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Expression of genes related to glucocorticoid action in human subcutaneous and omental adipose tissue☆,☆☆

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ABSTRACT

Adipose tissue glucocorticoid action relies on local enzymatic interconversion and glucocorticoid receptor (GR) availability. 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), 2 (11β-HSD2) and hexose-6-phosphate dehydrogenase (H6PDH) are likely involved in glucocorticoid activation/inactivation within adipose tissue. We examined adipose tissue mRNA expression of genes related to glucocorticoid action and their association with total and visceral adiposity. Messenger RNA was measured in paired subcutaneous and omental fat samples obtained from 56 women (age: 47.3 ± 4.8 years, BMI: 27.1 ± 5.2 kg/m^2) undergoing gynaecological surgery. Expression levels of 11β-HSD2, H6PDH and GRα were higher in omental adipose tissue while 11β-HSD1 expression was similar between fat compartments. Subcutaneous and omental 11β-HSD1 mRNA abundances were positively associated with total and visceral adiposity whereas omental H6PDH mRNA abundance was negatively associated with these measures. Only omental 11β-HSD1 mRNA expression remained significantly associated with visceral adipose tissue area following statistical adjustment for fat mass, age and menopausal status. Omental 11β-HSD1 mRNA expression explained 19.1% of the variance in visceral adipose tissue area. Omental fat tissue 11β-HSD-1 protein and cortisol levels were higher in visceral obese women, supporting findings obtained with 11β-HSD-1 mRNA. These results suggest that among the transcripts examined only omental 11β-HSD1 is independently associated with visceral obesity in women.

1. Introduction

Adipose tissue exposure to glucocorticoids is believed to be a significant determinant of adiposity and body fat distribution [1–7]. Unlike visceral obese patients with Cushing's syndrome, subjects with idiopathic visceral obesity may be characterized primarily by local alterations of active glucocorticoid levels [1]. While circulating glucocorticoid levels remain in the physiological range, locally enhanced glucocorticoid action is suggested in adipose tissue of these individuals [2,3–8]. In these cases, increased adipose tissue glucocorticoid exposure relies not only on glucocorticoid receptor (GR) availability but also on the local enzymatic interconversion of active and inactive hormones.

Adipose tissue 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) expression enables local production of active glucocorticoids [9]. Several studies observed a positive association between 11β-HSD1 mRNA expression or oxoreductase activity and adiposity measures in men and women [2–7]. We recently demonstrated that a relatively higher 11β-HSD1 reductase activity in omental compared to subcutaneous adipose tissue is associated with abdominal obesity and concomitant metabolic alterations in women [2].

Expression of 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) was recently reported in the adipose tissue stroma-vascular cell fraction [10–12]. This enzyme is also highly expressed in the kidney, where it drives the conversion of cortisol into cortisone and, therefore, protects cells from active glucocorticoid exposure [12]. Engeli et al. observed that 11β-HSD2 mRNA expression in subcutaneous adipose tissue is negatively associated with BMI [12]. Low expression levels observed in both subcutaneous and omen-

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tal adipose tissue may, however, limit the physiological impact of 11β-HSD2 on local concentrations of active glucocorticoids [10,12].

Recent studies pointed out hexose-6-phosphate dehydrogenase (H6PDH) as an important modulator of active glucocorticoid availability in adipose tissue [13–17]. H6PDH generates the NADPH cofactor needed for 11β-HSD1 oxoreductase activity [14,17]. Evidence for colocalization as well as interaction between 11β-HSD1 and H6PDH in the endoplasmic reticulum lumen support this hypothesis [17]. Preadipocyte H6PDH expression levels are negatively associated with BMI, although adipogenesis strongly induces its expression [14]. Available evidence supports a role of H6PDH activity in adipose tissue glucocorticoid exposure in mice [18]. However, further studies are needed in humans.

The glucocorticoid receptor alpha (GRα) is the only active receptor for glucocorticoids in human adipose tissue [19]. Messenger RNA levels of this receptor were found to be higher in omental than in subcutaneous adipose tissue in some, but not all, studies [6,10,19–21]. Negative associations between GRα mRNA expression and adiposity measures were previously reported [6,19]. These associations indirectly suggest that adipose tissue glucocorticoid action is reduced in obese individuals [19].

