Impaired Glucose Tolerance and Insulin Resistance Are Associated With Increased Adipose 11β-Hydroxysteroid Dehydrogenase Type 1 Expression and Elevated Hepatic 5α-Reductase Activity

Jeremy W. Tomlinson,[1] Joanne Finney,[2] Christopher Gay,[1] Beverly A. Hughes,[1] Susan V. Hughes,[1] and Paul M. Stewart[1]

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

RESEARCH DESIGN AND METHODS—A total of 101 obese patients (mean age 48 ± 7 years, BMI 34.4 ± 4.3 kg/m^2, 66 women, 35 men) underwent 75-g oral glucose tolerance testing (OGTT), body composition analysis (dual-energy X-ray absorptiometry), assessment of glucocorticoid metabolism (24-h urine steroid metabolite analysis by gas chromatography/mass spectrometry), and subcutaneous abdominal adipose tissue biopsies.

RESULTS—A total of 22.7% of women had IGT compared with 34.2% of men. Two women and five men were diagnosed with type 2 diabetes. In women, adipose 11β-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 glucocorticoid secretion was higher in men with and without IGT (normal 13,743 ± 863 vs. 7,453 ± 469 μg/24 h, $P < 0.001$; IGT 16,871 ± 2,113 vs. 10,133 ± 1,488 μg/24 h, $P < 0.05$), and in women, it was higher in those with IGT (7,453 ± 469 vs. 10,133 ± 1,488 μg/24 h, $P < 0.001$). In both sexes, 5αR 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 homeostasis model assessment of insulin resistance (men $R = 0.52$, $P = 0.004$; women $R = 0.33$, $P = 0.02$).

CONCLUSIONS—Increased adipose 11β-HSD1 expression in women may contribute to glucose intolerance. Enhanced 5αR activity in both sexes is associated with insulin resistance but not body composition. Augmented glucocorticoid inactivation may serve as a compensatory, protective mechanism to preserve insulin sensitivity. ***Diabetes*** **57:2652–2660, 2008**

From the [1]Division of Medical Sciences, Institute of Biomedical Research, University of Birmingham, Queen Elizabeth Hospital, Edgbaston, Birmingham, U.K.; and the [2]Wellcome Trust Clinical Research Facility, University Hospitals Birmingham NHS Foundation Trust, Birmingham, U.K.

Corresponding author: Jeremy W. Tomlinson, j.w.tomlinson@bham.ac.uk.

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Despite the severe health consequences of obesity (1) and the alarming rate at which its prevalence is increasing (2), many questions remain unanswered with regard 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 central not only to the development of obesity itself but also its complications, including dyslipidemia, 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 control subjects (4). The association between insulin resistance and obesity almost certainly explains the dramatic relationship between obesity and type 2 diabetes (5,6). Type 2 diabetes evolves from a state of insulin resistance with hyperinsulinema necessary to maintain glucose tolerance. Type 2 diabetes 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 2-h glucose concentration >7.8 mmol/l but <11.1 mmol/l after 75-g oral glucose testing (OGTT), significantly increases the risk of progression to type 2 diabetes (7), and although rates of progression to type 2 diabetes 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 in the development of obesity and insulin resistance has been widely speculated on based upon observation in patients with glucocorticoid excess (Cushing's syndrome). These patients develop a classical phenotype including central obesity, insulin resistance, and in some cases type 2 diabetes. However, simple obesity and insulin resistance are not caused by subclinical endogenous glucocorticoid excess; circulating cortisol levels in obese patients are not elevated (10). Tissue-specific glucocorticoid 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 glucocorticoid cortisol from inactive cortisone and therefore amplifies local glucocorticoid action (11). However, 11β-HSD1 is a bidirectional enzyme and is

TABLE 1
Clinical and serum biochemical characteristics of obese men and women with normal or IGT

