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Expression of 11β-hydroxysteroid dehydrogenase type 1 in visceral and subcutaneous adipose tissues of patients with polycystic ovary syndrome is associated with adiposity

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ABSTRACT

Polycystic ovary syndrome (PCOS) is characterized by insulin resistance (IR) and central obesity. The impact of adipose tissue cortisol reactivation by 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) on markers of obesity and IR was assessed in PCOS patients.

Eighty-five PCOS patients and 43 controls were enrolled for subcutaneous adipose tissue biopsy; 25/85 patients and 29/43 controls underwent also visceral adipose tissue biopsy. *HSD11B1* gene expression and expression of lipid metabolism genes were measured in subcutaneous and visceral adipose tissues. Anthropometric and biochemical markers of IR and PCOS were also assessed.

HSD11B1 expression in visceral and subcutaneous adipose tissue was increased in PCOS patients compared to controls ($p < 0.05$). After BMI adjustment, the difference was no longer significant. In PCOS patients, visceral *HSD11B1* expression correlated positively with waist circumference ($p = 0.001$), BMI ($p = 0.002$), plasma insulin ($p < 0.05$), systolic blood pressure ($p = 0.003$), and lipoprotein lipase (*LPL*), hormone-sensitive lipase (*LIPE*) and peroxisome-proliferator activated receptor γ gene expression. Subcutaneous *HSD11B1* expression correlated positively with BMI, waist circumference ($p < 0.001$ for both) and HOMA-IR ($p = 0.003$), and negatively with *LPL*, *LIPE*, adiponectin and glucose transporter GLUT4 gene expression. *HSD11B1* expression in both depots showed a negative correlation with plasma HDL-cholesterol ($p < 0.03$) and a positive one with C-reactive protein ($p < 0.001$). In multiple regression analysis, *HSD11B1* expression in visceral adipose tissue was most prominently associated with waist circumference, and that in subcutaneous adipose tissue with BMI ($p < 0.001$ for both).

Our results show that PCOS is not associated with increased *HSD11B1* expression once adiposity is controlled for. Increased expression of this gene correlates with markers of adiposity and predicts IR and an unfavorable metabolic profile, independently of PCOS.

1. Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous reproductive and metabolic disorder with a high prevalence of insulin resistance (IR) [1]. IR contributes to the pathogenesis of PCOS [2] and accounts for increased risk of type 2 diabetes and cardiovascular complications in these patients [1]. The central type of obesity which affects more than half of PCOS women seems to be an important factor in the development of IR and adverse metabolic consequences [3]. Nevertheless, IR can develop in lean PCOS patients, as well [4].

In the search for mechanisms implicated in the development of IR it has been hypothesized that increased cortisol activity in adipose tissue could be important [5]. The enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) interconverts glucocorticoids cortisone and cortisol in adipose and other tissues. *In vivo*, the reductase activity prevails, generating cortisol in an autocrine and paracrine manner (review in [6]). Various studies reported increased expression of 11β-HSD1 gene (*HSD11B1*) [7–10] and activity of this enzyme [11] in subcutaneous adipose tissue of obese people. However, there have been some controversies about the role of *HSD11B1* expression in visceral adipose tissue in obesity. Only few study groups demonstrated a positive correlation with obesity [8,12,13], while others that have studied both adipose depots found exclusive associations of subcutaneous adipose *HSD11B1* expression with obesity and IR [9,14–16].

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Studies from transgenic mice overexpressing the *HSD11B1* gene in adipose tissue revealed negative consequences of increased corticosteroid activity: visceral fat accumulation, IR, consequent hyperglycemia, and dyslipidemia [17]. Glucocorticoid receptor activation results in induction of various genes: the gene for lipoprotein lipase (*LPL*) [18], leptin [19] and angiotensinogen [20]. Glucocorticoids also stimulate lipolysis by inducing hormone-sensitive lipase gene expression [21] and activating the enzyme via cAMP [22].