In this context, variation in the expression of these genes may alter normal glucocorticoid homeostasis and subsequently modify adipose tissue metabolism or cell composition. The aim of the present study was to examine key glucocorticoid action-related genes expressed in subcutaneous and omental adipose tissue and assess their association with total and visceral adiposity measures in women. We tested the hypothesis that among genes related to glucocorticoid action, 11β-HSD1 mRNA expression would be the main correlate of total and visceral adiposity in women.

2. Materials and methods

2.1. Subjects

The study sample included 56 women aged from 40 to 62 years recruited through the elective surgery schedule of the Gynecology Unit of the Laval University Medical Center. The project was approved by the ethics committee of Laval University Medical Research Center. Written informed consent was obtained from all women. Abdominal gynaecological surgeries were scheduled for total ($n=53$) or subtotal ($n=3$) abdominal hysterectomies sometimes accompanied by salpingo-oophorectomy of one ($n=10$) or two ($n=19$) ovaries. Reasons for surgery included one or more of the following: myoma/fibroids ($n=40$), menorrhagia/menometrorrhagia ($n=26$), benign ovarian cyst ($n=13$), endometriosis ($n=11$), incapacitating dysmenorrhea ($n=10$), endometrial hyperplasia ($n=5$), pelvic adhesions ($n=4$), polyp ($n=3$), pelvic pain ($n=2$), excessive anaemia-causing uterine bleeding ($n=1$), adenomyosis ($n=1$), and/or ovarian thecoma ($n=1$). Menstrual history questionnaires as well as medical files were used to confirm menopausal status. Nine women were identified as postmenopausal, 16 were identified as perimenopausal while the remaining women ($n=31$) were recognized as premenopausal. Four women were using hormonal replacement therapy.

2.2. Total adiposity and body fat distribution measurements

We performed these measurements within a few days before or after the surgery. Total body fat mass and fat percentages were determined using dual-energy X-ray absorptiometry. Abdominal subcutaneous and visceral adipose tissue cross-sectional area measures were obtained at the L4–L5 vertebrae level using computed tomography as previously described [22,23].

2.3. Adipose tissue sampling and adipocyte isolation

During the surgical procedure, subcutaneous and omental adipose tissue samples were collected at the site of surgical incision (lower abdomen) and at the distal portion of the greater omentum, respectively. Both biopsies were generally performed within 15 min and were immediately carried to the laboratory. A portion of the tissue samples was immediately frozen in liquid nitrogen and stored at −80 °C for subsequent mRNA extraction. The remaining tissue was digested 45 min at 37 °C in the presence of type 1 collagenase (350 units/ml) in Krebs-Ringer-Henseleit buffer according to a modified version of the Rodbell method [24]. Adipocyte suspensions were washed and adipocyte diameter was measured by contrast phase microscopy as previously described [24].

2.4. RT-qPCR mRNA measurements

Total RNA was isolated from subcutaneous and omental adipose tissue using the RNeasy lipid tissue extraction kit and on-column DNase (Qiagen, CA, USA) treatment following the manufacturer's recommendations. RNA quality and concentration was assessed using the Agilent Technologies 2100 bioanalyzer (Agilent, CA, USA). Total RNA (5 μg) was denatured in the presence of random hexamers (Invitrogen, CA, USA), oligo-dT_{18} and dNTPs (Amersham Biosciences, UK). Complementary DNA was generated using the Superscript III RNase H- reverse transcriptase (Invitrogen, CA, USA). RNase-treated cDNA was then purified using QIAquick PCR Purification Kit (Qiagen, CA, USA). Real-time cDNA amplification was performed in duplicate or triplicate using the LightCycler 480 (Roche Diagnostics, IN, USA) and the SYBR Green I Master (Applied Biosystems, CA, USA) as follows: 95 °C for 10 s, 59–62 °C for 10 s, 72 °C for 12 s and then 76 °C for 5 s (reading) repeated 50 times. Target gene amplifications were normalized using expression levels of the following housekeeping genes: hypoxanthine guanine phosphoribosyl transferase 1 (HPRT1), ATP synthase O subunit (ATP5O), glucose-6-phosphate dehydrogenase (G6PD) or 18S ribosomal RNA. None of the four housekeeping gene expression levels examined were associated with age, adiposity or fat depot in our study sample. Similar results were obtained using each housekeeping gene. Only results normalized to G6PD expression are included in the present manuscript. Primer sequences for 11β-HSD1 (NM_005525; sense: 5′-TCATTCTCAACCACATCACCAACACT-3′; anti-sense: 5′-CCAGCCAGAGAGGAGACGACAAC-3′), 11β-HSD2 (NM_ 000196; sense: 5′-TCAGGCTGTGACTCTGGTTTTGGCA-3′; anti-sense: 5′-CGGGGCTGTTCAACTCCAATAC-3′), H6PDH (NM_004285; sense: 5′-TGCTTTTACCTCTCGTCCACTG-3′; anti-sense: 5′-GTGGGGTTTTTGTTTTATAGGGAGACT-3′), GRα (NM_001018077; sense: 5′-CCTGTCTGTACCTAACGCCCTAT-3′; anti-sense: 5′-GGGTGTCTAGCCATTTTTGCCATATT-3′), G6PDH (NM_000402; sense: 5′-GATGTCCCCTGTCCCACCAACTCTG-3′; anti-sense: 5′-GCAGGGCATTGAGGTTGGGAG-3′) were designed using GeneTools (Biotools Inc., FL, USA).