	Women			Men		
	Normal	IGT	*P*	Normal	IGT	*P*
n	48	15		17	12	
Age (years)	48 ± 7	46 ± 8	0.39	48 ± 8	53 ± 8	0.11
Systolic blood pressure (mmHg)	124 ± 17	127 ± 12	0.52	135 ± 16*	139 ± 22	0.60
Diastolic blood pressure (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 circumference (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-h 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-h 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^{-1} · h^{-1})	167.6 ± 91.8	173.7 ± 67.6	0.81	162.6 ± 75.5	158.9 ± 107.6	0.92
A1C (%)	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 (DEXA)						
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 fat–to–limb fat ratio	1.1 ± 0.2	1.2 ± 0.3	0.09	1.4 ± 0.14†	1.4 ± 0.23	0.71

Data are means ± SD unless otherwise indicated. *$P < 0.05$; †$P < 0.0001$; ‡$P < 0.01$ vs. women.

capable of inactivating cortisol to cortisone. Critically, the directionality of 11β-HSD1 activity is cofactor (NADPH) dependent, which is supplied by a tightly associated endolumenal enzyme, hexose-6-phopshate dehydrogenase (H6PDH). Decreases in H6PDH expression and activity decrease oxoreductase 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 glucocorticoid 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 [5αR1] and type 2 [5αR2] and 5β-reductase) inactivate cortisol, decreasing local glucocorticoid availability to bind and activate the glucocorticoid receptor (GR). 5αR1 is expressed in skin and adipose tissue (18,19) and 5αR2 in prostate, epididymis, and seminal vesicles; both isozymes are expressed in the liver (18).

Studies published to date have focused principally on the role of cortisol metabolism in the pathogenesis of obesity rather than that of 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 hypothesized that decreased local glucocorticoid 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 type 2 diabetes.

RESEARCH DESIGN AND METHODS

The study was approved by the South Birmingham Local Research Ethics Committee, and all subjects gave informed, written consent. A total of 101 obese volunteers (35 male, 66 female, mean age ± SD 48 ± 7 years, mean BMI 34.4 ± 4.3 kg/m^2) 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 9 A.M. for measurement of total cholesterol, triglycerides, cortisol, cortisone, glucose, insulin, and A1C. Measurements of BMI, waist circumference (measured supine, at the level of the umbilicus), hip circumference (at the level of the greater trochanter), and blood pressure (average of three readings, measured supine after 10 min rest using Dynamap [Critikon, Tampa, FL]) were also taken. Patients underwent a standard 75-g oral glucose tolerance test, with samples taken at 30-min intervals for 120 min for measurement of insulin and glucose.

In the fasting state, all patients had an abdominal subcutaneous adipose tissue biopsy (10–15 cm lateral to the umbilicus) performed under local anesthetic (1–2 ml of 1% lidocaine) in order to obtain ~250–500 mg of adipose tissue. The sample was immediately placed in RNALater (Ambion, Austin, TX) and, after initial storage for 24 h 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-h urine collection for corticosteroid metabolite analysis (20). Body composition analysis was performed using dual-energy X-ray absorptiometry (DEXA) with a total body scanner (QDR 45OO; Hologic, Bedford, MA). Coefficients of variation (CVs) for multiple

TABLE 2
Urinary corticosteroid metabolite analysis performed by GC/MS on 24-h urine samples from 73 obese volunteers divided by sex and glucose tolerance