The aim of this study was to explain the role of cortisol reactivation, assessed by *HSD11B1* expression, in metabolic derangements of PCOS patients. Therefore, *HSD11B1* expression was measured in subcutaneous and visceral adipose tissue in PCOS patients and healthy controls and correlated with anthropometric and metabolic markers. To elucidate the association of 11β-HSD1 with lipid metabolism and adipogenesis the expression of the following genes was also examined: the genes of lipid uptake (*LPL*) and lipolysis – hormone-sensitive lipase (*LIPE*), and an adipogenesis gene – peroxisome-proliferator activated receptor γ (*PPARG*). Additionally, gene expression of adiponectin (*ADIPOQ*) and glucose transporter GLUT4 (*SLC2A4*) were also analyzed.

2. Materials and methods

2.1. Subjects and experimental protocol

The study group consisted of 128 women. PCOS patients ($n = 85$) were recruited according to the National Institutes of Child Health and Human Development (NICHD) criteria [23], which better define the population of PCOS patients that are at higher risk for IR and cardio-metabolic complications. The PCOS patients had elevated plasma androgen levels or evidence of clinical hyperandrogenism. The latter was defined by the presence of hirsutism, represented by a Ferriman–Gallwey score of 7 or more, persistence of acne during the third decade of life or later, or the presence of androgenic alopecia. All patients had oligo- or amenorrhoea. They presented with normal serum prolactin concentrations and thyroid function; Cushing's disease and congenital adrenal hyperplasia were excluded. An additional exclusion criterion was the use of medications known or suspected to affect metabolic or reproductive functions, within 60 days prior to the study.

As controls, 43 healthy subjects, without clinical or laboratory evidence of PCOS were recruited from patients with a tubal factor of infertility, from the gynaecological department.

The study was conducted according to the Declaration of Helsinki and approved by the National Medical Ethics Committee. Subjects' written informed consents were obtained before entering the study.

Anthropometric and blood pressure measurements were done and fasting blood samples were drawn from all subjects for determination of glucose, insulin, androgen hormones, sex hormone-binding globulin (SHBG), plasma lipids and high sensitivity C-reactive protein (hsCRP). Subcutaneous adipose tissue samples were obtained from 60 patients and 14 controls by needle suction from the subumbilical abdominal region under local anesthesia with 2% lidocaine. In 25 PCOS patients and 29 controls, visceral adipose tissue from the omentum and subcutaneous adipose tissue from the abdominal wall were obtained during diagnostic or therapeutic laparoscopy performed for infertility reasons, under general anesthesia. In PCOS patients, laparoscopy was intended for ovarian electrocoagulation to enhance ovulation. In controls, laparoscopy was performed because of the tubal factor of infertility or unexplained infertility. Tissue samples were immediately frozen in liquid nitrogen and stored at −80 °C until analyzed.

Taken together, in 85 PCOS patients and 43 controls the subcutaneous adipose tissue was obtained, whereas in the subgroup of 25 PCOS patients and 29 controls, additionally, the visceral adipose tissue biopsy was done.

2.2. Assessment of biochemical markers and hormones

Glucose levels were assessed using a standard glucose oxidase method (Roche Hitachi 917, Roche, Mannheim, Germany). Plasma insulin was determined using a chemiluminescent immunoassay (Liaison Insulin, Diasorin, Salluggia, Italy). Androstenedione and dehydroepiandrosterone-sulphate (DHEAS) were measured by a specific double antibody radioimmunoassay using 125 I-labeled hormones (Diagnostic Systems Laboratories, Webster, TX, USA). Total and free testosterone levels were measured by a radioimmunoassay (DiaSorin and Siemens, Los Angeles, CA, USA, respectively). hsCRP and SHBG were assessed by a chemiluminescent immunoassay (Immulite, Siemens). Total cholesterol and triglyceride (TG) concentrations were measured by enzymatic–colorimetric methods; HDL-cholesterol was measured by a direct method (Roche Hitachi 917, Roche). LDL-cholesterol was determined by the Friedewald formula. Intraassay variations ranged from 1.6 to 6.3%, and interassay variations ranged from 5.8 to 9.6% for the applied methods.

