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Article type : Original Article - UK, Europe

Increased central adiposity and decreased subcutaneous adipose tissue 11β-hydroxysteroid dehydrogenase type 1 are associated with deterioration in glucose tolerance – a longitudinal cohort study

Running Title: Adiposity and glucocorticoid metabolism

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Acknowledgements

This work was supported by the Medical Research Council, UK (Senior Clinical Fellowship (G0802765) and programme grant (MR/P011462/1) to JWT); European Research Council (Advanced grant PMS), National Institute for Health Research (Senior Investigator award PMS and NIHR Oxford BRC principal investigator award JWT); the Royal College of Physicians (Samuel Leonard Simpson Fellowship RKC) and the Irish Endocrine Society (travelling fellowship grant CPW). The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, RCP, IES or the Department of Health.

The authors declare that there are no relevant conflicts of interest.

Summary

Objective & Context: Increasing adiposity, aging and tissue-specific regeneration of cortisol through the activity of 11β-hydroxysteroid dehydrogenase type 1 have been associated with deterioration in glucose tolerance. We undertook a longitudinal, prospective clinical study to determine if alterations in local glucocorticoid metabolism track with changes in glucose tolerance.

Design, Patients, Measurements: 65 overweight/obese individuals (mean age 50.3±7.3years), underwent oral glucose tolerance testing, body composition assessment, subcutaneous adipose tissue biopsy and urinary steroid metabolite analysis annually for up to 5 years. Participants were categorised into those in whom glucose tolerance deteriorated ('deteriorators') or improved ('improvers').

Results: Deteriorating glucose tolerance was associated with increasing total and trunk fat mass and increased subcutaneous adipose tissue expression of lipogenic genes. Subcutaneous adipose tissue 11β-HSD1 gene expression decreased in deteriorators and at study completion, was highest in the improvers. There was a significant negative correlation between change in area under the curve glucose and 11β-HSD1 expression. Global 11β-HSD1 activity did not change and was not different between deteriorators and improvers at baseline or follow-up.

Conclusion: Longitudinal deterioration in metabolic phenotype is not associated with increased 11β-HSD1 activity, but decreased subcutaneous adipose tissue gene expression. These changes may represent a compensatory mechanism to decrease local glucocorticoid exposure in the face of an adverse metabolic phenotype.

Keywords: Subcutaneous adipose tissue, 11β-HSD1, cortisol, glucose tolerance, metabolic function, gene expression.

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Introduction

Obesity is a global epidemic with severe health consequences [1]. Type 2 diabetes mellitus, insulin resistance and increased mortality [2] are all associated with weight gain and increased fat mass. Importantly, weight gain, independent of other metabolic risk factors, increases the risk of developing type 2 diabetes mellitus [3]. However, not all obese persons develop metabolic complications; a small sub-group of so called "healthy obese" individuals will remain healthy over time despite excess adiposity [4]. The description of metabolically healthy obese persons remains controversial and the mechanisms underpinning their apparent lack of metabolic dysfunction continue to be debated.

Based upon parallels with patients with glucocorticoid excess, Cushing's syndrome, who develop florid adverse metabolic features including insulin resistance, glucose intolerance and central obesity, there is an extensive body of literature that has examined the role of glucocorticoids in the development of common metabolic disease. Simple obesity is not characterised by circulating cortisol excess, but at a tissue-specific level, access of glucocorticoids to bind and activate the glucocorticoid receptor is governed by a series of 'pre-receptor' enzymes [5]. The isoforms of 11β-hydroxysteroid dehydrogenase (11β-HSD) interconvert active cortisol and inactive cortisone. 11β-HSD1 which is highly expressed in metabolic target tissues including adipose, liver and muscle, regenerates cortisol from cortisone requiring NADPH as a co-substrate which is generated by a tightly associated enzyme, hexose-6-phosphatre dehydrogenase (H6PDH). 11β-HSD2 inactivates cortisol to cortisone in mineralocorticoid target tissues (kidney, placenta and colon). The A-ring reductases, 5α-reductase type 1 and 2 and 5β-reductase metabolise cortisol and cortisone to inactive dihydro- with subsequent conversion to tetrahydro-metabolites.

Most, but not all cross-sectional studies have suggested that adipose tissue 11β-HSD1 expression is increased in obesity and dysglycaemia in parallel with decreased hepatic activity, as measured by urinary steroid metabolite analysis and / or cortisol generation from oral cortisone [6,7]. Furthermore, studies using selective 11β-HSD1 inhibitors have shown small but significant improvements in glycaemic control in patients with type 2 diabetes mellitus through a putative action to lower cortisol exposure in metabolic target tissues [8]. In addition, we, and others have demonstrated that baseline 5α-reductase activity can predict the future development of adverse metabolic features [9] and that these are worsened by 5α-reductase inhibition [10].