	Women			Men		
	Normal	IGT	*P*	Normal	IGT	*P*
n	35	11		14	12	
Corticosteroid metabolite production (μg/24h)						
Total glucocorticoid metabolites	7,453 ± 2,693	9,964 ± 4,741	0.03	13,743 ± 3,229*	16,871 ± 7,619†	0.17
Total cortisol metabolites (Fm)	3,088 ± 1,230	3,933 ± 2,103	0.10	5,481 ± 1,484*	6,679 ± 2,911†	0.19
Total cortisone metabolites (Em)	4,364 ± 1,697	6,031 ± 2,702	0.02	8,263 ± 1,971*	10,192 ± 4,833†	0.18
THF	1,440 ± 958	1,756 ± 910	0.32	2,387 ± 481‡	2,757 ± 1,346†	0.34
5αTHF	1,087 ± 597	1,382 ± 929	0.21	1,997 ± 1,176‡	2,553 ± 1,395†	0.27
THE	2,777 ± 1,670	3,677 ± 1,720	0.12	5,183 ± 1,562*	6,274 ± 3,421†	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	1,243 ± 516	1,612 ± 729	0.06	1,908 ± 534*	2,499 ± 830‡	0.04
β-Cortolone	524 ± 278	659 ± 328	0.17	1,046 ± 280*	1,267 ± 697†	0.28
Androsterone	1,054 ± 746	1,371 ± 1,009	0.25	2,246 ± 900*	2,602 ± 2,169	0.58
Etiocholanolone	1,020 ± 742	1,192 ± 737	0.49	2,075 ± 1,081*	2,296 ± 1,999	0.72
Corticosteroid metabolite ratios						
UFF-to-UFE	0.64 ± 0.53	0.63 ± 0.25	0.93	0.63 ± 0.11	0.66 ± 0.13	0.48
(THF + 5αTHF)-to-THF	0.98 ± 0.39	0.84 ± 0.18	0.24	0.86 ± 0.0.16	0.87 ± 0.19	0.88
Cortols-to-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)–to–11oxo-etio	3.17 ± 2.0	3.38 ± 1.87	0.76	3.23 ± 1.18	2.83 ± 1.29	0.41
Fm-to-Em	0.73 ± 0.23	0.64 ± 0.12	0.21	0.67 ± 0.12	0.66 ± 0.12	0.92
Androsterone-to-etiocholanolone	1.15 ± 0.55	1.14 ± 0.43	0.99	1.27 ± 0.58	1.24 ± 0.47	0.89
5αTHF-to-THF	0.84 ± 0.41	0.76 ± 0.26	0.53	0.84 ± 0.45	0.97 ± 0.41	0.47

Data are means ± SD. UFF, urinary free cortisol; UFE, urinary free cortisone; 11OH-and, 11hydroxyandrosterone; 11OH-etio, 11hydroxyetiocholanolone; 11oxo-etio, 11oxo-etiocholanolone; Fm, cortisol + THF + 5αTHF + α-cortol + β-cortol; Em, cortisone + THE + α-cortolone + β-cortolone. *$P < 0.0001$; †$P < 0.05$; ‡$P < 0.01$ vs. women.

scans were <3%. Regional fat mass (trunk and leg) was analyzed as previously described (21).

Biochemical assays. Blood counts, urea, creatinine and electrolytes, cholesterol, triglycerides, liver chemistry, glucose, and A1C were measured using standard laboratory methods (Roche Modular system; Roche, Lewes, U.K.). Insulin was measured using a commercially available colorimetric ELISA (Mercodia, Uppsala, Sweden) with an in-house CV of <5%. Cortisol was measured using a coat-a-count radioimmunoassay (Diagnostic Products, Los Angeles, CA) as per the manufacturer's guidelines. Cortisone was assayed after extraction from serum followed by radioimmunoassay of the extract with ^{125}I-cortisone and Sac-Cel (IDS, Tyne and Weir, U.K.) second antibody separation (22). The CV for 10 consecutive assays was <15% for values between 50 and 100 nmol/l and <10% for values over 100 nmol/l.

Calculated measures of pancreatic β-cell function and insulin resistance were made using the homeostasis model assessment (HOMA) 2 model (23). The HOMA of β-cell function (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 of insulin sensitivity (HOMA %S) values represent a measure of insulin sensitivity relative to a normal individual, again with a value of 100%. The HOMA of insulin resistance is a reciprocal measure of the %S values.

Urinary corticosteroid metabolites. Urinary corticosteroid metabolite analysis was performed by gas chromatography/mass spectrometry (GC/MS) as described previously (20). The sum of total cortisol metabolites (tetrahydrocortisol [THF], tetrahydrocortisone [THE], 5α-THF, α-cortolone, cortisone [E], cortisol [F], β-cortolone, β-cortol, and α-cortol) provides a reflection of cortisol secretion rate. The ratio of tetrahydrometabolites of cortisol (THF + 5αTHF) to those of cortisone (THE) provides a reflection of 11β-HSD1 activity when considered with the ratio of urinary free cortisol to cortisone, which more accurately reflects renal 11β-HSD2 activity (20). The ratios of cortols to cortolones and of 11β-hydroxy-etiocholanolone and 11β-hydroxy-androsterone combined to 11oxo-etiocholanolone also reflect 11β-HSD1 activity (24). The activities of 5α- and 5β-reductases can be inferred from measuring the ratio of 5α THF to THF and androsterone to etiocholanolone.