The homeostasis model assessment (HOMA-IR) score was used for determining IR using the formula: fasting serum insulin (mU/L) × fasting plasma glucose (mmol/L)/22.5 [24].

2.3. RNA analysis

Total RNA from adipose tissue was isolated using RNeasy Lipid Tissue Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Quantity and quality of isolated RNA were determined by Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), respectively. Total RNA was transcribed to cDNA in 800 ng reaction using TaqMan Reverse Transcription reagents (Applied Biosystems, Foster City, CA, USA). Real-time PCR was performed on ABI PRISM 7000 Sequence Detection System (Applied Biosystems) using primers and probes of inventoried or pre-designed assays for the mRNAs of the following genes: *HSD11B1* (Hs00194153_m1), *LPL* (Hs00173425_m1), *LIPE* (Hs00943410_m1), PPARγ2 (*PPARG*, Hs01115510), *ADIPOQ* (Hs00605917_m1) and GLUT4 (*SLC2A4*, Hs00168966_m1, all from Applied Biosystems). Phosphoglycerate kinase 1 (TaqMan Endogenous Controls, Applied Biosystems) was used as a housekeeping gene. Each measurement was run in duplicate in 20 μl reaction mixture with 10 ng total RNA converted to cDNA. Quantification was done using a calibration curve. Expression of target genes was reported in arbitrary units (AUs) relative to the housekeeping gene expression.

2.4. Statistical procedures

Some parameters in control and PCOS groups were not normally distributed. To compare biochemical markers between patients and controls, the Mann–Whitney test was used. For other parametric tests, all data were log-transformed: some data to normalize their skewed distributions and those with normal distribution for competent comparison. After transformation, all data distributed normally. For comparison of gene expression between PCOS and control group the Student's *t*-test was used. ANCOVA was applied for comparison of gene expression between the groups with BMI adjustment. To test between-depot differences the paired Student's *t*-test was used. For correlation between *HSD11B1* gene expression and metabolic markers Pearson's correlation test

Table 1
Clinical and biochemical characteristics of PCOS patients and controls.

	PCOS ($n = 85$)	Controls ($n = 43$)	p^*
Age (years)	25 (21–29)	28 (24–31)	0.010
BMI (kg/m^2)	26.8 (22.4–33.1)	20.9 (19.7–23.1)	<0.001
Waist circumference (cm)	84.0 (76.5–101.0)	75.3 (70.3–79.0)	<0.001
Systolic BP (mm Hg)	120 (110–130)	115 (100–120)	0.008
Diastolic BP (mm Hg)	70 (70–80)	73 (68–80)	0.320
Fasting glucose (mmol/L)	4.6 (4.3–5.1)	4.9 (4.4–5.3)	0.111
Fasting insulin (mU/L)	11.5 (7.9–18.0)	7.3 (4.3–9.4)	<0.001
HOMA-IR	2.6 (1.3–4.0)	1.6 (1.1–2.1)	<0.001
Total cholesterol (mmol/L)	4.5 (4.0–5.3)	4.5 (3.9–4.8)	0.190
HDL-cholesterol (mmol/L)	1.3 (1.1–1.6)	1.7 (1.4–1.9)	<0.001
LDL-cholesterol (mmol/L)	2.7 (2.3–3.4)	2.3 (2.0–2.7)	0.003
TG (mmol/L)	0.9 (0.7–1.4)	0.7 (0.6–1.1)	0.051
hsCRP (mg/L)	1.19 (0.51–5.80)	0.48 (0.33–1.11)	0.004
Total testosterone (nmol/L)	2.4 (1.7–3.2)	1.4 (1.2–1.8)	<0.001
Free testosterone (pmol/L)	7.0 (4.8–11.1)	3.8 (2.6–4.3)	<0.001
SHBG (nmol/L)	36.0 (25.0–58.5)	62.0 (43.0–76.0)	<0.001
DHEAS (μmol/L)	6.2 (4.7–7.6)	4.4 (2.8–6.8)	<0.001
Androstenedione (nmol/L)	8.7 (6.7–10.7)	6.7 (5.5–8.9)	<0.001