There is a lack of published studies of longitudinal changes in glucocorticoid metabolism and enzyme expression. We have undertaken a prospective clinical study to determine if improvement or deterioration in glucose tolerance over time is associated with global and tissue-specific changes in glucocorticoid metabolism. We aimed to test the hypothesis that in any given individual, increased activity and / or expression of 11β-HSD1 over time may drive the development of adverse metabolic features.

Research Design and Methods

The study was approved by South Birmingham Local Research Ethics Committee and subjects gave informed written consent (ref. 04/Q2707/278). Clinical investigations were performed at the National Institute for Health Research / Wellcome Trust Clinical Research Facility, Queen Elizabeth Hospital, Birmingham, UK. 65 obese / overweight unselected participants were prospectively recruited through local advertisement (40 women, 25 men). The overall sample size for the study, of which this report represents a post-hoc analysis, was based on the number expected to develop type 2 diabetes mellitus over a 5 year study. At enrolment, mean age was 50.3±7.3years and mean BMI was 33.7±4.3kg/m^2. All participants were investigated on an annual basis according to the clinical protocol and received written advice regarding diet and lifestyle. None received diabetes treatment during the study. Participants were included in this analysis if they attended for at least 2 assessment visits separated by ≥ 1 year; no missing visits were included. In order to minimise confounding factors participants had no significant medical history, were on no regular medications and had not received glucocorticoid treatment in the six months prior to taking part in the study (including inhaled therapy).

Clinical Protocol

At each visit, BMI, supine waist circumference at the level of the umbilicus, and hip circumference at the level of the greater trochanter were recorded. Blood pressure was measured using Dinamap® (Critikon, Tampa, FL, USA) while the patient was supine after a 10 minute rest; the average of three readings was recorded. The subjects fasted overnight before each visit. Blood samples were drawn for measurement of glucose, insulin, HbA1c, total cholesterol, and triglycerides. A 75g OGTT for 120 minutes was performed, with blood sampling at 30min intervals for measurement of glucose and insulin. Electrolytes, urea, creatinine, total cholesterol, triglycerides, glucose, and HbA1c were analysed on an automated platform (Roche Modular System, Roche). Insulin was measured using a commercially available colorimetric ELISA (Mercodia, Uppsala, Sweden) with an in-house CV of < 5%.

Body composition analysis was performed using Dual-energy x-ray absorptiometry (DXA) with a total body scanner (QDR 4500: Hologic, Bedford, MA). Coefficients of variation for multiple scans were 3%. Trunk: peripheral fat ratio was calculated by (trunk fat)/(arm fat + leg fat).

Participants performed a 24-hour urine collection for corticosteroid metabolite analysis [11]. The 24h urine volume was recorded, and aliquots stored at -20°C until analysis using GC-MS. Total glucocorticoid metabolites were defined as the sum of cortisol, cortisone, tetrahydrocortisol (THF), 5α-THF, tetrahydrocortisone (THE), α-cortolone, β-cortolone, α-cortol, and β-cortol [11]. Urinary cortisol/cortisone ratio (F/E) was used as a measure of 11β-HSD2 activity. The ratio of (THF+5αTHF)/THE and the ratio of cortols/cortolones were used to assess 11β-HSD1 activity.

Subcutaneous adipose tissue (SAT) biopsies were taken from the abdomen using an aseptic technique. Under local anaesthesia (5mL of 2% lignocaine), a 1cm incision was made approximately 10cm lateral and 5cm inferior to the umbilicus and 200-300mg of SAT removed. Adipose tissue was stored immediately in RNA later® at +4°C. The following day, the RNA later® (Thermo Fischer Scientific) was replaced and the samples frozen and stored at -20°C until analysis.

RNA extraction and Pre-PCR amplification

Total RNA was extracted from whole tissue using the Tri-Reagent (Trizol® Life technologies) single step system. Approximately 100mg of adipose tissue was homogenised in Trizol® for RNA extraction. RNA integrity was assessed by electrophoresis on 1% agarose gel. Concentration was determined spectrophotometrically at OD260.

Pre-PCR amplification reaction was performed in 5µL volumes, containing 2.5µL Taqman® PreAmp Master Mix (2x), 1.25µL of pooled Taqman® assay mix and 1.25µL cDNA. Pre-amplification PCR was carried out at one cycle +95°C for 10min followed by 14 cycles of +95°C for 15 seconds and then +60°C for 4 minutes. After pre-amplification, the product was diluted 1:5 and stored at -20°C.