2.5. Western blot

Following RNA extraction, 1 volume ethanol was added to the lower phase of the trizol extraction. Trace DNA was then removed by centrifugation at 5000 × *g* for 5 min. The supernatant was mixed by inversion with 2 volumes of isopropanol and incubated 5 min at room temperature. Proteins were precipitated by centrifugation at 5000 × *g* for 5 min. The pellet was then resuspended using heat (65 °C) and sonication (10 s) cycles in 20 mM Tris, pH 7.4, 3% SDS buffer containing protease inhibitors. A total of 20 μg of protein homogenate diluted in SDS buffer 4× (0.8 mM Tris, pH 6.8, 8% SDS, 5 mM EDTA, 40% glycerol, 0.2 mM DTT) was heated at 95 °C for 5 min and separated on a 12% SDS–polyacrylamide gel. Proteins

were transferred to nitrocellulose membranes (1 h at 100 V) and unspecific sites were blocked with 5% non-fat milk diluted in wash solution (50 mM Tris, pH 7.4, 150 mM NaCl, 0.1% Tween 20) for 1 h. Membranes were then incubated overnight at 4 °C with 10 μL rabbit antiserum against 11β-HSD1, washed 3 × 10 min and incubated 1 h with anti-rabbit immunoglobulin G conjugated to horseradish peroxidise (1:10,000). Finally, membranes were washed 3 × 10 min and proteins were visualized by chemiluminescence. Densitometric analysis was performed with ImageJ (NIH, USA).

2.6. Adipose tissue cortisol measurement

The supernatants of the protein extraction were evaporated and delipidated with methanol at −20 °C. They were then extracted using 2 volumes of diethylether and evaporated. The pellets containing glucocorticoids were resuspended in 500 μL PBS. Cortisol concentrations were measured using a cortisol radioimmunoassay (Diasorin, MN, USA) following the manufacturer's recommendations. Extraction efficiency was monitored using ^{14}C labelled cortisol and ranged from 71 to 83%. The inter-assay coefficient of variation for extraction and cortisol measurements was 17%.

2.7. Statistical analyses

Depot differences in adipose tissue mRNA expression levels in the entire sample were tested using paired *t*-tests. Repeated measures ANOVA were performed to compare mRNA levels according to adipose tissue depots and BMI categories. Associations between mRNA expression and adiposity measures were tested using general linear regression analysis. Adjustment for fat mass, age and menopausal status were performed using covariance analysis. Subcutaneous and omental mRNA abundance of each glucocorticoid action-related gene were included in the multivariate model to predict fat mass, visceral adipose tissue area and the visceral adipose tissue-to-fat mass ratio. Final models were obtained after stepwise exclusion of all non-significant variables ($p \leq 0.05$). The percent variance explained by independent variables was computed after adjustment for confounding variables (i.e. age and menopausal status). Based on a significant Shapiro-Wilk test ($p \leq 0.05$), non-normally distributed variables were $\log_{10}$- or Box–Cox-transformed. Statistical analyses were performed using SAS 9.1 (SAS Institute, NC, USA).

3. Results

Subcutaneous and omental adipose tissue mRNA expression of genes related to glucocorticoid action was measured in a sample of 56 women. Women were overweight since their mean BMI was greater than 25 kg/m^2. However, a wide range of adiposity values and various body fat distribution patterns were represented in this sample of women (Table 1).

Table 1
Characteristics of the study sample (n = 56 women).