Values represent medians (lower–upper quartile).
* Significance of difference in Mann–Whitney test; BMI, body mass index; BP, blood pressure; TG, triglycerides; hsCRP, high sensitivity C-reactive protein; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone-sulphate.

was done. The stepwise multiple linear regression analysis was used to identify independent anthropometric/biochemical parameters associated with *HSD11B1* expression in all participants of the study together. Data for gene expressions and anthropometric/biochemical parameters are shown as medians (lower–upper quartile). Statistical analyses were performed using SPSS software version 15.0 (Chicago, IL, USA). A p value ≤ 0.05 was considered statistically significant.

3. Results

3.1. HSD11B1 expression in PCOS patients and controls in subcutaneous and visceral adipose tissue

HSD11B1 expression in subcutaneous adipose tissue was measured in 128 subjects: 85 PCOS patients and 43 controls, of whom anthropometric, clinical and biochemical characteristics are summarized in Table 1. Expression of *HSD11B1* in visceral adipose tissue was measured in 54 subjects: 25 PCOS patients and 29 controls.

In PCOS patients, *HSD11B1* expression was higher as compared to controls, both in subcutaneous ($p = 0.046$) and visceral adipose tissue ($p = 0.028$), Fig. 1. After adjustment for BMI, *HSD11B1* expression in subcutaneous or visceral adipose tissue was no longer significantly different between PCOS patients and controls.

To compare the expression level of *HSD11B1* between visceral and subcutaneous adipose tissue within PCOS patients, a trend for higher expression in visceral than in subcutaneous adipose tissue was observed (1.07 (0.65–2.0) vs. 0.90 (0.76–1.48), respectively, $p = 0.063$, $n = 25$), whereas in controls there was no difference (data not shown). Only in PCOS patients, but not in controls, expression of *HSD11B1* in visceral adipose tissue positively correlated with that of subcutaneous adipose tissue ($r = 0.729$, $p < 10^{-4}$).

3.2. Correlations of adipose tissue HSD11B1 expression with metabolic markers in PCOS patients and controls

HSD11B1 expression in both adipose tissues and separately for PCOS and control group was tested for possible associations with markers of obesity, IR, dyslipidemia, inflammation and PCOS.

In PCOS patients, *HSD11B1* expression in visceral and subcutaneous adipose tissue correlated positively with BMI, waist circumference, hsCRP and negatively with HDL-cholesterol (Table 2). Subcutaneous *HSD11B1* expression showed a strong correlation

