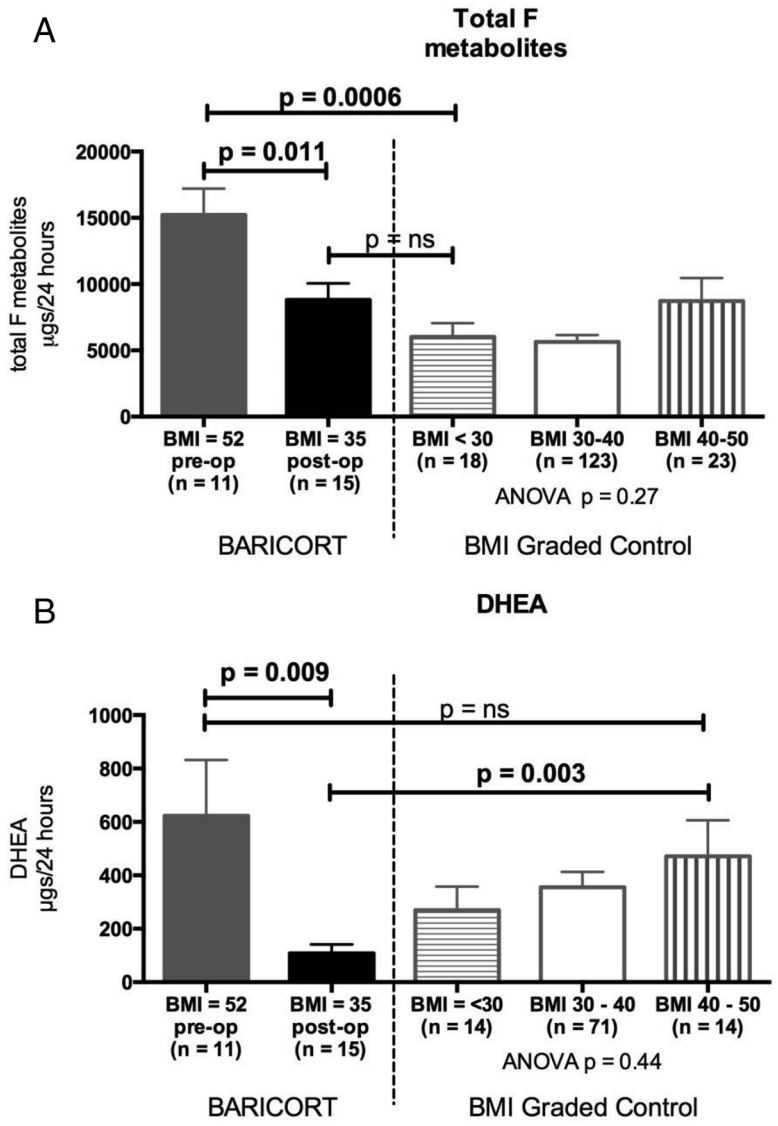

Figure 3. Urinary corticosteroid metabolites from BARICORT patients and control data. A, Total urinary corticosteroid metabolites in severely obese patients undergoing bariatric surgery shows a significant reduction in total F metabolites after weight loss. Control data from nonobese and obese participants demonstrate a stepwise increase in total F metabolites with increasing BMI category. Panel B shows urinary DHEA significantly reduced after bariatric surgery. Data expressed as means ± SEM. Pre-op BMI range 37–64 kg/m^2. Post-op BMI range 27–40 kg/m^2.

pression in sc adipose tissue compared to preoperative levels (0.41 ± 0.28 vs 0.17 ± 0.1 AUs, $P = .03$). Expression of GR in sc adipose tissue (Figure 4D) was lower in nonobese controls compared to severely obese patients, but this was not statistically significant (0.2 ± 0.14 vs 0.34 ± 0.23 AUs, $P = .08$).

When results of archived samples from previous bariatric surgery patients are added, analysis shows a positive correlation between 11β-HSD1 expression in omental adipose tissue and BMI (spearman r = + 0.52, $P = .0001$, Figure 5A) but not sc adipose tissue (spearman r = + 0.28, $P = .17$, Figure 5B). Hepatic 11β-HSD1 expression negatively correlated with BMI (Pearson r = −0.37, $P = .04$, Figure 5C).

