

Impaired glucose tolerance and insulin resistance are associated with increased adipose 11 β -hydroxysteroid dehydrogenase type 1 expression and elevated hepatic 5 α -reductase activity

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Objective: The precise molecular mechanisms contributing to the development of insulin resistance, impaired glucose tolerance (IGT) and type 2 diabetes (T2DM) are largely unknown. Altered endogenous glucocorticoid (GC) metabolism, including 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) that generates active cortisol from cortisone, and 5 α -reductase (5 α R) that inactivates cortisol have been implicated.

Research Design and Methods: 101 obese patients (mean age 48 \pm 7years, BMI 34.4 \pm 4.3kg/m², 66 women, 35 men) underwent 75g oral glucose tolerance testing (OGTT), body composition analysis (DXA), assessment of GC metabolism (24h urine steroid metabolite analysis by GC/MS) and subcutaneous abdominal adipose tissue biopsies.

Results: 22.7% of women had IGT compared with 34.2% of men. 2 women and 5 men were diagnosed with T2DM. In women, adipose 11 β -HSD1 expression was increased in patients with IGT and correlated with glucose levels across the OGTT (R=0.44, p<0.001), but was independent of fat mass. Total GC secretion was higher in men with and without IGT (Normal: 13743 \pm 863 vs. 7453 \pm 469 μ g/24h, p<0.001; IGT: 16871 \pm 2113 vs. 10133 \pm 1488 μ g/24h, p<0.05) and in women was higher in patients with IGT (7453 \pm 469 vs. 10133 \pm 1488 μ g/24h, p<0.001). In both sexes, 5 α R activity correlated with fasting insulin (Men: R=0.53, p=0.003; Women: R=0.33, p=0.02), insulin secretion across an OGTT (Men: R=0.46, p=0.01; Women: R=0.40, p=0.004), and HOMA-R (Men: R=0.52, p=0.004; Women: R=0.33, p=0.02).

Conclusion: Increased adipose 11 β -HSD1 expression in women may contribute to glucose intolerance. Enhanced 5 α R activity in both sexes is associated with insulin resistance, but not body composition. Augmented GC inactivation may serve as compensatory, protective mechanism to preserve insulin sensitivity.

Despite the severe health consequences of obesity (1) and the alarming rate at which its prevalence is increasing (2), many questions remain unanswered with regards to its pathogenesis and etiology. Insulin resistance and obesity are tightly associated, yet the molecular mechanisms that underpin this relationship are unknown. Insulin resistance has been implicated as being central not only to the development of obesity itself, but also its complications including dyslipidaemia, thromboembolic disease and hypertension (3). Furthermore, it is a feature of many other clinical conditions including polycystic ovary syndrome (PCOS) where the degree of insulin resistance exceeds that observed in body composition matched controls (4). The association between insulin resistance and obesity almost certainly explains the dramatic relationship between obesity and type 2 diabetes (T2DM) (5;6). T2DM evolves from a state of insulin resistance with hyperinsulinemia necessary to maintain glucose tolerance. T2DM is manifest when insulin secretion fails to meet demands with consequent hyperglycemia and eventual pancreatic β -cell failure. Impaired glucose tolerance (IGT), defined as a 2h glucose concentration $>7.8\text{mmol/L}$ but $<11.1\text{mmol/L}$ after 75g oral glucose, significantly increases the risk of progression to T2DM (7) and although rates of progression to T2DM are variable, they may exceed 10% per year (8;9). Although the benefits of lifestyle intervention are clear (9), the molecular mechanisms that cause IGT and similarly the mechanisms by which IGT progresses to type 2 diabetes are largely unknown.

The role of endogenous glucocorticoids (GC) in the development of obesity and insulin resistance has been widely speculated based upon observation in patients with GC excess (Cushing's syndrome). These patients develop a classical phenotype including central obesity, insulin resistance and in some cases T2DM. However, simple obesity and insulin resistance are not caused by 'sub-clinical' endogenous GC excess; circulating cortisol levels in obese patients are not

elevated (10). Tissue specific GC metabolism however may be important. The enzyme 11β -hydroxysteroid dehydrogenase type 1 (11β -HSD1) is highly expressed in liver, adipose tissue and muscle and generates the active GC, cortisol from inactive cortisone and therefore amplifies local GC action (11). However, 11β -HSD1 is a bi-directional enzyme and is capable of inactivating cortisol to cortisone. Critically, the directionality of 11β -HSD1 activity is cofactor (NADPH) dependent which is supplied by a tightly associated endo-luminal enzyme, Hexose-6-phosphate dehydrogenase (H6PDH). Decreases in H6PDH expression and activity decrease oxo-reductase and increase dehydrogenase activity (12;13). Recently, much attention has focused on pharmacological manipulation of cortisol metabolism as a therapeutic strategy. Selective 11β -HSD1 inhibitors administered to rodents and primates decrease local GC generation, improve glucose tolerance, increase insulin sensitivity and may promote weight loss (14-17). In contrast to the action of 11β -HSD1, the A-ring reductases (5α -reductase type 1 and 2, (5α R1 and 2) and 5β -reductase) inactivate cortisol, decreasing local GC availability to bind and activate the GC receptor (GR). 5α R1 is expressed in skin and adipose tissue (18;19) and 5α R2 in prostate, epididymis and seminal vesicles; both isozymes are expressed in the liver (18).