Variables	Mean ± SD	Range (min–max)
Age (year)	47.3 ± 4.8	40.4–61.7
Postmenopausal status (%)	16.1%	–
Weight (kg)	71 ± 15	49–111
BMI (kg/m^2)	27.1 ± 5.2	17.2–41.3
Fat mass (kg)	25.6 ± 9.4	10.0–50.8
Body fat percentage (%)	35.2 ± 6.1	19.6–47.5
Abdominal adipose tissue (cm^2)		
Total	429 ± 184	137–991
Visceral	97 ± 47	34–233
Subcutaneous	333 ± 144	103–759

Mean mRNA levels of selected genes measured in subcutaneous and omental adipose tissue are shown in Table 2. Relatively high expression levels were observed for 11β-HSD1, H6PDH and GRα in both adipose tissue compartments. As previously reported by Lee et al. and Engeli et al., we detected the presence of 11β-HSD2 mRNA in subcutaneous and omental adipose tissue [11,12]. However, 11β-HSD2 expression levels were 22- and 8-fold lower than that of 11β-HSD1 in subcutaneous and omental adipose tissue, respectively. While no regional difference in 11β-HSD1 expression was observed between fat depots, we found significantly higher expression levels in omental vs. subcutaneous adipose tissue for 11β-HSD2, H6PDH and GRα. The regional difference was especially marked for 11β-HSD2 as mRNA levels were 2.5-fold higher in omental than in subcutaneous adipose tissue.

Messenger RNA levels in subcutaneous and omental adipose tissue were further considered according to BMI of the patients. In the whole sample, no regional difference in 11β-HSD1 mRNA expression level was found (Fig. 1A). However, we observed higher 11β-HSD1 mRNA levels in subcutaneous adipose tissue of obese compared to normal weight women (Fig. 1A). In a similar manner, significantly higher 11β-HSD1 mRNA levels were observed in omental adipose tissue of overweight and obese compared to normal weight women (Fig. 1A). The overall regional difference in 11β-HSD2 mRNA expression was maintained in all BMI categories (Fig. 1B). Within each adipose tissue compartment, 11β-HSD2 mRNA expression was not related to adiposity (Fig. 1B). H6PDH mRNA levels were higher in omental compared to subcutaneous adipose tissue of normal weight and overweight women but not of obese women (Fig. 1C). The lack of regional difference in obese women resided in a significantly decreased of omental H6PDH mRNA expression in these women compared to normal weight and overweight women (Fig. 1C). GRα mRNA expression was similar between subcutaneous and omental adipose tissue of normal weight and obese women. In fact, the higher GRα mRNA levels observed in omental adipose tissue of the entire sample were apparent in overweight women but not in normal weight or obese women (Fig. 1D).

The relationship between adiposity and the studied transcripts was further confirmed by correlation analysis. We observed positive associations between 11β-HSD1 mRNA expression and BMI

Table 2
Glucocorticoid action-related measures in subcutaneous and omental adipose tissue.

Measures	Subcutaneous		Omental		n	p-Values
	Mean ± SD	Range (min–max)	Mean ± SD	Range (min–max)		
11β-HSD1mRNA	2.23 ± 1.15	0.42–5.15	2.03 ± 0.98	0.49–4.52	55	NS
11β-HSD2 mRNA	0.10 ± 0.05	0.01–0.27	0.25 ± 0.12	0.05–0.53	55	0.0001
H6PDH mRNA	0.81 ± 0.28	0.35–1.65	1.33 ± 0.48	0.27–2.38	55	0.0001
GRα mRNA	1.64 ± 0.50	0.50–2.80	1.98 ± 0.53	0.52–3.59	55	0.0001
11β-HSD1 protein	135 ± 118	48–666	190 ± 126	66.2–593	43	0.001
Cortisol levels[a]	34.4 ± 29.9	5.4–139	47.4 ± 44.4	10.6–219	49	0.05

[a] Cortisol levels are shown in nmol/kg.