Real-time PCR

Real time PCR was performed using the Fluidigm® platform [12]. Reactions were performed in singleplex in 10µL volumes on 96-well plates in reaction buffer containing 2xTaqMan® Universal PCR Master Mix (Applied Biosystems, UK.) All reactions were normalised against the house keeping gene 18s rRNA, provided as a pre-optimised control probe. 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) and used to determine ΔCt values (ΔCt = (Ct of the target gene) – (Ct of the reference gene).

Analytical approach and statistical methods

Participants with data from at least two visits were included in the study. In those individuals with more than two assessments, the data from the most recent study visit were included in the analysis and compared with baseline data.

The cohort was divided into two groups: those whose glucose tolerance worsened (increased glucose AUC across the OGTT) - designated as 'deteriorators', and those whose glucose tolerance improved (decreased glucose AUC across the OGTT) - 'improvers'

Data analysis was performed using JMP 8 (SAS Ca.) and GraphPad Prism 6 (La Jolla, Ca.). Normally-distributed data were described using mean and standard deviation. Data that were not normally distributed were described using median and IQR. AUC analysis was performed using the trapezoidal method. For comparison of baseline and follow up data, paired t-tests were used. For comparisons between 'deteriorators' and 'improvers', t-tests were used (or non-parametric tests for data that were not normally distributed). Significance was accepted at a p value of <0.05. All data generated or analysed during this study are included in this published article (and its supplementary information files).

Results

The metabolic characteristics of the entire cohort (n=65) at study entry (baseline) and at last follow-up visit are presented in Table 1. The mean follow-up was 3.8±1.5years. Overall, BMI HbA1c, total body fat, total lean mass and the ratio of trunk: peripheral fat increased with time. Fasting glucose and insulin remained unchanged over time, as did the AUC glucose across the OGTT (Table 1).

Metabolic variables associated with changes in glucose tolerance

Analysis of the cohort stratified as either ‘deteriorators’ (35) or ‘improvers’ (30) in glucose tolerance over the duration of the study is presented in Table 2. In the 'deteriorator' cohort at the second timepoint of testing, 1 subject had a fasting glucose in the diabetes range and 1 had impaired fasting glucose, 3 subjects had a 2-hour glucose of > 11.1mmol and 10 had a 2 hour glucose of 7.8-11mmol. In the 'improver cohort' at the second timepoint of testing, 1 had impaired fasting glucose, 2 subjects had a 2-hour glucose of > 11.1mmol, and 3 had a 2-hour glucose of 7.8-11mmol. At baseline, total and trunk lean mass were lower in deteriorators, whilst total and trunk fat mass were not different. Interestingly, AUC glucose was higher at baseline in those individuals whose glucose tolerance subsequently improved (Table 2).

Longitudinally, in the deteriorators, weight and BMI increased significantly as did HbA1c, total body fat, trunk fat, waist circumference, fasting insulin and waist to hip ratio. In contrast, in the improvers, there was no significant change in weight, BMI or body composition DXA. In the entire cohort (both improvers and deteriorators), change in total fat mass was the only factor that correlated with increasing AUC glucose ($r=0.28$, $p=0.03$).

Glucocorticoid metabolism

Data from urinary corticosteroid metabolites were available in 61/65 participants. During the study, there were significant increases in F/E and 5αTHF/THF ratios consistent with decreased 11β-HSD2 and increased 5α-reductase activity respectively. Total F and E metabolites decreased whilst 11β-HSD1 activity (THF+5αTHF/THE) remained unchanged (supplementary table 1). There was no relationship between 11β-HSD1 activity and longitudinal change in AUC glucose ($r=0.17$, $p=0.8$)

There were no significant differences in corticosteroid metabolites or ratios between 'deteriorators' and 'improvers' either at baseline or at the end of the study (table 3). There was no relationship between change in total glucocorticoids and change in AUC glucose in either deteriorators or improvers. The patterns of change in steroid metabolites were similar in both the deteriorators and the improvers with the exception of 5αTHF and cortisone, both decreased in the improvers (table 3). There was no difference in the THF+5αTHF/THE ratio between deteriorators and improvers suggesting that global (principally hepatic) 11β-HSD1 activity did not change (figure 1a, table 3).