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

One microgram of total RNA was initially denatured by heating to 70°C for 5 min. Thirty units of avian myeloblastososis virus, 200 ng of random primers, 20 units of ribonuclease inhibitor, and 40 nmol of deoxy–nucleoside triphosphates with 5× reaction buffer were added to the RNA, and the reverse transcriptase reaction was carried out at 37°C for 1 h. The reaction was terminated by heating the cDNA to 95°C for 5 min.

Real-time PCR. mRNA levels of genes of interest were determined using an ABI 7500 sequence detection system (Perkin-Elmer and Applied Biosystems, Warrington, U.K.). Reactions were performed in 25-μl volumes on 96-well plates in reaction buffer containing 2× TaqMan Universal PCR Master mix (Applied Biosystems, Foster City, CA). Probes and primers for all genes were supplied by assay on demand (Applied Biosystems). All reactions were normalized against the housekeeping gene 18S rRNA, provided as a preoptimized control probe.

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

Statistical analysis. Data are presented as means ± SE 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 nonparametric 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 SigmaStat 3.1 (Systat Software, Point Richmond, CA).

RESULTS

A total of 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. Two women (3.0%) and three men

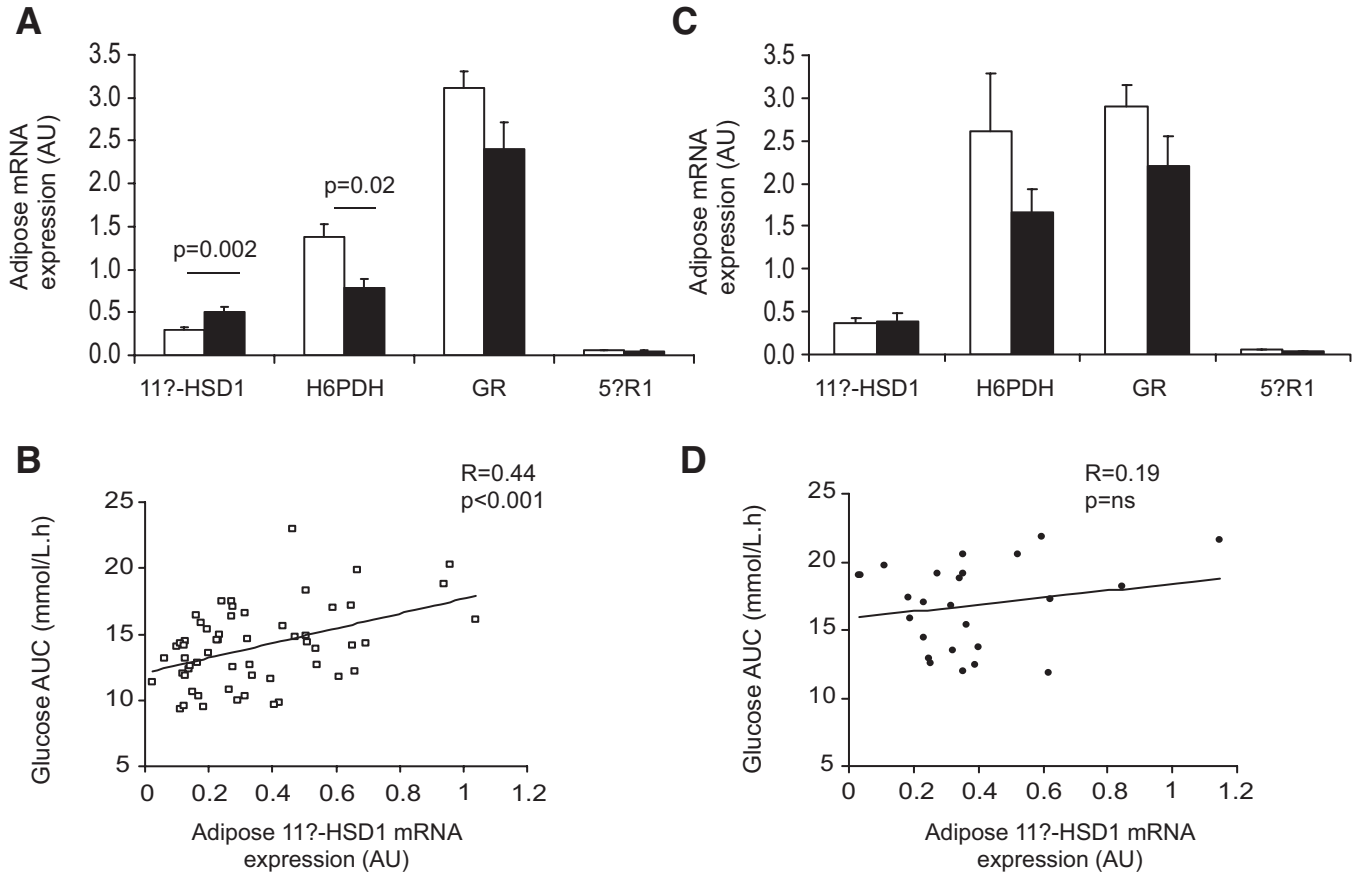

FIG. 1. In women only, IGT is associated with increased adipose tissue 11β-HSD1 but decreased H6PDH mRNA expression (*A*) without alteration in fat mass; 11β-HSD1 mRNA levels correlate with glucose AUC across an OGTT (*B*). However, in men with IGT, adipose 11β-HSD1, H6PDH, GR, and 5αR1 mRNA expression is not dysregulated (*C* and *D*). □, normal glucose tolerance; ■, IGT.