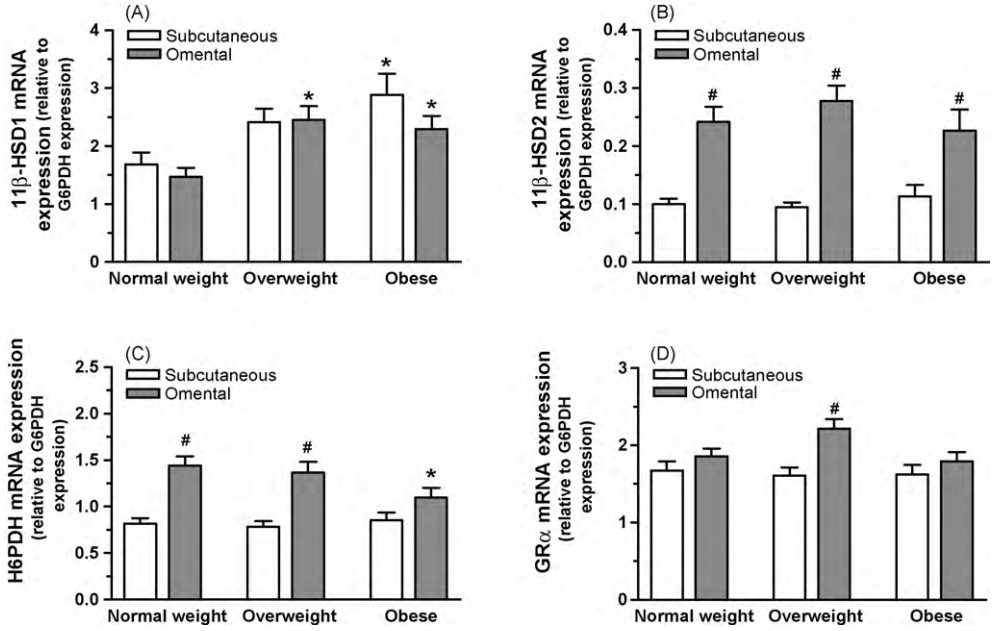

Fig. 1. Messenger RNA expression of genes related to glucocorticoid action according to BMI categories. 11β-HSD1 (A), 11β-HSD2 (B), H6PDH (C) and GRα (D) mRNA levels in subcutaneous and omental adipose tissue of normal weight ($n = 22$), overweight ($n = 21$) and obese ($n = 12$) women. Messenger RNA levels relative to G6PDH expression are shown on the graph (Mean ± SEM). $^{\#}p \leq 0.05$ compared to subcutaneous adipose tissue within the same BMI categories, $^{*}p \leq 0.05$ compared to normal weight women.

(SC: $r = 0.47$, $n = 55$, $p < 0.001$; OM: $r = 0.50$, $n = 56$, $p < 0.0001$), body fat mass (SC: $r = 0.47$, $n = 53$, $p < 0.001$; OM: $r = 0.57$, $n = 54$, $p < 0.0001$), adipocyte size (SC: $r = 0.53$, $n = 52$, $p < 0.0001$; OM: $r = 0.59$, $n = 51$, $p < 0.0001$), visceral adipose tissue area (SC: $r = 0.47$, $n = 51$, $p < 0.001$; OM: $r = 0.56$, $n = 52$, $p < 0.0001$) as well as the visceral adipose tissue area-to-fat mass ratio (SC: $r = 0.32$, $n = 51$, $p < 0.05$; OM: $r = 0.39$, $n = 52$, $p < 0.005$). Omental H6PDH mRNA expression was negatively associated with BMI ($r = -0.27$, $n = 56$, $p < 0.05$) and OM adipocyte size ($r = -0.34$, $n = 51$, $p < 0.01$); trends were observed with body fat mass ($r = -0.25$, $n = 54$, $p = 0.07$), visceral adipose tissue area ($r = -0.25$, $n = 52$, $p = 0.07$) and the visceral adipose tissue area-to-fat mass ratio ($r = -0.26$, $n = 52$, $p = 0.06$). On the other hand, mRNA expression of H6PDH in subcutaneous adipose tissue and of 11β-HSD2 as well as GRα in both depots were not related to total or visceral adiposity.

Associations of visceral adipose tissue area with 11β-HSD1 and H6PDH mRNA expression were adjusted to account for confounding variables, namely fat mass, menopausal status and age (Table 3). Omental 11β-HSD1 mRNA expression remained significantly associated with visceral adipose tissue area following adjustment for fat mass and also following further adjustment for menopausal status and age. However, subcutaneous 11β-HSD1 and omental H6PDH mRNA levels were no longer associated with visceral adipose tissue area following adjustment for either confounding variables.

Contribution of the selected transcripts to variation in total and visceral adiposity was corroborated using multivariate analysis (Table 4). Subcutaneous and omental mRNA expression as well as all confounding variables were included in the model to predict fat mass, visceral adipose tissue area and the visceral adipose tissue-to-fat mass ratio. Fat mass and visceral adipose tissue area variances were partly explained by omental expression of 11β-HSD1 and H6PDH. The visceral adipose tissue area-to-fat mass ratio, a specific marker of visceral fat distribution, was solely predicted by omental 11β-HSD1 mRNA expression and age.