In contrast to the global urinary markers, SAT 11β-HSD1 mRNA expression changed across the duration of the study. At baseline, mRNA expression levels were similar in deteriorators and improvers. However, in the deteriorators, expression decreased whilst increasing in the improvers such that at the end of the study, 11β-HSD1 was the only gene (amongst the 52 genes examined) that was differentially expressed with higher expression in the improvers compared to deteriorators (16.8 ± 2.1 vs. 14.4 ± 2.7, $p<0.05$, figure 1b, supplementary data table 2). Furthermore, there was a significant negative correlation between the change in AUC glucose across OGTT and the change in SAT 11β-HSD1 mRNA expression such that decreasing expression over time was associated with increasing AUC glucose ($r=-0.4$,

p=0.04, figure 1c). The expression of both the glucocorticoid receptor (NR3C1) and H6PDH did not change over time and was not different between deteriorators and improvers (figure 1d and e, supplementary data table 2).

When analysing the cohort as whole, both systolic BP and diastolic BP increased over time (table 1). However, when comparing improvers and deteriorators, there was no difference in systolic blood pressure, and significant increase in diastolic blood pressure in the improvers only (table 2). Increasing F/E ratios over time (reflecting decreased 11β-HSD2 activity) were associated with higher final systolic BP (r=0.28, p=0.04) (figure 2a). The relationship between change in systolic blood pressure and change in F/E ratio failed to reach statistical significance (r=0.25, p=0.06) (figure 2b). There were no significant relationships with diastolic blood pressure (data not shown).

Gene expression profiles in SAT

SAT gene expression profiles at baseline and follow-up were only available in 31 individuals, due to integrity of biopsy material. Clinical characteristics of this cohort at baseline, and longitudinal changes stratified by changes in glucose tolerance were not different when compared against the entire cohort (supplemental table 3).

Gene expression profiles were similar at baseline in improvers (n=14) and deteriorators (n=17). Baseline adipose gene expression did not predict longitudinal changes in glucose tolerance (data not shown). In the deteriorators, there were significant changes in the expression level of 30/52 genes from baseline to follow-up. SAT genes were clustered into those that were involved in lipid metabolism (ACLS1, CD36, C3, AQP7, FABP4, FASN, LPL, MGLL and PNPLA2), genes involved in hepatic steatosis (LOX1 and 2, AOC3),

adipokines (ADIPO1, ADQOR1, LEP and RARRES2), adipose inflammation (CD14 and TNFα), adipocyte differentiation (CCND1, CEBPA, CEBPB, GPD, HOX). In the improvers, the expression of 6/52 genes changed significantly between baseline and follow-up: DGAT1, AR, SIRT1, BCKDH4, IRS2, HSPA5 and XBP1 (figure 3, supplementary table 2).

Discussion

In this study of an obese/overweight cohort, we have demonstrated longitudinal changes in metabolic phenotype. Increasing fat mass, and in particular central fat accumulation, was specifically associated with deterioration in glucose tolerance over time. Perhaps surprisingly, SAT 11β-HSD1 mRNA expression decreased in those with deterioration in glucose tolerance and was significantly lower than in those in whom glucose tolerance improved. This appears to be specific to SAT as more global measures of 11β-HSD1 activity (urinary steroid metabolites analysis) remained unchanged.

Alterations in glucocorticoid metabolism have been implicated in the pathogenesis of metabolic disease [13]. Many cross-sectional studies have been reported and for the most part these have demonstrated increased 11β-HSD1 expression and activity in SAT. The vast majority of studies have examined SAT expression; observations in visceral adipose tissue (VAT) have been less consistent [6,14–16]. To date, longitudinal studies that have examined changes in 11β-HSD1 expression in adipose tissue over time without a specific intervention have not been published. Our data show that with worsening glucose tolerance, 11β-HSD1 expression levels fall, suggesting that this is not a primary pathogenic mechanism but perhaps a compensatory response to limit local glucocorticoid exposure and potential weight gain. It is interesting to speculate that increased 11βHSD1 expression (as was seen in the 'improvers'), would potentially increase cortisol half-life and therefore decrease total

glucocorticoid production rate through classical negative feedback. In patients with decreased 11β-HSD1 activity the converse is true, with reduced cortisol half-life and increased glucocorticoid production rates [17,18]. Studies using weight loss interventions have shown variable results. Very low-calorie diets have also shown that 11β-HSD1 expression can both increase or decrease with weight loss and that activity (either measured using urinary steroid metabolites or cortisol generation from oral cortisone) may not change [19–21]. In contrast, bariatric surgery results in reduced SAT 11β-HSD1 expression and activity, and increased global (hepatic) measures of 11β-HSD1 activity [6,22]. Clinical studies that have used highly selective 11β-HSD1 inhibitors to inhibit 11β-HSD1 and not 11β-HSD2 have shown modest improvements in glycaemic control in patients with type 2 diabetes mellitus taking metformin therapy, and some reductions in circulating lipids and blood pressure. Interestingly, in all studies, treatment with these compounds was associated with a modest weight loss in comparison with placebo [8,23]. One interpretation of this study is that the contribution of 11β-HSD1 activity to the development of adverse metabolic features is limited; this may explain modest improvements with selective pharmacologic inhibitors.