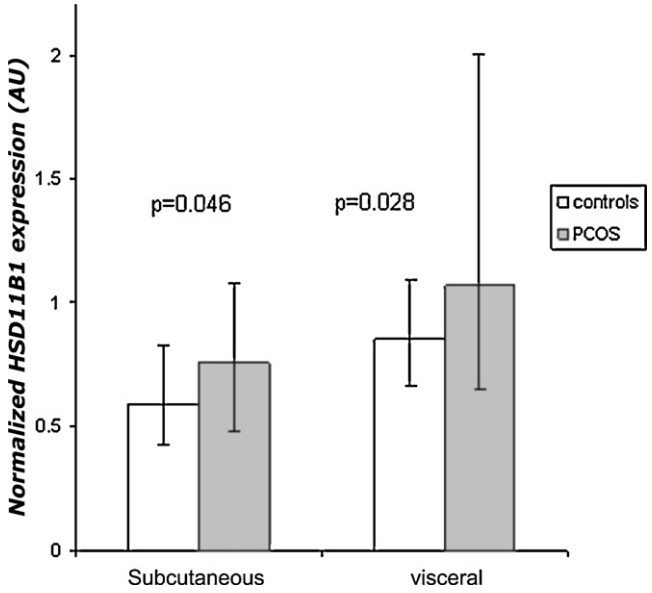

Fig. 1. Differences in *HSD11B1* expression between PCOS patients and controls for subcutaneous and visceral adipose tissues. Gene expression is measured in arbitrary units (AUs) relative to housekeeping gene expression. The bars represent medians (lower–upper quartile).

with fasting insulin, glucose and HOMA-IR, whereas for visceral *HSD11B1* expression there was a borderline positive significance of correlation with insulin and HOMA-IR and none with glucose (Table 2). Further, visceral *HSD11B1* expression showed positive correlations with systolic BP and TG, and a negative correlation with SHBG. These were not observed for subcutaneous *HSD11B1* expression. When the correlations of subcutaneous *HSD11B1* expression were tested on the smaller group of PCOS patients with both adipose tissue measurements ($n = 25$) the results were similar (data not shown).

In controls, visceral adipose tissue *HSD11B1* expression correlated positively only with free testosterone and androstenedione. Subcutaneous *HSD11B1* expression of controls correlated positively with markers of obesity and IR, and with diastolic pressure (Table 2).

Additionally, a stepwise multiple regression analysis was performed on all subjects combined, with subcutaneous or visceral

Table 2
Pearson's correlation coefficients and significance levels between *HSD11B1* expression and metabolic phenotype in PCOS patients and controls.

	Visceral adipose tissue				Subcutaneous adipose tissue			
	PCOS ($n=25$)		Controls ($n=29$)		PCOS ($n=85$)		Controls ($n=43$)	
	r	p	r	p	r	p	r	p
BMI	0.591	**0.002**	0.350	0.062	0.399	**<0.001**	0.340	**0.025**
Waist circumference	0.614	**0.001**	0.238	0.222	0.469	**$<10^{-5}$**	0.524	**$<10^{-3}$**
Systolic BP	0.563	**0.003**	0.258	0.176	0.006	0.563	0.224	0.148
Diastolic BP	0.339	0.097	0.205	0.285	0.103	0.349	0.424	**0.005**
Fasting glucose	0.110	0.601	0.140	0.467	0.296	**0.006**	0.334	**0.028**
Fasting insulin	0.409	**0.042**	0.300	0.114	0.299	**0.006**	0.308	**0.045**
HOMA-IR	0.393	0.052	0.312	0.099	0.323	**0.003**	0.331	**0.030**
Total cholesterol	−0.054	0.797	0.224	0.241	−0.119	0.278	0.132	0.397
HDL-cholesterol	−0.566	**0.003**	−0.014	0.941	−0.244	**0.024**	−0.075	0.634
LDL-cholesterol	0.088	0.677	0.219	0.253	−0.099	0.366	0.131	0.401
TG	0.488	**0.013**	0.125	0.518	0.182	0.093	0.061	0.699
hsCRP	0.622	**<0.001**	0.176	0.360	0.418	**$<10^{-4}$**	0.158	0.350
Total testosterone	0.259	0.210	0.234	0.221	−0.006	0.953	0.052	0.772
Free testosterone	0.361	0.076	0.476	**0.009**	0.144	0.190	−0.056	0.720
SHBG	−0.432	**0.031**	−0.195	0.309	−0.069	0.530	−0.061	0.699
DHEAS	0.129	0.538	0.110	0.568	−0.127	0.248	−0.012	0.938
Androstenedione	−0.151	0.472	0.392	**0.035**	−0.239	**0.028**	0.116	0.458

All the parameters were log transformed for the test. Significant correlations are shown in bold. BMI, body mass index; BP, blood pressure; TG, triglycerides; hsCRP, high sensitivity C-reactive protein; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone-sulphate.