Studies published to date have focussed principally on the role of cortisol metabolism in the pathogenesis of obesity rather than insulin resistance. We have performed a detailed clinical study in a large cohort of obese patients exploring the concept that cortisol metabolism is an important regulator of insulin sensitivity and that this may be independent of fat mass. We have hypothesised that decreased local GC availability (through decreased 11β -HSD1 and / or increased A-ring reductase activity) will represent a novel physiological adaptive response to preserve insulin sensitivity in a cohort of patients predisposed to the development of insulin resistance, IGT and T2DM.

RESEARCH DESIGN AND METHODS

The study was approved by South Birmingham Local Research Ethics Committee and all subjects gave their informed, written consent. 101 obese volunteers (35 male, 66 female, mean age \pm s.d. 48 \pm 7years, mean BMI 34.4 \pm 4.3kg/m²) were recruited following local advertisement and underwent the clinical protocol described below. Patients had no significant past medical history and none had received glucocorticoid therapy (oral, topical or inhaled) within the last 12 months. All patients had normal blood counts and renal function.

Subjects were investigated in the fasting state. Blood samples were drawn at 09.00h for measurement of total cholesterol, triglycerides, cortisol, cortisone, glucose, insulin and HbA1C. Measurements of BMI, waist circumference (measured supine, at the level of the umbilicus) and hip circumference (at the level of the greater trochanter) and blood pressure (average of three readings, measured supine after 10 minutes rest using Dynamap®, Critikon, Tampa, USA) were also taken. Patients underwent a standard 75g oral glucose tolerance test, with samples taken at 30-minute intervals for measurement of insulin and glucose for 120 minutes.

In the fasting state, all patients had an abdominal subcutaneous (sc) adipose tissue biopsy (10-15cm lateral to the umbilicus) performed under local anesthetic (1-2mL of 1% lidocaine), in order to obtain approximately 250-500mg of adipose tissue. The sample was immediately placed in RNALater® (Ambion inc., Austin, TX, USA) and after initial storage for 24h at room temperature was stored at -20°C for subsequent total RNA extraction, reverse transcription and real-time PCR analysis.

In addition, all patients performed a 24-hour urine collection for corticosteroid metabolite analysis (20). Body composition analysis was performed using dual energy X-ray absorptiometry (DXA) with a total body scanner (QDR 4500, Hologic inc., Bedford, MA). Coefficients of variation for multiple scans were less than 3%. Regional fat mass

(trunk and leg) was analyzed as previously described (21).

Biochemical assays. *Serum.* Blood counts, urea, creatinine and electrolytes, cholesterol, triglycerides, liver chemistry, glucose and HbA1C were measured using standard laboratory methods (Roche Modular system, Roche Ltd, Lewes, UK). Insulin was measured using a commercially available colorimetric ELISA (Mercodia, Uppsala, Sweden) with an in-house coefficient of variation of <5%. Cortisol was measured using a coat-a-count radio-immuno assay (Diagnostic Products Corporation, Los Angeles, CA) as per the manufacturers' guidelines. Cortisone was assayed after extraction from serum followed by radioimmunoassay (RIA) of the extract with ¹²⁵I-Cortisone and Sac-Cel® (IDS Ltd., Tyne and Weir, UK) second antibody separation (22). The coefficient of variation for 10 consecutive assays was less than 15% for values between 50 and 100nmol/L and less than 10% for values over 100nmol/L.

Calculated measures of pancreatic β -cell function and insulin resistance were made using the HOMA2 model (23). HOMA %B values provide an index of pancreatic islet β -cell function relative to a normal individual with a value set at 100%. Similarly, HOMA %S values represent a measure of insulin sensitivity relative to a normal individual, again with a value of 100%. The HOMA-R is a reciprocal measure of the %S values.

Urinary corticosteroid metabolites. Urinary corticosteroid metabolite analysis was performed by GC/MS as described previously (20). The sum of total cortisol metabolites (THF (tetrahydrocortisol), THE (tetrahydrocortisone), 5 α -THF, α -cortolone, cortisone (E), cortisol (F), β -cortolone, β -cortol, α -cortol) provides a reflection of cortisol secretion rate. The ratio of tetrahydro-metabolites of cortisol (THF + 5 α -THF) to those of cortisone (THE) provides a reflection of 11 β -HSD1 activity when considered with the ratio of urinary free cortisol (UFF) to cortisone (UFE) which more accurately reflects renal 11 β -HSD2 activity (20). In addition, the ratios of cortols:cortolones and

of (11 β -hydroxy-etiocholanolone + 11 β -hydroxy-androsterone)/11oxo-etiocholanolone also reflect 11 β -HSD1 activity (24). The activities of 5 α and 5 β -reductases can be inferred from measuring the ratio of 5 α THF/THF and androsterone/etiocholanolone.