Discussion

In this study we characterize the discordance in tissue specific cortisol metabolism between adipose tissue depots and liver in a severely obese cohort before and after significant weight loss. Serum cortisol generation, reflecting hepatic 11β-HSD1 activity (due to first pass metabolism of oral cortisone acetate), was significantly increased after weight loss. There was an increase in cortisol generation at each time point postoperatively (Figure 2A) and AUC showed a statistically significant increase in total cortisol generated (Figure 2, A and B). Serum F/E AUC was also significantly raised after bariatric surgery (Figure 2E). There was a significant increase in AUC of cortisone postoperatively (Figure 2D). This may reflect altered and increased absorption of orally ingested cortisone acetate following gastric surgery or alterations in metabolism.

Previous studies assessing a very low calorie diet (VLCD) to achieve weight loss, failed to show a change in hepatic 11β-HSD1 activity (19), but this is the first study to our knowledge to analyze hepatic 11β-HSD1 activity in a severely obese cohort before and after bariatric surgery. Reduction in hepatic 11β-HSD1 activity may be a protective mechanism in obesity to protect against excessive hepatic cortisol exposure (45) from omental adipose tissue cortisol generation which is delivered via the portal vein. In contrast to the increased hepatic 11β-HSD1 activity with weight loss, reduced 11β-HSD1 activity and expression was seen in sc tissue following weight loss. Subcutaneous adipose tissue microdialysis revealed a threefold reduction in cortisol/cortisone ratio in postoperative patients, suggesting a reduction in sc adipose tissue activity of 11β-HSD1 following weight loss. In a study examining weight loss using a VLCD, Tomlinson et al (29) did not demonstrate a significant change in AUC cortisol production in sc adipose tissue although this was a lower BMI group and relied on VLCD to achieve significant weight loss.

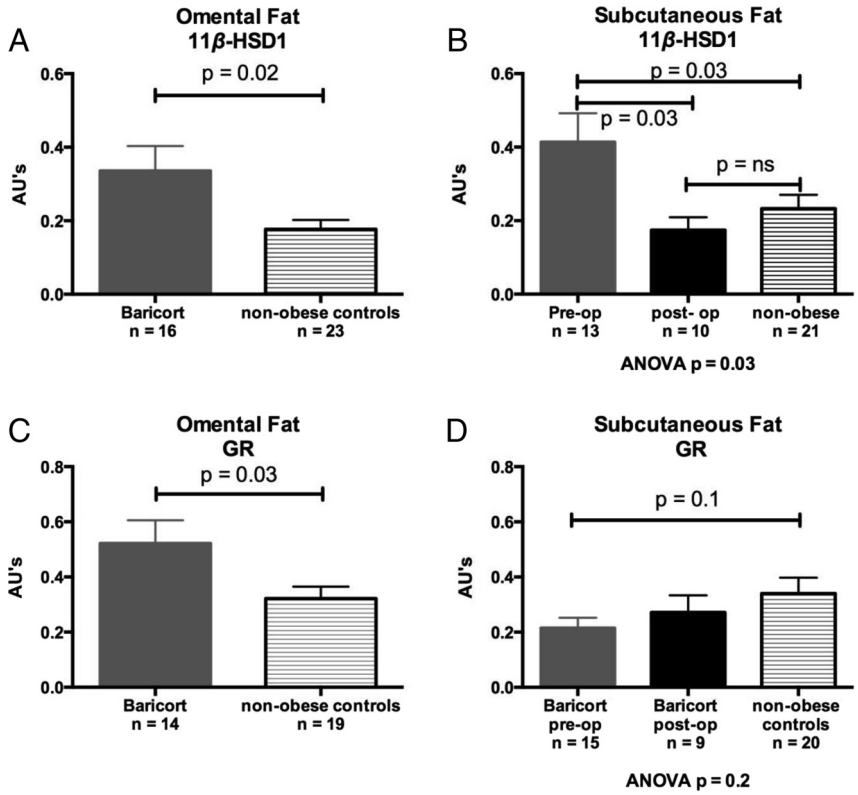

Figure 4. Expression of 11β-HSD1 and GR from omental and sc adipose tissue in patients undergoing bariatric surgery compared to nonobese controls. Data expressed in arbitrary units (AUs) and as means ± SEM. Unpaired *t*-test used to compare parametric data sets and Mann Whitney U test used to compare nonparametric data. Adipose tissue from nonobese patients undergoing elective abdominal surgery was used as controls. Severely obese patients undergoing bariatric surgery had a significantly higher expression of 11β-HSD1 and GR in omental adipose tissue (A and C). Severely obese patients had a significantly higher expression of 11β-HSD1 in sc adipose tissue (B). After weight loss, Baricort sc tissue had similar expression of 11β-HSD1 compared to nonobese controls (B) but not in GR expression.