HSD11B1 expression set as a dependent variable and previously significant biochemical markers and control/PCOS status as independent variables. BMI was a variable far more strongly associated with *HSD11B1* expression in subcutaneous adipose tissue (standardized $\beta = 0.324$, $p < 0.001$) than HOMA (standardized $\beta = 0.186$, $p = 0.049$), p of the model = 2.3×10^{-6}. For *HSD11B1* expression in visceral adipose tissue, waist circumference (standardized $\beta = 0.452$, $p < 0.001$) and systolic pressure (standardized $\beta = 0.260$, $p = 0.048$) were the significantly associated variables, p of the model = 3.9×10^{-6}.

3.3. Correlation of HSD11B1 expression with expression of other genes in adipose tissues of PCOS patients and controls

HSD11B1 expression in both adipose tissues was tested for possible correlations with expression of genes of lipid uptake (*LPL*), lipolysis (*LIPE*), adipogenesis (*PPARG*), and the genes of two insulin sensitivity factors: adiponectin (*ADIPOQ*) and GLUT4 (*SLC2A4*).

In PCOS patients, visceral adipose tissue *HSD11B1* expression showed a positive correlation with the expression of *LPL* ($r = 0.476$, $p = 0.016$), *LIPE* ($r = 0.450$, $p = 0.024$) and *PPARG* genes ($r = 0.640$, $p = 0.001$) and no correlation with *ADIPOQ* gene expression. The expression of GLUT4 gene in visceral adipose tissue was not analyzed. In subcutaneous adipose tissue of PCOS patients, *HSD11B1* expression was negatively correlated with *LPL* ($r = -0.327$, $p = 0.011$), *LIPE* ($r = -0.301$, $p = 0.019$), *ADIPOQ* ($r = -0.439$, $p = 0.028$) and GLUT4 gene expression ($r = -0.285$, $p = 0.027$), but was not correlated with *PPARG* gene expression.

In controls, the only significant association of *HSD11B1* expression was a negative one with *SLC2A4* (GLUT4 gene) expression ($r = -0.625$, $p = 0.017$) in subcutaneous adipose tissue.

When expressions of other genes were compared between controls and patients, expression of *LIPE* in subcutaneous adipose tissue was significantly lower in PCOS patients (0.96 (0.78–1.13) in PCOS and 1.04 (0.86–1.24) in controls; $p = 0.05$). Again, after BMI adjustment there was no association of *LIPE* expression with PCOS state. Additionally, gene expression of GLUT4 was lower in subcutaneous tissue of PCOS patients (0.54 (0.31–1.07)) compared to controls (1.83 (1.55–2.66), $p < 10^{-6}$), the significance remaining after BMI adjustment ($p < 10^{-4}$).

4. Discussion

Our study is the first to analyze the expression of 11β-HSD1 and lipid metabolism genes in subcutaneous and visceral adipose tissues of PCOS patients and controls. We found *HSD11B1* expression in both adipose depots of PCOS patients to be associated with markers of adiposity and IR as well as with expression of the genes for lipid uptake (*LPL*), lipolysis (*LIPE*), adipogenesis (*PPARG*), and the insulin sensitivity factors adiponectin (*ADIPOQ*) and GLUT4 (*SLC2A4*).

The *HSD11B1* gene encodes a glucocorticoid reactivating enzyme, 11β-hydroxysteroid-dehydrogenase type 1. Glucocorticoids are implicated in the development of central obesity in Cushing's and metabolic syndromes [6]. As central adiposity has a high prevalence in PCOS [3], *HSD11B1* expression was measured in subcutaneous and visceral adipose tissues of PCOS patients.