(8.6%) had impaired fasting glucose (IFG) (>5.6 mmol/l), and within this group, two men and no women had IGT. IGT with normal fasting glucose concentrations was observed in 15 women (22.7%) and 10 men (28.6%). Type 2 diabetes was diagnosed in two women (3.0%) and five men (14.3%), and all subjects with type 2 diabetes had IFG. Taking into account the sexual dimorphism described in body composition and glucocorticoid metabolite production rates, subsequent analysis was performed separately in men and women.

Normal glucose tolerance versus IGT and IFG. IGT was associated with increased waist circumference (103 ± 1.3 vs. 111 ± 2 cm, $P < 0.002$) and waist-to-hip ratio (WHR) (0.87 ± 0.01 vs. 0.90 ± 0.01, $P < 0.05$) in women but not in men (waist circumference 114 ± 3 vs. 116 ± 3 cm, $P = 0.57$; WHR 0.98 ± 0.02 vs. 0.99 ± 0.01, $P = 0.46$).

A1C was increased in both men and women with IGT, although this only reached statistical significance in men (men 5.3 ± 0.1 vs. 5.9 ± 0.3%, $P < 0.05$; women 5.4 ± 0.1 vs. 5.7 ± 0.1%, $P = 0.08$). Surprisingly, measures of insulin resistance (HOMA-R, fasting and 2-h insulin concentrations) were not different between groups (Table 1). Furthermore, body composition analysis by DEXA failed to show differences in fat-free mass or total or regional fat mass between those patients with normal glucose tolerance or IGT (Table 1). Although men and women were not different in terms of insulin resistance (as measured by HOMA, fasting insulin, 2-h insulin or insulin AUC across the 75-g OGTT, percent 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 glucocorticoid metabolite production rates in men compared with women for subjects with and without IGT (Table 2). When analyzed according to glucose tolerance, total glucocorticoid production rates were higher in women with IGT compared with women with normal glucose tolerance (10,133 ± 1,488 vs. 7,453 ± 469, respectively, $P < 0.05$), but this relationship was not observed in men (16,871 ± 2,113 vs. 13,743 ± 863, respectively, $P = 0.17$). Furthermore, there were no differences in absolute production rates for individual metabolites or activities of metabolizing enzymes (in particular 11β-HSD1 or 5αR) when comparing normal and IGT subjects (Table 2).