Other pre-receptor enzyme systems that regulate cortisol availability have been implicated in the pathogenesis of metabolic disease. Increased 5α-reductase activity has been demonstrated in obesity and insulin resistance that falls following weight loss [20]. In addition, small detailed metabolic studies have demonstrated hepatic and peripheral insulin resistance associated with combined inhibition of 5α-reductase type 1 and 2 [10]. We observed a reduction in the 5α-reduced metabolite, 5αTHF, only in improvers. In both improvers and deteriorators, 5β-reduced metabolites (THF and THE) fell significantly with time. Our finding complements the finding of a recent metabolomics study in which plasma THF glucuronide was associated with higher insulin resistance [24]; these studies suggest that higher 5α reductase activity and

clearance of these metabolites is present in metabolically unhealthy individuals and falls with improvement in insulin sensitivity.

Whilst we have focussed on specific aspects of glucocorticoid metabolism, accrual of fat mass (particularly centrally) was the largest determinant of deterioration in glucose tolerance. In cross–sectional studies, VAT mass correlates more closely than SAT with insulin resistance [25] although in an analysis based on the Framingham Heart Study cohort, both VAT and SAT were associated with insulin resistance [26]. Our analysis does not allow us to quantify changes in VAT and our gene expression profiling was performed in SAT biopsies. However, SAT mass has been associated with aspects of metabolic phenotype and SAT reductions (when corrected for total weight loss) are associated with reductions in blood glucose, which justifies the exploration of gene expression profiles in this depot [27]. Clearly obtaining VAT biopsies in otherwise healthy individuals is not feasible.

Another important consideration is the role of lean mass in the regulation of metabolic phenotype. 11β-HSD1 is expressed in skeletal muscle, but muscle expression and activity was not examined in this study. We have previously shown that 11β-HSD1 can regulate insulin sensitivity in skeletal muscle; increased expression is associated with increasing age and lower grip strength [28,29]. Total lean mass at baseline was lower in the deteriorators (although did not change significantly over time in either group) and it is plausible that this may have contributed to their worsening glucose tolerance. Jang et al demonstrated lower 11β HSD1 activity in skeletal muscle of people with type 2 diabetes compared to controls and also suggested that this was a compensatory mechanism [30]. We did not take skeletal muscle biopsies as part of the protocol, so we are unable to comment as to whether our findings would replicate those of Jang et al. However, it is possible that the more subtle disturbance in glucose tolerance that we observed (the Jang cohort had diabetes), may not

have caused down-regulation of 11β HSD1 in skeletal muscle. This is relevant as both liver and muscle are believed to contribute significantly to the urinary steroid pool that helps to define 11βHSD1 activity [31]. Therefore a lack of change in skeletal myocyte or hepatocyte 11β HSD1 expression could result in unchanged urinary steroid metabolites.

The changes in SAT gene expression profiles were more marked in the participants in whom glucose tolerance deteriorated (significant changes in 30/52 vs. 6/52 genes, $p<0.0001$). The clusters of dysregulated genes in deteriorators were involved in lipid metabolism, hepatic steatosis, adipose tissue inflammation, and adipocyte differentiation. The most consistent patterns of differential regulation between deteriorators and improvers were in gene clusters related to lipid metabolism and adipocyte differentiation (figure 3). Many of the specific genes within these clusters have been linked to alterations in metabolic phenotype. Patatin-like phospholipase domain-containing protein 2 (PNPLA2, also known as Adipose triglyceride lipase, ATGL) is regarded as the principal enzyme responsible for initial hydrolysis of triglyceride within the adipocyte. Expression increased 28.6-fold in the deteriorators as compared with 6.2-fold in the improvers. Its role in the pathogenesis of obesity and metabolic dysfunction continues to be debated; some studies have shown increased expression in association with obesity, metabolic disease and diabetes, whilst others have demonstrated reduced expression [32]. Studies in rodent models have shown that genetic deletion leads to lipid accumulation within adipocytes (both white and brown) and the development of obesity, but with improved glucose tolerance [33]. Adipose tissue-specific over-expression improves insulin insensitivity during hyperinsulinaemic euglycaemic clamp studies [34]. Other significant changes in gene expression in deteriorators might be expected to increase lipid accumulation within adipocytes through enhanced lipid synthesis (eg fatty acid

synthase and fatty acid binding protein 4) and adipocyte differentiation (PPARγ, CEPBα and β) and in combination these changes might explain the increased fat mass observed in this group.