RNA extraction and reverse transcription. Following sc adipose tissue biopsy, total RNA was extracted using a single step extraction method (Magmax™96, Applied Biosystems, Foster City, CA, USA) as per the manufacturer's guidelines. RNA integrity and quantity were assessed using a Nanodrop® spectrophotometer (Wilmington, DE, USA).

1 μ g of total RNA was initially denatured by heating to 70°C for 5 minutes. 30 units of avian myeloblastosis virus, 200ng of random primers, 20U ribonuclease inhibitor and 40nmol deoxy-NTPs with 5 \times reaction buffer were added to the RNA and the reverse transcriptase reaction carried out at 37°C for 1 hour. The reaction was terminated by heating the cDNA to 95°C for 5 minutes.

Real-time PCR (RT-PCR). mRNA levels of genes of interest were determined using an ABI 7500 sequence detection system (Perkin-Elmer Applied Biosystems, Warrington, UK). Reactions were performed in 25 μ l volumes on 96 well plates in reaction buffer containing 2 \times TaqMan Universal PCR Master mix (Applied Biosystems, Foster City, CA, USA). Probes and primers for all genes were supplied by applied biosystems 'assay on demand' (Applied Biosystems). All reactions were normalised against the house keeping gene 18S rRNA, provided as a preoptimized control probe.

Data were obtained as ct values (ct=cycle number at which logarithmic PCR plots cross a calculated threshold line) and used to determine Δ ct values (Δ ct = (ct of the target gene) – (ct of the housekeeping gene)). Data were expressed as arbitrary units using the following transformation [expression = $10^{5-(2^{-\Delta$ ct)} arbitrary units (AU)].

Statistical analysis. Data are presented as mean \pm s.e. unless otherwise stated. Area under the curve (AUC) analysis was performed using the trapezoidal method. For

comparison of single variables, t-tests have been used (or non-parametric equivalents where data were not normally distributed). 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, Inc. Point Richmond, CA).

RESULTS

101 (66 women and 35 men) volunteers were recruited into the study; 48 women (72.7%) and 17 men (48.6%) had normal fasting glucose levels and normal glucose tolerance. 2 women (3.0%) and 3 men (8.6%) had impaired fasting glucose (IFG) (>5.6mmol/L) and within this group, 2 men, but no women had IGT. IGT with normal fasting glucose concentrations were observed in 15 women (22.7%) and 10 men (28.6%). T2DM was diagnosed in 2 women (3.0%) and 5 men (14.3%) and all had impaired fasting glucose.

Taking into account the sexual dimorphism described in body composition and GC metabolite production rates, subsequent analysis was performed separately in men and women.

Normal vs. impaired glucose tolerance / impaired fasting glucose. IGT was associated with increased waist circumference (103 \pm 1.3 vs. 111 \pm 2cm, p <0.002) and waist:hip ratio (WHR) (0.87 \pm 0.01 vs. 0.90 \pm 0.01, p <0.05) in women, but not men (waist circ: 114 \pm 3 vs. 116 \pm 3cm, p =0.57; WHR: 0.98 \pm 0.02 vs. 0.99 \pm 0.01, p =0.46).

HbA1C was increased in both men and women with IGT although this only reached statistical significance in men (men: 5.3 \pm 0.1 vs. 5.9 \pm 0.3%, p <0.05; women: 5.4 \pm 0.1 vs. 5.7 \pm 0.1%, p =0.08). Surprisingly, measures of insulin resistance (HOMA-R, fasting and 2h insulin concentrations) were not different between groups (table 1). Furthermore, body composition analysis by DXA failed to show differences in fat free mass or total or regional fat mass between those patients with normal or IGT (table 1). Although men and women were not different in terms of insulin

resistance (as measured by HOMA, fasting insulin, 2h insulin or insulin AUC across the 75g OGTT), % trunk fat and absolute total and trunk fat mass were higher in women with correspondingly lower fat free masses (Table 1).

Urinary steroid metabolite analysis by GC/MS demonstrated clear sexual dimorphism with increased total and individual GC metabolite production rates in men compared with women, with and without IGT (Table 2). When analysed according to glucose tolerance, total GC production rates were higher in women with IGT compared with normal glucose tolerance (10133 ± 1488 (IGT) vs. 7453 ± 469 (normal), $p < 0.05$), but this was not observed in men (16871 ± 2113 (IGT) vs. 13743 ± 863 (normal), $p = 0.17$). Furthermore, there were no differences in absolute production rates for individual metabolites or activities of metabolizing enzymes (in particular 11β -HSD1 or 5α R) when comparing normal and IGT (Table 2).

Adipose tissue biopsy gene expression. Successful isolation of total RNA and generation of cDNA from sc adipose tissue biopsies was achieved in 90 volunteers. 11β -HSD1, H6PDH, GR and 5α R1 were expressed in all samples. In contrast, 5α R2 was only expressed in 5/90 samples and only at very low levels (data not shown).