Adipose tissue biopsy gene expression. Successful isolation of total RNA and generation of cDNA from subcutaneous adipose tissue biopsies was achieved in 90 volunteers. All samples expressed 11β-HSD1, H6PDH, GR, and 5αR1. In contrast, 5αR2 was only expressed in 5 of 90 samples and only at very low levels (data not shown).

Whereas 11β-HSD1, GR, and 5αR1 mRNA expression levels were similar in men and women, H6PDH expression was higher in biopsies from male volunteers (2.3 ± 0.4 vs. 1.6 ± 0.5 AU, $P < 0.05$).

In women only, adipose tissue 11β-HSD1 expression was higher in those with IGT than in 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 two groups (Fig. 1*A*). Moreover, expression correlated with glucose AUC across the OGTT ($R = 0.44$, $P < 0.005$) (Fig. 1*B*) but not with fat mass (total fat mass

TABLE 3
Correlation of metabolic parameters with 5α-reductase activity as assessed by the urinary 5αTHF-to-THF and androsterone-to-etiocholanolone ratios analyzed by GC/MS

	Women (n = 49)				Men (n = 30)			
	Androsterone-to-etiocholanolone ratio		5αTHF-to-THF ratio		Androsterone-to-etiocholanolone ratio		5αTHF-to-THF ratio	
	Correlation coefficient (R)	P	Correlation coefficient (R)	P	Correlation coefficient (R)	P	Correlation coefficient (R)	P
Insulin sensitivity and glucose tolerance								
Fasting glucose	0.082	0.58	0.065	0.66	0.26	0.17	0.22	0.26
2-h 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-h 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
A1C	−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
Anthropometry								
BMI	0.14	0.35	−0.02	0.87	0.28	0.13	0.32	0.08
Waist circumference	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
Body composition (DEXA)								
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 fat–to–limb fat ratio	0.10	0.51	0.10	0.52	0.27	0.17	0.26	0.18

R = 0.12, P = NS; trunk fat mass R = 0.15, P = 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 5αR1 expression did not differ (Fig. 1*A*). In contrast to our findings in women, expression of 11β-HSD1, H6PDH, GR, and 5αR1 in adipose tissue biopsies from men did not differ between those with and those without IGT and did not relate to glucose levels across an OGTT (Fig. 1*C* and *D*).

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

Activity of 5αR increased with increasing total and regional fat and fat-free mass in men but not in women (Table 3). While 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 fat–to–limb fat ratio via DEXA.

Total glucocorticoid secretion rate increased with total and regional fat and fat-free mass in women but not in men and was weakly associated with 2-h insulin concentration after 75 g oral glucose (Table 4). Global 11β-HSD1 activity as measured by the 5α THF + THF–to–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. This was unrelated to metabolic parameters with the exception of 2-h 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 modeling to correct for total and regional fat mass, 5αR activity remained an independent predictor of insulin secretion across the OGTT (2-h insulin $P < 0.005$; insulin AUC $P < 0.005$) in women. In men, 5αR 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 the prevalence of type 2 diabetes 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, subcutaneous adipose tissue 11β-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 11β-HSD1 mRNA expression and activity in adipose tissue in the context of fat mass with variable results. Within the subcutaneous depot, most studies, although not all, have suggested increased expression with indexes of obesity (25–29), but relative expression within the intra-abdominal depot remains controversial. Importantly, the expression of 11β-HSD1 in adipose tissue and its relationship with