Adiponectin and adiponectin receptor 1 gene expression were increased 9.9-fold and 10.4-fold respectively in deteriorators (compared with 3.9-fold and 2.9-fold respective in improvers). Adiponectin receptor expression has been shown to be increased in patients with impaired glucose tolerance and type 2 diabetes mellitus in comparison with normoglycaemic controls [35], and this is consistent with our data. However, there are also conflicting data in the published literature; adolescents with hepatic steatosis [36], glucose intolerance and insulin resistance have decreased adiponectin expression in SAT biopsies [37]. Within our study we did not measure circulating adiponectin levels and the functional importance of these changes in SAT gene expression remains to be determined.

The data from this study also provide evidence to suggest that changes in 11β-HSD2 activity may have a role to regulate blood pressure longitudinally in overweight and obese individuals. Genetic defects and pharmacological inhibition of 11β-HSD2 result in severe hypertension [38] and it is possible that a mild defect in 11β-HSD2 (as reflected by the observed increased F/E ratio) could play an important role; the mechanisms underlying this are not clear. Within the cohort, 23% (5/35 deteriorators & 10/30 improvers) were on anti-hypertensive medications, none of which are known to influence 11β-HSD2 activity.

Our study is not without limitations and the associations that we describe do not necessarily infer causality. We have only examined gene expression profiles in SAT and not VAT (nor muscle) and it is possible that the observed changes arise as a consequence, rather than a driver, of increased fat mass. In addition, our assessments of glucocorticoid metabolism are limited to the analysis of urinary steroid metabolites and SAT gene expression; we have not

assessed functional activity in adipose tissue (although published data has shown a relatively tight relationship between activity and expression [6,21,39]) nor looked at the dynamic generation of cortisol from cortisone. However, the urinary steroid metabolite profile suggested that there was no significant alteration in hepatic (and perhaps skeletal muscle) 11β-HSD1 activity / expression.

In conclusion, we have demonstrated that SAT 11β-HSD1 expression decreased with deterioration in glucose tolerance over time; this was accompanied by increased fat mass. It would therefore seem unlikely that dysregulation of local tissue-specific cortisol availability through the activity of 11β-HSD1 within adipose tissue is a primary mechanism driving adverse metabolic phenotype. The changes observed may represent a compensatory mechanism to limit local cortisol exposure as adiposity increases.

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Table 1. Metabolic and anthropometric characteristics in a cohort of overweight / obese individuals (n=65) at baseline and follow-up. Mean length of time to follow-up=3.9±1.5 years. Data are expressed as means ± SD.

Parameter	Baseline	Follow-up	*P*
Age (years)	50.3 ± 7.3	54.2 ± 7.6	**< 0.001**
Weight (kg)	94.9 ± 16.4	97.8 ± 17.7	**0.003**
BMI (kg/m^2)	33.7 ± 4.3	35.1 ± 5.0	**< 0.001**
Systolic BP (mmHg)	128.7 ± 17.9	134.2 ± 16.9	**0.03**
Diastolic BP (mmHg)	73.9 ± 11.4	78.1 ± 9.6	**0.01**
Waist circumference (cm)	107.6 ± 10.7	108.0 ± 13.2	0.8
Hip circumference (cm)	116.9 ± 9.2	116.5 ± 12.1	0.8
Waist / Hip Ratio	0.92 ± 0.07	0.93 0.08	0.2
Total cholesterol (mmol/L)	5.1 ± 1.0	5.1 ± 0.9	0.9
Triglycerides (mmol/L)	1.5 ± 1.1	1.5 0.7	0.9
Area under curve glucose (mmol/L.h)	14.9 ± 3.2	15.1 ± 3.8	0.5
Area under curve insulin (mu/L.h)	151.8 ± 83.8	145.8 ± 87.5	0.3
Fasting glucose (mmol/L)	4.8 ± 0.6	5.0 ± 0.7	0.07
Fasting insulin (mU/L)	9.8 ± 6.6	11.1 ± 6.2	0.2
HbA1c (mmol/mol) (%)	38 ± 7 (5.6 ± 0.6)	40 ± 8 (5.9 ± 0.7)	**<0.001**
Total body fat (g)	36377 ± 9311	37993 ± 10940	**0.004**
Trunk: peripheral fat	1.18 ± 0.27	1.22 ± 0.28	**0.03**
Trunk fat mass (g)	18682 ± 4674	19694 ± 5557	**0.008**
Total lean mass (g)	54402 ± 11835	55771 ± 11216	**0.03**
Trunk lean mass (g)	28607 ± 6186	28830 ± 5902	0.06