11β -HSD1, GR and 5α R1 mRNA expression levels were similar in men and women, however, H6PDH expression was more highly expressed in biopsies from male volunteers (2.3 ± 0.4 vs. 1.6 ± 0.5 arbitrary units (AU), $p < 0.05$).

In women only, adipose tissue 11β -HSD1 expression was higher in those with IGT compared to those with normal glucose tolerance (0.5 ± 0.06 vs. 0.29 ± 0.03 AU, $p < 0.005$) despite fat mass (total and regional) being similar between the 2 groups (figure 1A); Moreover, expression correlated with glucose AUC across the OGTT ($R = 0.44$, $p < 0.005$) (figure 1B), but not with fat mass (total fat mass, $R = 0.12$, $p = \text{ns}$; trunk fat mass, $R = 0.15$, $p = \text{ns}$). H6PDH expression was lower in female volunteers with IGT (0.79 ± 0.1 vs. 1.38 ± 0.14 AU, $p < 0.05$), but GR and 5α R1 expression did not differ (Figure 1A).

In contrast to our findings in women, expression of 11β -HSD1, H6PDH, GR and 5α R1 in adipose tissue biopsies from men did not differ between those with and without IGT and did not relate to glucose levels across an OGTT (Figures 1C and D).

Regression analyses. Univariate regression analysis revealed positive relationships between 5α R activity (as measured by both the urinary androsterone/etiocholanolone and 5α THF/THF ratios) and markers of insulin resistance. In women, significant correlations were observed with fasting insulin, 2h-insulin levels after 75g oral glucose, AUC insulin across the OGTT and HOMA-R (Table 3, Figure 2 A-D). In men, a similar pattern was observed (Table 3, Figure 2 A-D), but in addition, increased 5α R activity was associated with decreased pancreatic β -cell function as calculated from the HOMA %B (Table 3).

5α R activity increased with increasing total and regional fat and fat free mass in men, but not in women (Table 3). Whilst these relationships remained significant, they were weaker than those observed with markers of insulin resistance. There was no relationship in either sex with fat distribution as measured by waist circumference, WHR or the trunk/limb fat ratio on DEXA.

Total GC secretion rate increased with total and regional fat and fat free mass in women but not in men and was weakly associated with 2h insulin concentration after 75g oral glucose (Table 4). Global 11β -HSD1 activity as measured by the (5α THF+THF)/THE ratio was unrelated to metabolic or anthropometric variables in women and only weakly related to HOMA-R in men ($R = 0.38$, $p = 0.04$). 11β -HSD2 inactivates cortisol to cortisone in mineralocorticoid target tissues, notably the kidney. Activity is reflected in the ratio of urinary free cortisol to cortisone (UFF:UFE). This was unrelated to metabolic parameters with the exception of 2h-insulin levels in men only ($R = 0.389$, $p = 0.03$), and inversely related to WHR in women only ($R = -0.30$, $p = 0.04$).

Using multivariate modelling to correct for total and regional fat mass, 5 α R activity remained an independent predictor of insulin secretion across the OGTT (2h-insulin $p < 0.005$; insulin AUC $p < 0.005$) in women. In men, 5 α R activity and not fat mass independently predicted fasting insulin levels ($p < 0.05$), HOMA %B ($p < 0.05$), HOMA %S ($p < 0.05$) and HOMA-R ($p < 0.05$).

DISCUSSION

In this unselected cohort of obese patients, we have identified the prevalence of IGT to be 22.7% in women and 34.2% in men and T2DM to be 3.0% in women and 14.3% in men. This was associated with central fat distribution (but not absolute fat mass) in women and unrelated to fat mass or distribution in men.

In this cohort sc adipose tissue 11 β -HSD1 mRNA expression was higher in women with IGT and correlated with glucose secretion across the OGTT, but was unrelated to fat mass, consistent with our previous observations (25). Previous studies have examined 11 β -HSD1 mRNA expression and activity in adipose tissue in the context of fat mass with variable results. Within the sc depot, most studies, although not all have suggested increased expression with indices of obesity (25-29), but relative expression within the intra-abdominal depot remains controversial. Importantly, the expression of 11 β -HSD1 in adipose tissue and its relationship with insulin resistance and glucose tolerance has not been examined before. Whilst expression studies were only performed in sc adipose tissue, enhanced cortisol generation through increased 11 β -HSD1 mRNA expression may contribute to glucose intolerance through increased lipolysis (30;31) and generation of free fatty acids which impair peripheral glucose uptake (32). However, due to the small volumes of tissue obtained, activity studies were not performed and it is possible that decreased H6PDH expression that we also observed in the IGT group may decrease 11 β -HSD1 oxo-reductase activity by limiting cofactor supply. Sexually dimorphic activity and expression of 11 β -HSD1 has been described in some

studies (33), but not all (34). In our cohort, urinary GC/MS analysis was similar in men and women. Studies examining sex specific regulation of expression within adipose tissue are lacking. In non-adipose tissue, estradiol has been shown to regulate expression levels and it is possible that this may contribute to our observations, however the published data are often contradictory with studies showing both increased and decreased expression (35-38). With the exception of DHEA (39), there is little evidence to support a role for regulation by androgens (35;38). Sexually dimorphic expression of H6PDH has not been described previously and the observed increase in expression in men will need to be endorsed with dedicated activity studies to see if changes in H6PDH expression translate into alterations in GC availability. However, it is possible that this could contribute to differences in fat distribution between men and women with the consequent increase in cardiovascular risk (40).