To confirm the finding of increased 11β-HSD1 mRNA expression in women with visceral obesity, we have assessed other indicators or 11β-HSD1 action in adipose tissue, namely 11β-HSD1 protein and tissue cortisol levels (Table 2, Fig. 2). Protein expression levels were higher in omental compared to subcutaneous adipose tissue of women with a high visceral adipose tissue area-to-fat mass ratio (Fig. 2A). However, these regional differences were not observed in women with a low visceral adipose tissue-to-fat mass ratio. Similarly, cortisol concentrations in both adipose tissue compartments were similar in women with a low visceral adipose tissue-to-fat

Table 3
Adjusted partial correlation coefficients of visceral adipose tissue area and mRNA expression of genes related to glucocorticoid action.

Transcripts	Depot	*N*	Unadjusted		Adjusted for FM		Adjusted for FM, MS and age	
			r	*p*-Values	*r*	*p*-Values	*r*	*p*-Values
11β-HSD1 mRNA	OM[a]	52	0.56	0.0001	0.30	0.015	0.31	0.014
	SC[a]	51	0.47	0.001	0.24	NS	0.20	NS
11β-HSD2 mRNA	OM[a]	52	−0.11	NS	−0.07	NS	−0.11	NS
	SC[a]	51	−0.05	NS	−0.02	NS	0.01	NS
H6PDH mRNA	OM	52	−0.27	0.05	−0.06	NS	−0.05	NS
	SC	51	0.01	NS	0.03	NS	0.02	NS
GRα mRNA	OM	52	0.03	NS	0.15	NS	0.18	NS
	SC	51	−0.06	NS	−0.18	NS	−0.08	NS

SC: subcutaneous adipose tissue; OM: omental adipose tissue; FM: fat mass; MS: menopausal status; NS: non-significant. Unadjusted and adjusted partial correlation coefficient (*r*) and *p*-values are provided.

[a] Correlation coefficient of $\log_{10}$-transformed variables.

Table 4
Multivariate regression analyses predicting fat mass, visceral adipose tissue area and visceral adipose tissue area-to-fat mass ratio.

Variables	Depot	Fat mass[a]		Visceral adipose tissue area[a]		Visceral adipose tissue area-to-fat mass ratio[a]	
		Partial ($r^2 \times 100$)	p-Values	Partial ($r^2 \times 100$)	p-Values	Partial ($r^2 \times 100$)	p-Values
11β-HSD1 mRNA	OM	16.1	0.001	19.1	0.0002	7.1	0.03
	SC	–	NS	–	NS	–	NS
11β-HSD2 mRNA	OM	–	NS	–	NS	–	NS
	SC	–	NS	–	NS	–	NS
H6PDH mRNA	OM	5.3	0.05	6.3	0.03	–	NS
	SC	–	NS	–	NS	–	NS
GRα mRNA	OM	–	NS	–	NS	–	NS
	SC	–	NS	–	NS	–	NS
Age		–	NS	4.7	0.05	12.2	0.01
Menopausal status		6.9	0.08	–	NS	–	NS

SC: subcutaneous adipose tissue, OM: omental adipose tissue, NS: non-significant. Final model was obtained using a stepwise exclusion procedure.
[a] Log_{10}-transformed dependent variables.

mass ratio, but were significantly higher in omental vs. subcutaneous fat of women with a high visceral adipose tissue area-to-fat mass ratio (Fig. 2B). Omental adipose tissue cortisol levels were also significantly increased compared to women with a low visceral adipose tissue-to-fat mass ratio. Regional differences in protein and cortisol levels were less apparent when women were stratified into normal weight and overweight or obese subgroups (data not shown).