Table 2. Metabolic characteristics of the study participants stratified into *'improvers'* and *'deteriorators'* in glucose tolerance as assessed by area under the curve (AUC) analysis for glucose across a 75g oral glucose tolerance test. Data expressed as means ± SD. Quoted p-values represent within group baseline to follow-up comparisons. * = p<0.05 comparisons between *improvers* and *deteriorators* at baseline; † = p <0.05, †† = p < 0.005 comparisons between *deteriorators* and *improvers* at follow up. (NS=non-significant)

	AUC Glucose					
	Deteriorators (n=35)			**Improvers (n=30)**		
Parameter	**Baseline**	**Follow-up**	***P***	**Baseline**	**Follow-up**	***P***
Age (years)	50.6 ± 7.3	54.5 ± 7.7	**<0.0001**	50 ± 7.3	53.9 ± 7.6	**<0.0001**
Weight (Kg)	91.5 ± 17.1	97.5 ± 18.8	**<0.0001**	98.8 ± 14.9	98.1 ± 16.6	NS
BMI (kg/m^2)	33.5 ± 4.3	36.0 ± 4.9	**<0.0001**	34 ± 4.4	34 ± 5.1	NS
Systolic BP (mmHg)	128.1 ± 18.4	134.3 ± 17.2	NS	129.5 ± 17.7	134.1 ± 16.7	NS
Diastolic BP (mmHg)	73.3 ± 12.2	78.0 ± 10.0	**0.02**	74.5 ± 10.4	78.3 ± 9.3	NS
Waist circumference (cm)	106.3 ± 12.8	110.4 ± 15.0	**0.004**	109.1 ± 7.5	105.3 ± 10.3	**0.01**
Hip circumference (cm)	116.7 ± 8.7	118.5 ± 11.3 †	**0.04**	117.2 ± 10.0	114.2 ± 12.8 †	**0.04**
Waist / Hip Ratio	0.91 ± 0.08	0.94 ± 0.08	**0.01**	0.94 ± 0.07	0.92 ± 0.07	NS
Total Cholesterol (mmol/L)	4.9 ± 0.9	5.1 ± 1.1	NS	5.3 ± 1.1	5.0 ± 0.8	NS
Triglycerides (mmol/L)	1.4 ± 0.8	1.5 ± 0.6	NS	1.7 ± 1.3	1.5 ± 0.7	NS
AUC glucose(mmol/L/hour)	13.8 ± 3.0 **	16.1 ± 4.0 †	**<0.0001**	16.1 ± 3.0 **	14.0 ± 3.2 †	**<0.0001**
AUC insulin (mU/L/hour)	154.5 ± 90.2	171.6 ± 101 †	NS	148.6 ± 77.4	118.1 ± 60.7 †	**0.007**
Fasting glucose (mmol/L)	4.7 ± 0.7	5.1 ± 0.9	**0.002**	4.9 ± 0.4	4.9 ± 0.5	NS
Fasting insulin (mU/L)	9.5 ± 6.8	11.4 ± 6.1	**0.04**	10.1 ± 6.4	10.4 ± 6.6	NS

HbA1c (mmol/mol) (%)	38 ± 9 (5.6 ± 0.8)	42 ± 9 (6.0 ± 0.8)	**<0.0001**	38 ± 5 (5.6 ± 0.4)	39 ± 5 (5.7 ± 0.5)	NS
Total body fat (g)	36325 ± 8134	39588 ± 10190	**0.0005**	36436 ± 10676	36114 ± 11666	NS
Total lean (g)	50727 ± 14058 *	53761 ± 10797	**0.05**	59038 ± 11000 *	58141 ± 11429	NS
Trunk Fat (g)	18766 ± 4786	20753 ± 5713	**<0.0001**	18587 ± 4624	18447 ± 5192	NS
Trunk Lean (g)	27049 ± 6029 *	27858 ± 5711	**0.006**	30372 ± 5976 *	29975 ± 6020	NS

Table 3. Urinary corticosteroid metabolite analysis performed by gas chromatography/mass spectrometry on 24-h urine samples from participants at baseline and follow up (n=61). Results are expressed as medians with IQRs in parentheses and stratified according to change in area under the curve glucose across a 75g oral glucose tolerance test. P-values represent baseline to follow-up analysis within groups; there were no significant differences between groups either at baseline or follow-up.