In our study, in both sexes 5 α R activity as measured by urinary GC metabolite ratios increased with indices of insulin resistance, an observation that was independent of fat mass. The role of 5 α R in the control of body composition and insulin sensitivity has not been explored in detail in the published literature. The global measures of activity that we have used in this study do not allow us to distinguish between 5 α R1 and 2, and whilst patients with 5 α R2 mutations have clear evidence of abnormal hepatic cortisol metabolism (41), the contribution of 5 α R1 is yet to be clarified. Sexual dimorphism of 5 α R activity has been described with increased activity in males (10;34). In our study, absolute levels of 5 α and 5 β -reduced metabolites were higher in men (as was total GC production rate), but the relative balance of 5 α R and 5 β R activity was not different. Furthermore, 5 α R1 mRNA expression in sc adipose tissue was similar in men and women. Studies in small cohorts of patients have shown enhanced 5 α R activity with obesity (42) and T2DM (43) and regulation of 5 α R and 5 β R activity by dietary macronutrient composition; high-fat, low-carbohydrate and

moderate-fat, moderate-carbohydrate diets decrease 5 α R and 5 β R activity in the context of weight loss (44). Thiazolidinedione treatment of obese Zucker rats decreases 5 α R1 expression in the liver (45), but similar experiments in humans have not been performed. However, we have recently shown that following weight loss with consequent insulin sensitization, 5 α R activity decreased (46). 5 α R has been implicated in the pathogenesis of the insulin resistance associated with PCOS in that patients with PCOS are more insulin resistant compared with body composition matched controls (4) and have increased 5 α R activity (47). Moreover, within a cohort of patients with PCOS, 5 α R activity correlates with insulin resistance (48). Our findings are consistent with these data and extend this observation to healthy obese women and men, and demonstrate that the relationship (in both sexes) is independent of fat mass. In the context of insulin resistance, we postulate that increased 5 α R activity represents a compensatory mechanism to decrease local GC availability and occurs in conjunction with the decrease in 11 β -HSD1 activity (as evidenced by reduced cortisol generation following oral cortisone administration and decreased urinary 5 α -THF/THE ratios) that we and others have previously described in obesity (22;26). These processes act together (decreased generation of cortisol through a reduction in 11 β -HSD1 and increased inactivation of cortisol through enhanced 5 α R activity) to decrease local GC availability and decrease GR activation specifically within the liver with the aim of maintenance of hepatic insulin sensitivity. A further impact of these changes will be to drive the HPA axis in order to maintain circulating cortisol levels. Whilst we believe that the changes that we have observed largely reflect hepatic GC metabolism, a direct effect upon insulin secretion can not be excluded. However, this remains highly speculative bearing in mind that evidence of

A-ring reductase expression in human pancreatic islets is lacking.

Pharmacological manipulation of GC availability through pre-receptor metabolism adds credence to our observations. Selective 11 β -HSD1 inhibitors that decrease local GC availability remain an exciting therapeutic prospect. Significant improvements in insulin sensitivity, glucose tolerance and lipid profiles following short-term administration in rodents and primates have been observed. Human clinical studies are still eagerly awaited. Conversely, Finasteride (5 α R2 inhibitor) and Dutasteride (combined 5 α R1 and 2 inhibitor) are widely used in the treatment of prostatic hypertrophy (49) based upon their action upon androgen metabolism. However, by decreasing GC inactivation they may have a detrimental impact upon insulin sensitivity, but the studies to address this question have not been performed.

In conclusion, this study adds to the growing body of evidence that supports a role for 5 α R in the control of insulin sensitivity in simple obesity. Whilst little is currently known about the regulation of 5 α R activity, limiting local GC availability through enhanced 5 α R and / or inhibition of 11 β -HSD1 activity may have significant clinical benefits. Finally, prospective studies examining the changes in activity and expression of these enzymes over time may begin to shed light on their potential role in the development of IGT and T2DM.