4. Discussion

Several lines of evidence demonstrated that adipose tissue glucocorticoid exposure modulates fat accumulation as well as body fat distribution. This exposure mainly relies on the local generation of active glucocorticoids and glucocorticoid receptor (GR) availability rather than on circulating levels of active glucocorticoids. We designed this study to examine the association between transcripts related to glucocorticoid action (11β-HSD1, 11β-HSD2, H6PDH and GRα) and total or visceral fat accumulation. We described regional differences in mRNA expression of 11β-HSD2, H6PDH and GRα in normal weight, overweight and obese women. Moreover, we observed positive associations between total adiposity and body fat distribution measures and 11β-HSD1 expression in subcutaneous and omental adipose tissue. On the other hand, H6PDH expression in omental adipose tissue was negatively associated with total adiposity and visceral adipose tissue area. Expression levels of 11β-HSD2 and GRα in subcutaneous and omental adipose tissue as well as expression of H6PDH in subcutaneous adipose tissue were not associated with adiposity measures in this sample of women. Of all the transcripts examined, omental 11β-HSD1 mRNA expression was the most important determinant of visceral fat accumulation in women. Omental 11β-HSD1, but not subcutaneous 11β-HSD1 or omental H6PDH, were independently associated with visceral adipose tissue area. Moreover, a significant proportion of the variance in the visceral adipose tissue area-to-fat mass ratio was explained by omental 11β-HSD1 mRNA expression. These results were confirmed by higher 11β-HSD1 protein and cortisol levels in omental adipose tissue than in subcutaneous adipose tissue of women with proportionally higher visceral adipose tissue accumulation. Our study points towards the existence of slightly distinct glucocorticoid dynamics in subcutaneous and omental adipose tissues. Furthermore, our findings reinforce the notion that omental 11β-HSD1 is an important predictor of visceral adipose tissue accumulation.

Our conclusions are supported by several studies [2,3,6,7,11]. Positive correlations between 11β-HSD1 mRNA expression in subcutaneous [2–7,11] and omental [2,3,6,7,11] adipose tissue and adiposity have been described in most reports. We had demonstrated that 11β-HSD1 oxoreductase activity in omental adipose tissue of women is positively associated with visceral fat accumulation and metabolic alterations [2]. We had also underscored the relation between the omental-to-subcutaneous 11β-HSD1 oxoreductase activity ratio and body fat distribution in women [2]. It is noteworthy that the present study, which did not assess cortisone reduction but 11β-HSD1 mRNA and protein expression as well as cortisol concentration, generated results that are consistent with our previous study [2]. Taken together, both studies sup-

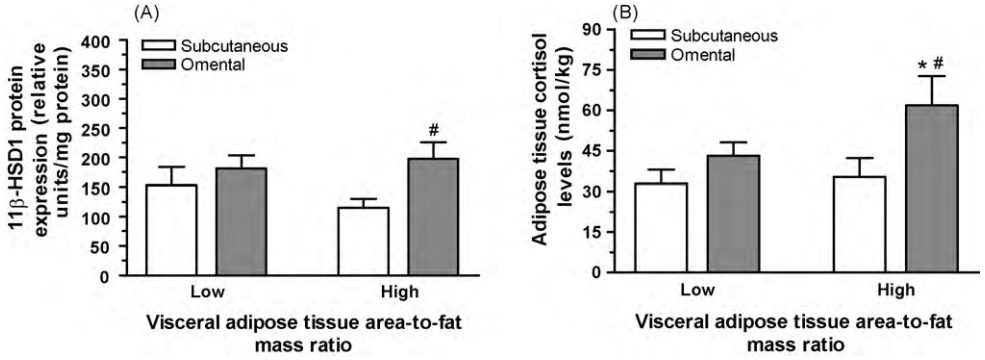

Fig. 2. 11β-HSD1 protein and cortisol level in subcutaneous and omental adipose tissue in subgroups stratified according to the visceral adipose tissue-to-fat mass ratio median. (A) Protein levels are shown in relative units (mean ± SEM). (B) Cortisol levels are shown in nmol/kg of fat. $^{\#}p \leq 0.05$ compared to subcutaneous adipose tissue within the same visceral adipose tissue area-to-fat mass category. $^{*}p \leq 0.05$ compared to women with a low visceral adipose tissue area-to-fat mass ratio within the same adipose tissue depot.

port the notion that omental 11β-HSD1 is the only glucocorticoid action-related gene independently associated with a preferential accumulation of fat in the visceral adipose tissue compartment. Our results are however at variance with those of Goedecke et al. [25] showing that subcutaneous but not omental adipose tissue was associated to visceral adipose tissue volume in South African women. We also failed to observe an association between measures of insulin resistance and omental 11β-HSD1 mRNA expression levels, as previously reported [26]. The fact that none of our women had previously been diagnosed with type 2 diabetes may explain this discrepancy.