Corticosteroid Metabolite (µg/24h)	***Deteriorators (n=32)***			***Improvers (n=29)***		
Parameter	**Baseline**	**Follow-up**	***P***	**Baseline**	**Follow-up**	***P***
THF (tetrahydrocortisol)	1780 (1133, 2723)	1307 (759, 1910)	**0.003**	1639 (1168, 2586)	1489 (902, 1943)	**0.001**
THE (tetrahydrocortisone)	2908 (1963, 5706)	2758 (1948, 3938)	**0.025**	3939 (2260, 5161)	3133 (1839, 3878)	**0.014**
5αTHF	1077 (735, 2476)	1061 (813, 1831)	0.2	1472 (924, 2187)	1168 (814, 1907)	**0.004**
Cortisone (E)	86 (58, 134)	63 (54, 87)	0.12	105 (57, 141)	76 (52, 98)	**0.01**
Cortisol (F)	54 (30, 91)	44 (31, 69)	0.8	54 (36, 96)	50 (34, 68)	0.132
F/E (cortisol/cortisone)	0.6 (0.5, 0.7)	0.7 (0.6, 0.8)	**0.005**	0.6 (0.5, 0.7)	0.7 (0.6, 0.8)	**0.006**
THF + 5αTHF/THE	0.9 (0.7, 1.1)	0.9 (0.7, 1.1)	0.69	0.9 (0.8, 1.1)	0.9 (0.8, 1.2)	0.83
Total F metabolites (Fm)	3969 (2380, 6089)	3495 (2298, 4588)	**0.04**	4061 (3219, 6929)	3702 (2730, 4964)	**0.001**
Total E metabolites (Em)	4991(3341, 8806)	5182 (3405, 6419)	**0.04**	6717 (4006, 8838)	5124 (3437, 6648)	**0.03**
Fm/Em	0.68 (0.56, 0.82)	0.67 (0.60, 0.85)	0.555	0.71 (0.64, 0.90)	0.71 (0.59, 0.86)	0.97
Glucocorticoids	8743 (6762, 14523)	8701 (5742, 11197)	**0.04**	11216 (7493,16183)	8879 (6248, 11952)	**0.01**
Mineralocorticoids	576 (298, 894)	493 (340, 623)	0.120	650 (463, 1003)	610 (377, 870)	0.066
5αTHF/THF	0.8 (0.5, 1.2)	0.9 (0.7, 1.4)	**0.0002**	0.9 (0.6, 1.1)	1.1 (0.6, 1.3)	**0.01**

Figure Legends

Figure 1. Urinary steroid metabolites and adipose tissue gene expression in obese / overweight individuals in whom glucose tolerance either improved (white bars) or deteriorated (black bars) over a mean duration of 3.9±1.5years. 11β-HSD1 activity as measured by urinary steroid metabolite ratio, THF+5αTHF/THE, did not change with improvement or deterioration in glucose tolerance (A). However, 11β-HSD1 mRNA expression in subcutaneous adipose tissue (SAT) decreased in those individuals in whom glucose tolerance worsened and was significantly lower than in patients in whom glucose tolerance improved (B). Change in SAT 11β-HSD1 mRNA expression negatively correlated with change in AUC glucose over time. Change in 11β-HSD1 expression over time is expressed as ddCT (baseline dCT minus the dCT at follow-up) (C). Glucocorticoid receptor expression and H6PDH were not differentially expressed (D and E) (* $p<0.05$). Data in figures B, D & E expressed as arbitrary units (AU), using the following transformation, [expression=$1000x(2^{-Ct})$].

Figure 2. The change in urinary steroid metabolite ratio of cortisol to cortisone (F/E) (follow-up minus baseline) significantly correlated with systolic blood pressure at follow-up (Pearson r=0.28, p=0.04) (A). The positive association between change in F/E ratio and change in systolic BP failed to reach statistical significance (Spearman r=0.25, p=0.06) (B).

Figure 3. Fold-change in mRNA expression of selected genes in subcutaneous adipose tissue biopsies taken from overweight / obese individuals in whom glucose tolerance either improved (white bars) or deteriorated (black bars) over a mean duration of 3.9±1.5 years. * $p<0.05$ baseline to follow up comparison of expression level within group.