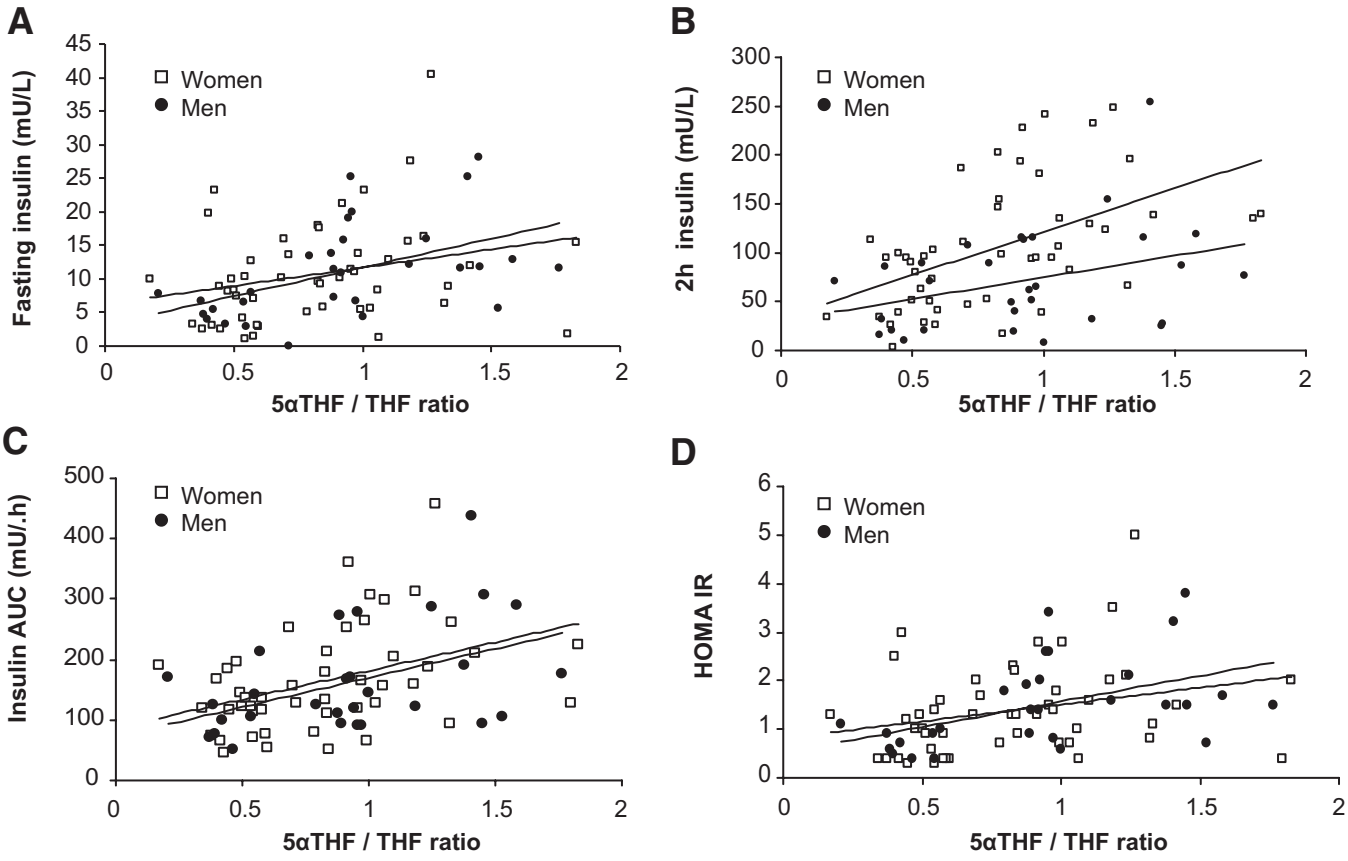

FIG. 2. Increasing 5α-reductase activity is associated with increased insulin secretion across an OGTT in men (●) and women (□)—fasting insulin (*A*), 2-h insulin (*B*), area under curve insulin (*C*), and increased insulin resistance as measured by HOMA-IR (*D*).

insulin resistance and glucose tolerance has not been examined before. While expression studies were only performed in subcutaneous adipose tissue, enhanced cortisol generation through increased 11β-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 11β-HSD1 oxo-reductase activity by limiting cofactor supply.