Adipose tissue 11β-HSD1 activity has been suggested to be tightly linked to NADPH production by H6PDH. This hypothesis is supported by several reports of colocalization and interaction between 11β-HSD1 and H6PDH [13–15,17]. Moreover, H6PDH mRNA expression is induced following adipogenesis in parallel with 11β-HSD1 expression and activity [14]. In the present study, surprisingly, we observed a lower H6PDH mRNA expression in omental adipose tissue of obese women. While Bujalska et al. observed a negative correlation between preadipocyte expression of H6PDH and BMI, our study challenges the postulated link between H6PDH and visceral obesity [13,14,17]. Production of the NADPH cofactor in adipose tissue by H6PDH is definitely essential to maintain 11β-HSD1 oxoreductase activity [13–15,17]. Nevertheless, our results did not support a coordinated regulation 11β-HSD1 and H6PDH mRNA expression. Indeed, we observed only weak negative associations between omental H6PDH mRNA expression and adiposity measures. Reduced NADPH production presumably triggered by decreased H6PDH expression should lead to a decreased glucocorticoid activation capacity in adipose tissue. We therefore propose that while H6PDH may be essential to maintain 11β-HSD1 oxoreductase activity, only 11β-HSD1 mRNA expression in omental fat tracks with obesity and increased active glucocorticoid production as shown by this and our previous report [2].

Few studies have reported on 11β-HSD2 expression in human adipose tissue [11,12]. In the present study, 11β-HSD2 expression levels were 8- to 22-fold lower than 11β-HSD1 expression levels found in adipose tissue. As previously shown by Engeli et al., we did not observe associations between adiposity measures and 11β-HSD2 mRNA expression [12]. If cortisol inactivation rates are related to abundance of the 11β-HSD2 transcript, we postulate that this enzyme barely affects glucocorticoid action in subcutaneous and omental adipose tissue. Specific expression of 11β-HSD2 in the adipose tissue stroma-vascular cell fraction may, however, suggest that this enzyme protects non-mature adipocytes from active glucocorticoid spillover [12].

As a result of this study, the involvement of H6PDH, GRα and 11β-HSD2 in adipose tissue glucocorticoid homeostasis cannot be ruled out and should not be excluded. Indeed, 11β-HSD1, H6PDH, GRα and possibly 11β-HSD2 are required for glucocorticoid interconversion or signal transduction. Lack of association between expression levels of some of these transcripts and adiposity measures therefore only suggests that increased adipose tissue glucocorticoid exposure in obese women is not triggered by changes in their expression levels. Despite their lack of association with adiposity, regional differences observed in the expression of glucocorticoid action-related genes may suggest a distinct glucocorticoid homeostasis and dynamics in each adipose tissue compartment. Indeed, higher 11β-HSD2, H6PDH and GRα mRNA expression levels as well as higher 11β-HSD1 oxoreductase activity in omental adipose tissue point towards enhanced local glucocorticoid turnover as well as increased response to active glucocorticoids in this adipose tissue compartment [2].

Measures of mRNA expression performed in this study were confirmed through assessment of more direct markers of 11β-HSD1 action. Elevated 11β-HSD1 protein expression and cortisol levels were observed in omental adipose tissue of women with a high visceral fat accumulation relative to their overall adiposity. Therefore, the link between visceral obesity and omental 11β-HSD1 is now based on measures of mRNA, protein, cortisol levels and oxoreductase activity [2]. Enzymatic activities of 11β-HSD2 and H6PDH and the glucocorticoid binding capacity of GRα were not assessed in the present study. We cannot exclude that these parameters could be modulated by adiposity or the local cellular environment independent of their mRNA expression levels and impact local glucocorticoid homeostasis as well as adipose tissue metabolism.

Other limitations need to be acknowledged. Measures of mRNA, protein and cortisol were performed in whole adipose tissue samples. The regional differences observed may have been modulated by differences in the cell composition of each tissue. Another limitation is that these results are cross-sectional, which prevents us from concluding on cause-and-effect relationships between glucocorticoid action-related genes and visceral fat accumulation. We also acknowledge that individual drugs used during the surgery may have confounded our analysis. However, we observed that the main drugs of the surgery cocktail were generally similar among women. Finally, similar to most previous studies [2–4,6,27,28], only women were examined because of the difficulty of setting-up similar studies including lean to moderately obese men [7].

In conclusion, our results support our hypothesis that 11β-HSD1 mRNA expression and protein levels in omental adipose tissue is a significant correlate of fat mass accumulation and, more importantly, of specific visceral fat accumulation. Alterations in adipose tissue glucocorticoid dynamics reflected by relatively elevated cortisol levels in omental adipose tissue of women with visceral obesity may, therefore, partly rely on an increase in 11β-HSD1 expression rather than changes in other glucocorticoid action-related genes.

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