Few studies have been published assessing 11β-HSD1 expression and activity in humans specifically around bariatric surgery. The bulk of data to date reflects women undergoing bariatric surgery in Sweden (21, 27). In rodent models, increased 11β-HSD1 expression and activity is associated with obesity and metabolic dysfunction. 11β-HSD1 knockout mice are resistant to the metabolic side effects of a high-fat diet compared to wild type (46). Transgenic mice, with overexpression of 11β-HSD1 specifically in adipose tissue, gain more weight and develop metabolic complications compared to the wild type (47).

In our cohort, omental 11β-HSD1 expression was significantly higher in severely obese persons compared to nonobese controls (Figure 4A). Leyvraz et al (48) showed similar higher omental expression of 11β-HSD1 compared to controls however Michalaki et al (49) did not show an increase in 11β-HSD1 omental expression. Torrecilla et al (26) similarly did not find associations between 11β-HSD1 omental expression in patients with severe obesity and metabolic syndrome. Gene expression of 11β-HSD1 in subcutaneous adipose tissue in our cohort showed elevated levels compared to nonobese controls. 11β-HSD1 expression in subcutaneous adipose tissue was significantly reduced after weight loss, similar to previous studies (21, 48). Leyvraz et al (48) showed severely obese patients preoperatively have elevated subcutaneous adipose tissue 11β-HSD1 gene expression levels compared to nonobese controls and after RYGB surgery this gradually reduced similar to nonobese controls. However, Tomlinson et al (19) found an increase in expression of 11β-HSD1 in sc adipose tissue after low calorie induced weight loss. Unlike previous studies we also show a matched reduction in 11β-HSD1 activity in sc depot using in vivo microdialysis which has not been shown before in this cohort type. In our results, subcutaneous 11β-HSD1

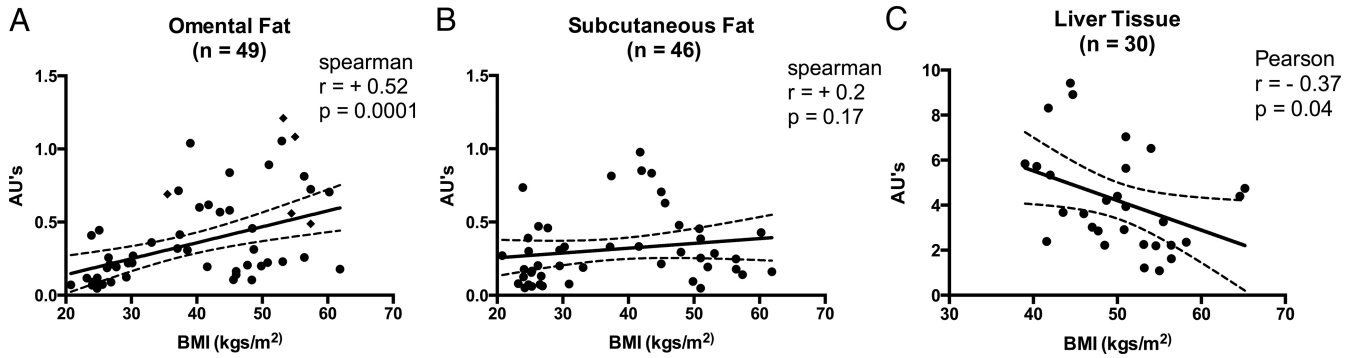

Figure 5. Correlations between BMI and 11β-HSD1 gene expression in all patients including archived tissue samples. A, Significant statistical positive correlation between BMI and omental 11β-HSD1 expression. C, Significant negative correlation between hepatic 11β-HSD1 expression and BMI.