Sexually dimorphic activity and expression of 11β-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 nonadipose 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 dehydroepiandrosterone (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 glucocorticoid 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, 5αR activity as measured by urinary glucocorticoid metabolite ratios increased with indexes of insulin resistance, an observation that was independent of fat mass. The role of 5αR 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 5αR1 and 5αR2, and whereas patients with 5αR2 mutations have clear evidence of abnormal hepatic cortisol metabolism (41), the contribution of 5αR1 is yet to be clarified. Sexual dimorphism of 5αR activity has been described with increased activity in male subjects (10,34). In our study, absolute levels of 5α- and 5β-reduced metabolites were higher in men (as was total glucocorticoid production rate), but the relative balance of 5αR and 5βR activity was not different. Furthermore, 5αR1 mRNA expression in subcutaneous adipose tissue was similar in men and women. Studies in small cohorts of patients have shown enhanced 5αR activity with obesity (42) and type 2 diabetes (43) and regulation of 5αR and 5βR activity by dietary macronutrient composition; high-fat, low-carbohydrate and moderate-fat, moderate-carbohydrate diets decrease 5αR and 5βR activity in the context of weight loss (44). Thiazolidinedione treatment of obese Zücker rats decreases 5αR1 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, 5αR activity decreased (46). 5αR has been implicated in the pathogenesis of the insulin resistance associated with PCOS, in that patients with PCOS are more insulin resistant

TABLE 4
Correlation of metabolic parameters with glucocorticoid secretion rate and 11β-HSD1 activity (as measured by the urinary 5αTHF + THF–to–THE ratio) analyzed by GC/MS

	Women (n = 49)				Men (n = 30)			
	Total glucocorticoid secretion rate		(5αTHF + THF)-to-THF ratio		Total glucocorticoid secretion rate		(5αTHF + THF)-to-THF ratio	
	Correlation coefficient (R)	P	Correlation coefficient (R)	P	Correlation coefficient (R)	P	Correlation coefficient (R)	P
Insulin sensitivity and glucose tolerance								
Fasting glucose	0.06	0.70	−0.05	0.74	0.07	0.73	0.28	0.14
2-h 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-h 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
A1C	−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
Anthropometry								
BMI	0.32	0.03	0.13	0.38	0.21	0.28	0.14	0.46
Waist circumference	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
Body composition (DEXA)								
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	031	0.11
Total % fat	0.19	0.20	0.21	0.16	0.23	0.23	0.14	0.49
Trunk fat–to–limb fat ratio	0.19	0.22	−0.23	0.13	−0.005	0.98	0.31	0.11

compared with body composition–matched control subjects (4) and have increased 5αR activity (47). Moreover, within a cohort of patients with PCOS, 5αR activity correlates with insulin resistance (48). Our findings are consistent with these data and extend this observation to healthy obese women and men, as well as demonstrate that the relationship (in both sexes) is independent of fat mass. In the context of insulin resistance, we postulate that increased 5αR activity represents a compensatory mechanism to decrease local glucocorticoid availability and occurs in conjunction with the decrease in 11β-HSD1 activity (as evidenced by reduced cortisol generation following oral cortisone administration and decreased urinary 5α- + THF–to–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 11β-HSD1 and increased inactivation of cortisol through enhanced 5αR activity) to decrease local glucocorticoid 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 hypothalamo-pituitary-adrenal axis in order to maintain circulating cortisol levels. While we believe that the changes that we have observed largely reflect hepatic glucocorticoid metabolism, a direct effect upon insulin secretion cannot 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 glucocorticoid availability through prereceptor metabolism adds credence to our observations. Selective 11β-HSD1 inhibitors that decrease local glucocorticoid 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 (5αR2 inhibitor) and Dutasteride (combined 5αR1 and 5αR2 inhibitor) are widely used in the treatment of prostatic hypertrophy (49) based upon their action upon androgen metabolism. However, by decreasing glucocorticoid 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 5αR in the control of insulin sensitivity in simple obesity. While little is currently known about the regulation of 5αR activity, limiting local glucocorticoid availability through enhanced 5αR and/or inhibition of 11β-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 type 2 diabetes.

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