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Table 1. Clinical and serum biochemical characteristics of obese men and women with normal or impaired glucose tolerance (mean±s.d.) (*p<0.05, **p<0.01, ***p<0.0001 vs. women)

	Women			Men		
	Normal (n=48)	IGT (n=15)	p	Normal (n=17)	IGT (n=12)	p
Age (years)	48±7	46±8	0.39	48±8	53±8	0.11
Systolic BP (mmHg)	124±17	127±12	0.52	135±16*	139±22	0.60
Diastolic BP (mmHg)	72±11	76±12	0.28	80±14*	79±10	0.88
Anthropometry						
BMI (kg/m ²)	34.3±4.4	36.4±3.8	0.09	33.4±5.1	34.7±3.9	0.47
Waist circ (cm)	103±9.0	111±9	0.002	114±13	116±10	0.57
WHR	0.87±0.05	0.90±0.05	0.05	0.98±0.07***	0.99±0.04***	0.46
Serum biochemistry						
Fasting glucose (mmol/L)	4.6±0.6	5.0±0.4	0.02	4.8±0.5	5.4±0.6*	0.009
2-hour glucose (mmol/L)	6.0±1.0	8.8±0.9	<0.001	5.4±1.4*	9.2±1.5	<0.001
Fasting insulin (IU/L)	10.0±7.2	12.3±6.3	0.27	8.7±3.9	12.1±7.2	0.12
2-hour insulin (IU/L)	97.8±66.0	128±58.0	0.12	52.0±40.9*	97.6±56.7	0.02
AUC insulin (IU/L.h)	167.6±91.8	173.7±67.6	0.81	162.6±75.5	158.9±107.6	0.92
HbA1C (%)	5.4±0.5	5.7±0.4	0.08	5.3±0.3	5.9±0.9	0.03
HOMA %B	141.6±52.2	143.5 ±52.6	0.90	119.2 ±32.6	118.9 ±44.9	0.98
HOMA %S	104.6±71.5	78.2 ±61.8	0.20	101.5 ±60.1	75.7 ±42.1	0.20
HOMA-R	1.5 ±1.0	1.8 ±0.9	0.25	1.3 ±0.6	1.3 ±1.1	0.10
Total cholesterol (mmol/L)	5.0±1.0	5.3±1.0	0.29	5.3±0.9	5.0±0.7	0.48
Triglycerides (mmol/L)	1.3±0.6	1.6±0.6	0.11	2.0±1.8*	1.7±0.8	0.57
Cortisol (nmol/L)	216±103	204±102	0.70	320±163**	239±80	0.12
Cortisone (nmol/L)	64±26	55±22	0.30	66±20	73±19*	0.42
Body composition (DXA)						
Trunk fat mass (kg)	19.5±5.0	21.7±4.3	0.14	16.8±4.7	19.2±4.8	0.22
Trunk fat free mass (kg)	25.2±3.2	26.1±4.3	0.39	34.6±4.4***	35.7±3.9***	0.50
Total fat mass (kg)	38.8±9.2	41.7±9.3	0.30	29.9±7.7***	34.4±8.9**	0.17
Total fat free mass (kg)	48.6±5.7	50.2±8.4	0.39	66.9±8.1***	68.7±7.3***	0.54
Total % fat (%)	43.0±4.3	44.1±5.0	0.41	29.7±4.2***	32.2±5.6***	0.21
Trunk:limb fat ratio	1.1±0.2	1.2±0.3	0.09	1.4±0.14***	1.4±0.23	0.71

Table 2. Urinary corticosteroid metabolite analysis performed by GC/MS on 24hr urine samples from 73 obese volunteers divided by sex and glucose tolerance (mean±s.d.). (THE=tetrahydrocortisone, THF=tetrahydrocortisol, UFF=urinary free cortisol, UFE = urinary free cortisone, 11OH-androst=11hydroxyandrosterone, 11OH-etio=11hydroxyetiocholanolone, 11oxo-etio=11oxo-etiocholanolone, Fm=cortisol+THF+5αTHF+α-cortol+β-cortol, Em=cortisone+THE+α-cortolone+β-cortolone) (*p<0.05, **p<0.01, ***p<0.0001 vs. women)

	Women			Men		
	Normal (n=35)	IGT (n=11)	p	Normal (n=14)	IGT (n=12)	p
Corticosteroid metabolite production (µg/24h)						
Total glucocorticoid metabolites	7453±2693	9964±4741	0.03	13743±3229***	16871±7619*	0.17
Total cortisol metabolites (Fm)	3088±1230	3933±2103	0.10	5481±1484***	6679±2911*	0.19
Total cortisone metabolites (Em)	4364±1697	6031±2702	0.02	8263±1971***	10192±4833*	0.18
THF	1440±958	1756±910	0.32	2387±481**	2757±1346*	0.34
5αTHF	1087±597	1382±929	0.21	1997±1176**	2553±1395*	0.27
THE	2777±1670	3677±1720	0.12	5183±1562***	6274±3421*	0.29
UFF	56±76	48±22	0.74	80±29	98±40**	0.20
UFE	79±37	83±35	0.72	127±43***	152±71**	0.27
α-cortol	303±155	351±179	0.37	415±112*	535±185*	0.05
β-cortol	379±183	395±157	0.79	603±232**	737±366**	0.26
α-cortolone	1243±516	1612±729	0.06	1908±534***	2499±830**	0.04
β-cortolone	524±278	659±328	0.17	1046±280***	1267±697*	0.28
Androsterone	1054±746	1371±1009	0.25	2246±900***	2602±2169	0.58
Etiocholanolone	1020±742	1192±737	0.49	2075±1081***	2296±1999	0.72
Corticosteroid metabolite ratios						
UFF/UFE	0.64±0.53	0.63 ±0.25	0.93	0.63±0.11	0.66±0.13	0.48
(THF+ 5αTHF)/THF	0.98±0.39	0.84±0.18	0.24	0.86±0.16	0.87±0.19	0.88
Cortols/cortolones	0.39±0.1	0.33±0.05	0.06	0.35±0.07	0.34±0.09	0.94
(11OH-and + 11OH-etio)/11oxo-etio	3.17±2.0	3.38±1.87	0.76	3.23±1.18	2.83±1.29	0.41
Fm/Em	0.73±0.23	0.64±0.12	0.21	0.67±0.12	0.66±0.12	0.92
Androsterone / etiocholanolone	1.15±0.55	1.14±0.43	0.99	1.27±0.58	1.24±0.47	0.89
5αTHF/THF	0.84±0.41	0.76±0.26	0.53	0.84±0.45	0.97±0.41	0.47