expression is slightly higher than in omental tissue, which broadly speaking is in keeping with studies to date. Some studies have shown similar expression of 11β-HSD1 between visceral and subcutaneous compartments (9, 50, 51). However, they demonstrated different levels of expression of H6PDH and GR between depots (9), whereas we show a difference only in GR in the omental depot (Figure 4C). In subcutaneous adipose tissue GR is reduced in preoperative samples but not significantly reduced compared to nonobese controls (Figure 4D). When analyzing our samples with archived tissue samples, we found a positive correlation with BMI and 11β-HSD1 in omental adipose tissue but not subcutaneous adipose tissue. Hepatic 11β-HSD1 expression also correlated inversely with BMI. Baudrand et al (24) found an inverse correlation with BMI and hepatic 11β-HSD1 expression in patients undergoing bariatric surgery. However, Tomlinson et al in 2001 did not find a correlation with adipose tissue 11β-HSD1 expression and BMI (18) (Figure 5).

By assessing urinary corticosteroid metabolites, we have shown that global 11β-HSD1 and 11β-HSD2 activity, remains unchanged before and after significant weight loss. However, in different metabolic tissues, we show different changes (increases and decreases) in 11β-HSD1 activity and expression, in response to significant weight loss after bariatric surgery. Global 11β-HSD1 activity, as reflected by [(THF+5αTHF)/THE], in our study was not significantly different before and after surgery in keeping with earlier short studies using low calorie diets (22, 29). In a study assessing women undergoing RYGB surgery, Rask et al (27), demonstrated a significant total reduction in urinary glucocorticoid metabolites after weight loss, as reflected by the ratio (THF+5αTHF)/THE. Global 11β-HSD1 activity, includes 11β-HSD1 activity in adipose, hepatic, and skeletal muscle as well as several other tissues. Global 11β-HSD1 activity remains similar before and after bariatric surgery. Given our findings of increased hepatic 11β-HSD1 activity and reduced subcutaneous 11β-HSD1 activity after weight loss, the unchanged global 11β-HSD1 activity may be explained by a combination of altering 11β-HSD1 activity in liver, adipose tissue, and possibly muscle to compensate each other. Cortisol and cortisone secretion were not statistically different before and after bariatric surgery. The ratio of cortisol/cortisone (UFF/UFE), which represents total body 11β-HSD2 activity, also remained unchanged following weight loss, again in contrast to Rask et al (27).

Interestingly, a significant reduction in total cortisol metabolites is seen, including DHEA. This suggests a reduction in ACTH drive and reduction in HPA axis activity, which perhaps compensates for the increased hepatic 11β-HSD1 activity. There were nonsignificant reductions in androsterone, etiocholanolone, and 17-hydroxyprogesterone. DHEA is under the influence of ACTH and the observed reduction supports our theory of a reduction in pituitary ACTH secretion following weight loss in this bariatric cohort. This altered HPA axis following weight loss has been suggested by previous authors (22).

Our data suggest alterations in glucocorticoid metabolism in severe obesity, with tissue specific changes following weight loss due to bariatric surgery. 11β-HSD1 expression and activity is increased in adipose tissue in severely obese patients with a reduction in liver 11β-HSD1 activity, potentially as a protective mechanism (in order not to exacerbate the significant hepatic cortisol delivery from omental adipose tissue). Importantly, weight loss leads to dynamic changes in omental, hepatic, and total body glucocorticoid metabolism.

Specific inhibitors of 11β-HSD1 have been investigated in small clinical trials, primarily in the area of type 2 diabetes and the metabolic syndrome (33, 34). All published data have demonstrated modest but significant weight loss in patients who received 11β-HSD1 inhibitors. However, given our findings in this study (and those of others), highlighting the tissue specific changes in glucocorticoid metabolism after weight loss, an adipose tissue specific inhibitor of 11β-HSD1 may potentially be more efficacious than a global specific 11β-HSD1 inhibitor.

In summary, we have shown that in severely obese individuals undergoing bariatric surgery, hepatic 11β-HSD1 activity increases after weight loss and subcutaneous 11β-HSD1 activity is reduced after weight loss. Omental and liver tissue 11β-HSD1 expression correlates with BMI. Compared to nonobese controls, 11β-HSD1 expression was higher in adipose tissue, both omental and subcutaneous and these were reduced post-RYGB. Global F metabolites in urine were reduced after weight loss indicating a reduction in HPA axis activity. This study further elucidates the role 11β-HSD1 has in different metabolic tissues in obesity.

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