HSD11B1 expression in both adipose compartments was higher in PCOS patients than in non-BMI matched controls. However, after correction for BMI, the significance of the difference was lost. Additionally, the absence of positive association between *HSD11B1* and androgens in PCOS patients suggests that the higher *HSD11B1* expression was due to adiposity rather than PCOS *per se*. Several recent studies found increased *HSD11B1* expression in subcutaneous [7–10,15,25] and visceral adipose tissue [7,8] of patients with obesity or the metabolic syndrome. In contrast to our results, a study comparing seven obese PCOS patients with eight obese women without PCOS found higher *HSD11B1* expression in visceral adipose tissue of the patients. The increased *HSD11B1* expression was associated with the hyperandrogenism of PCOS [26]. However, we found a positive correlation of *HSD11B1* expression in visceral adipose tissue with plasma free testosterone and androstenedione in (lean) controls only, which is in agreement with the hypothesis that androgens might somehow stimulate *HSD11B1* expression, the effect probably being attenuated as obesity develops and other regulators take over in parallel to the degree of adiposity [27]. Higher *HSD11B1* expression was found in lean PCOS patients ($n = 17$) compared to lean controls ($n = 8$) [27]. Obese men had similar levels of *HSD11B1* expression as obese women, while lean men had higher *HSD11B1* expression when compared to lean women [7]. A very recent study demonstrated that testosterone induced *HSD11B1* expression in visceral but not in subcutaneous adipose tissue of young boys [28].

HSD11B1 expression in both adipose tissue compartments of our PCOS patients correlated positively with markers of visceral (waist circumference) and overall obesity (BMI). 11β-HSD1 converts inactive cortisone to active cortisol in adipose and other tissues. Increased cortisol activity promotes preadipocyte differentiation to adipocytes and fat tissue accumulation [29]. Three previous studies found that *HSD11B1* expression in visceral [12,13] and subcutaneous adipose tissue [13,30] was associated with local adipocyte hypertrophy and fat mass/area. Two additional factors promoting lipid accumulation are LPL and PPARγ. LPL mediates free fatty acid uptake into adipose tissue, and PPARγ is the main transcription factor for preadipocyte differentiation. Indeed, *HSD11B1* expression correlated positively with *LPL* and *PPARG* expression in visceral adipose tissue of our PCOS women. However, *HSD11B1* expression correlated negatively with *LPL* expression in the patients' subcutaneous adipose tissue. This is in line with the cortisol induced increase in *LPL* transcription seen in cultured adipose tissue from the visceral but not from the subcutaneous depot [18]. Glucocorticoids also induce *PPARG* expression in cultured human adipocytes [31].

In our study, *HSD11B1* expression was also associated with markers of IR (plasma glucose, insulin and HOMA-IR). GLUT4 gene expression was in negative correlation with *HSD11B1* expression in subcutaneous adipose tissue of our PCOS patients and controls. However, GLUT4 gene expression is not directly affected by glucocorticoids [32]. Nevertheless, increased glucocorticoid reactivation can enhance IR by direct inhibition of *ADIPOQ* expression [33] and stimulation of lipolysis. Adiponectin gene expression was negatively correlated with *HSD11B1* expression in subcutaneous adipose tissue of our PCOS patients. Cortisol-induced lipolysis increases the free fatty acid flux from visceral adipose tissue to the liver and muscle, thus worsening IR. In our patients, a positive correlation of *HSD11B1* expression with *LIPE* – hormone sensitive lipase gene was found in visceral adipose tissue. Dexamethasone increased *LIPE* expression in rat visceral adipocytes [21]. Our findings in PCOS patients are in agreement with those in a population of 36 lean to obese healthy women in whom a positive correlation of visceral 11β-HSD activity with visceral lipolysis, LPL activity and HOMA-IR, and a negative correlation with plasma adiponectin were observed [34].