Table 3. Correlation of metabolic parameters with 5 α -reductase activity as assessed by the urinary 5 α THF/THF and androsterone/etiocholanolone ratios analysed by GC/MS.

	Women (n=49)				Men (n=30)			
	Androsterone / Etiocholanolone		5 α THF/THF		Androsterone / Etiocholanolone		5 α THF/THF	
	Correlation coefficient (R)	p	Correlation coefficient (R)	p	Correlation coefficient (R)	p	Correlation coefficient (R)	p
<i>Insulin sensitivity and glucose tolerance</i>								
Fasting glucose	0.082	0.58	0.065	0.66	0.26	0.17	0.22	0.26
2-hour glucose	0.14	0.35	0.13	0.37	0.13	0.51	0.81	0.67
Fasting insulin	0.33	0.022	0.27	0.07	0.53	0.003	0.50	0.006
2-hour insulin	0.51	0.0002	0.51	0.0002	0.14	0.48	0.35	0.06
AUC insulin	0.38	0.007	0.40	0.004	0.31	0.10	0.46	0.01
HBA1C	-0.11	0.47	-0.09	0.58	0.39	0.04	0.14	0.47
HOMA %B	0.26	0.07	0.22	0.13	0.47	0.01	0.55	0.003
HOMA %S	-0.27	0.06	-0.23	0.11	-0.43	0.02	-0.53	0.003
HOMA-R	0.33	0.02	0.26	0.07	0.52	0.004	0.49	0.008
<i>Anthropometry</i>								
BMI	0.14	0.35	-0.02	0.87	0.28	0.13	0.32	0.08
Waist circ.	0.15	0.33	0.02	0.90	0.03	0.89	0.14	0.46
WHR	0.09	0.56	0.16	0.27	0.09	0.62	0.05	0.81
<i>Body composition (DXA)</i>								
Trunk fat mass	0.06	0.67	-0.04	0.78	0.44	0.02	0.46	0.01
Trunk fat free mass	0.11	0.45	-0.006	0.97	0.41	0.03	0.41	0.03
Total fat mass	0.02	0.90	-0.10	0.51	0.36	0.06	0.38	0.04
Total fat free mass	0.09	0.56	-0.02	0.87	0.493	0.008	0.43	0.02
Total % fat	-0.02	0.89	-0.07	0.62	0.18	0.37	0.24	0.23
Trunk:limb fat ratio	0.10	0.51	0.10	0.52	0.27	0.17	0.26	0.18

Table 4. Correlation of metabolic parameters with glucocorticoid (GC) secretion rate and 11 β -HSD1 activity (as measured by the urinary 5 α THF+THF/THF ratio) analysed by GC/MS.

	Women (n=49)				Men (n=30)			
	Total GC secretion rate		(5 α THF+THF)/THF		Total GC secretion rate		(5 α THF+THF)/THF	
	Correlation coefficient (R)	p	Correlation coefficient (R)	p	Correlation coefficient (R)	p	Correlation coefficient (R)	p
<i>Insulin sensitivity and glucose tolerance</i>								
Fasting glucose	0.06	0.70	-0.05	0.74	0.07	0.73	0.28	0.14
2-hour glucose	0.22	0.15	-0.01	0.97	-0.10	0.59	0.34	0.07
Fasting insulin	0.25	0.09	-0.01	0.95	0.04	0.83	0.37	0.05
2-hour insulin	0.34	0.02	0.07	0.64	0.12	0.53	0.03	0.88
AUC insulin	0.17	0.25	-0.08	0.61	0.07	0.70	0.01	0.96
HBA1C	-0.00	1.0	-0.12	0.47	-0.16	0.43	0.45	0.17
HOMA %B	0.19	0.20	-0.04	0.78	0.08	0.67	0.22	0.26
HOMA %S	-0.18	0.24	0.10	0.49	-0.16	0.42	-0.35	0.06
HOMA-R	0.25	0.09	-0.00	0.98	0.04	0.85	0.38	0.04
<i>Anthropometry</i>								
BMI	0.32	0.03	0.13	0.38	0.21	0.28	0.14	0.46
Waist circ.	0.32	0.03	-0.03	0.87	0.07	0.73	0.28	0.14
WHR	-0.04	0.70	-0.18	0.23	-0.05	0.78	-0.02	0.92
<i>Body composition (DXA)</i>								
Trunk fat mass	0.44	0.002	0.05	0.76	0.22	0.27	0.35	0.07
Trunk fat free mass	0.44	0.002	-0.06	0.68	0.28	0.15	0.33	0.09
Total fat mass	0.41	0.006	0.14	0.37	0.25	0.20	0.25	0.20
Total fat free mass	0.38	0.01	-0.06	0.67	0.12	0.56	0.31	0.11
Total % fat	0.19	0.20	0.21	0.16	0.23	0.23	0.14	0.49
Trunk:limb fat ratio	0.19	0.22	-0.23	0.13	-0.005	0.98	0.31	0.11

FIGURE LEGENDS

Figure 1. In women only, IGT is associated with increased adipose tissue 11 β -HSD1, but decreased H6PDH mRNA expression (A) without alteration in fat mass; 11 β -HSD1 mRNA levels correlate with glucose AUC across an OGTT (B). However, in men with IGT, adipose 11 β -HSD1, H6PDH, GR and 5 α R1 mRNA expression is not dysregulated (C and D) (normal glucose tolerance - open bars, IGT - black bars).

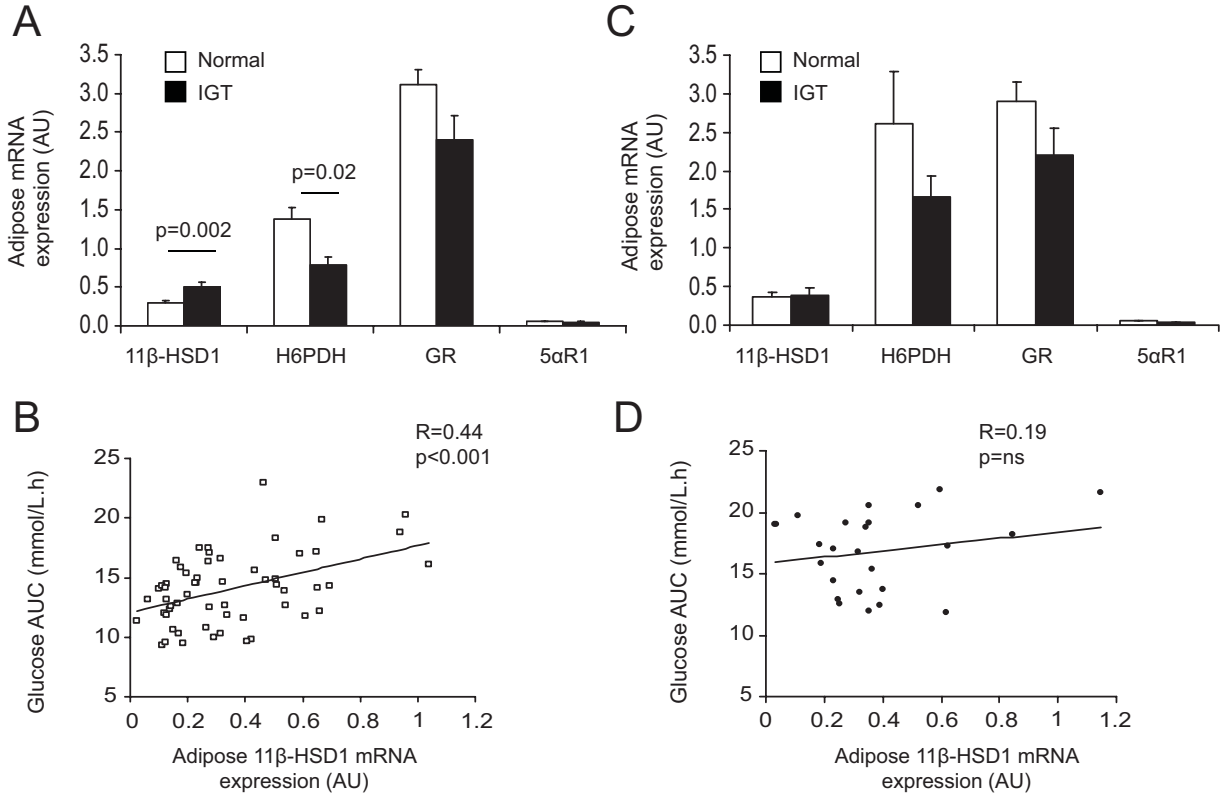


Figure 2. Increasing 5 α -reductase activity is associated with increased insulin secretion across an oral glucose tolerance test in men and women (A, fasting insulin; B, 2-hour insulin; C, area under curve insulin) and increased insulin resistance as measured by HOMA IR (D) (women are shown in the open squares and men in the filled circles).