It is of interest that in PCOS, *HSD11B1* expression in visceral adipose tissue positively correlated with *LPL* and *LIPE* expression, but in subcutaneous fat this correlation was negative. In subcutaneous adipose tissue, the regulation of *HSD11B1* on one side and *LPL* and *LIPE* on the other side seems to be in opposite directions. For example, thiazolidinediones suppress *HSD11B1* expression [35] while stimulating *LPL* and *LIPE* by PPARγ activation [36].

Additionally, an obesity associated decrease of *LIPE* expression in the subcutaneous adipose depot was observed in our PCOS patients. This confirms previous reports of attenuated mRNA and protein expression of *LIPE* in IR and obesity [37,38] and could be an adaptive response to restrain further increase of plasma free-fatty acid levels and subsequent worsening of IR [37]. As expected, the gene expression of GLUT4 in subcutaneous adipose tissue was lower in PCOS patients compared to controls both before and after BMI adjustment [39].

In PCOS, we demonstrated a positive correlation of *HSD11B1* expression in adipose tissues with systolic BP, plasma TG, and hsCRP, and a negative one with HDL-cholesterol, parameters typical of a proatherogenic constellation of visceral adiposity and the metabolic syndrome. The fewer *HSD11B1* correlations with biochemical markers found in the control group could be attributed to less variance in BMI in this group.

In the multiple regression analysis of control and PCOS groups combined, subcutaneous adipose tissue *HSD11B1* expression was independently associated with BMI and weakly with HOMA-IR, whereas *HSD11B1* expression in visceral adipose tissue was significantly associated with waist circumference and weakly with systolic BP. This again indicates that 11β-HSD activity is predominantly associated with obesity, rather than with the intrinsic IR of PCOS. Glucocorticoids induce angiotensinogen gene expression [20], which can at least partially explain the association between *HSD11B1* expression and BP.

The negative association of *HSD11B1* expression in visceral adipose tissue with plasma SHBG levels in PCOS patients is in line with the positive correlation of *HSD11B1* with insulin levels, as SHBG synthesis is suppressed by insulin in the liver [40].

Comparing *HSD11B1* expression in PCOS between two different adipose depots, we found a trend for higher *HSD11B1* expression in visceral adipose tissue, concordant with higher expression of other "unfavorable" genes (for PAI-1, IL-6, VEGF [41] and glucocorticoid receptor [12]) in this adipose tissue compartment, whose deleterious metabolic and cardio-vascular effects are manifested as visceral fat mass increases.

The limitations of our study should be mentioned. The correlation of *HSD11B1* expression with biochemical and anthropometric markers was assessed by numerous tests. As 17 variables were used for each set (patients, controls; subcutaneous, visceral adipose tissue), one false positive result is expected in each set ($17 \times 0.05 = 1$). At least for PCOS patients, with 8 significant bivariate correlations obtained for every tissue, we believe that a substantial number of significant correlations exist in reality. If approached with a more stringent Bonferroni correction, the whole number of tests (90) gives a new significance level of 0.001, which implies that the most important correlations in the multiple regression test, namely that of waist circumference with visceral *HSD11B1* expression, and that of BMI with subcutaneous *HSD11B1* expression, are valid.

Protein expression and 11β-HSD enzyme activity were not measured in our study; however, a tight association between *HSD11B1* gene expression and enzyme activity had been well documented in previous reports [34,42,43].

In summary, in PCOS patients, as long as they are lean, there is no increase in *HSD11B1* expression in adipose tissues. However, in obesity, being a common complication of PCOS, we found substantial positive correlations of *HSD11B1* expression in visceral and subcutaneous adipose tissues with markers of adiposity and IR, which were supported by correlations with genes implicated in lipid metabolism and adipogenesis. We can conclude that increased *HSD11B1* expression, leading to augmented auto- and paracrine glucocorticoid reactivation, is associated with an unfavorable metabolic profile and could play a role in the pathogenesis of visceral adiposity and consequent IR, independent of PCOS status.

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