Cortisol in tissue and systemic level as a contributing factor to the development of metabolic syndrome in severely obese patients

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Abstract

Context: Adrenal and extra-adrenal cortisol production may be involved in the development of metabolic syndrome (MetS).

Objective: To investigate the activity of the hypothalamic–pituitary–adrenal (HPA) axis and the expression of HSD11B1, nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptors) α (NR3C1α) and β (NR3C1β) in the liver, subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) of severely obese patients with and without MetS.

Methods: The study included 37 severely obese patients (BMI ≥ 40 kg/m²), 19 with MetS (MetS+ group) and 18 without (MetS− group), studied before and during bariatric surgery. Before the day of surgery, urinary free cortisol (UFC) and diurnal variation of serum and salivary cortisol were estimated. During surgery, biopsies of the liver, VAT and SAT were obtained. The expression of HSD11B1, NR3C1α and NR3C1β was evaluated by RT-PCR.

Results: UFC and area under the curve for 24-h profiles of serum and salivary cortisol were lower in the MetS− group. In the MetS− group, mRNA levels of HSD11B1 in liver exhibited a negative correlation with liver NR3C1α (LNR3C1α) and VAT expression of HSD11B1 was lower than the MetS+ group.

Conclusions: We observed a downregulation of the NR3C1α expression and lower VAT mRNA levels of HSD11B1 in the MetS− group, indicating a lower selective tissue cortisol production and action that could protect these patients from the metabolic consequences of obesity. In the MetS− group, a lower activity of the HPA axis was also detected. Taken together, cortisol in tissue and systematic level might play a role in the development of MetS in severely obese patients.

Introduction

After Reaven (1) first described syndrome X, 26 years ago, metabolic syndrome (MetS), as it is defined nowadays, demonstrates a notable increasing prevalence, due to a worldwide epidemic of obesity and diabetes (2). Thus, justifiably, it consists, along with obesity, a major field of research and controversies. As it is well known, MetS is recognised as a major and prevalent risk factor for cardiovascular disease (CVD) by the World Health Organization (WHO) (3), the National Cholesterol Education Program–Adult Treatment Panel III (NCEP–ATP III) (4) and the International Diabetes Federation (IDF) (5), pointing out the growing necessity to clarify its pathophysiology and subsequently to establish effective treatment.

During the last few years, the resemblance of the clinical signs, symptoms and biochemical findings between Cushing’s syndrome and MetS (6, 7, 8) has been highlighted, suggesting that cortisol may play a key role in all these entities. However, idiopathic obese patients have normal circulating levels of cortisol, indicating that...
research needs to focus on the tissue level and the intracellular metabolism of glucocorticoids.

It is well known that the intracellular glucocorticoid metabolism is regulated at a pre-receptor level by the activity of two isoforms of the 11β-hydroxysteroid dehydrogenase enzyme: Hydroxysteroid (11-beta) dehydrogenase 1 and 2 (HSD11B1 and HSD11B2). The enzyme HSD11B1 promotes the extra-adrenal production of cortisol by catalysing the regeneration from inactive cortisone and thus amplifying glucocorticoid action at the pre-receptor level. HSD11B1 is widely expressed throughout the body, mainly in the liver as well as in other tissues such as the visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT) (9, 10, 11, 12, 13).

The pathogenic role of HSD11B1 in the development of visceral obesity and key features of MetS has been demonstrated in transgenic mice models. In these studies, mice with selective overexpression of HSD11B1 in adipose tissue had increased adipose levels of corticosterone and therefore developed visceral obesity, also exhibiting pronounced insulin-resistant diabetes and hyperlipidaemia (14). Furthermore, in another study, transgenic mice, which were expressing increased HSD11B1 activity selectively in the liver, developed MetS without obesity, which was associated with fatty liver, dyslipidaemia, mild insulin resistance (IR) and increased angiotensinogen expression (15). In the opposite, Hsd11b1-knockout mice did not develop IR or glucose intolerance on hypercaloric diets. They demonstrated an improved lipid and lipoprotein profile, hepatic insulin sensitivity and glucose tolerance, and they resisted hyperglycaemia on obesity or stress (16, 17, 18).

Fat depots can be distinguished, based on their anatomical distribution as VAT (omentum and mesenteric) and SAT. Visceral fat is considered to have a stronger association with the different features of MetS (19, 20, 21, 22).

Obese patients do not always have MetS, thus constituting an intriguing metabolically ‘healthy’ obese phenotype, which is linked with a lower risk of all-cause mortality and CVD compared with obese subjects with MetS (23, 24). Therefore, questions arise about the factors that contribute to the appearance of the metabolic features of MetS in obese patients. Moreover, data regarding the expression of HSD11B1 in adipose tissue of obese patients remains conflicting.

The aim of this study was to evaluate the activity of the hypothalamic–pituitary–adrenal (HPA) axis and the expression of HSD11B1 and nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptors) α (NR3C1α) and β (NR3C1β) in SAT, VAT and the liver of severely obese patients with or without MetS, and to investigate the possible associations and discrepancies relating to MetS.

Patients and methods

Patients

Before bariatric surgery, 37 severely obese patients (BMI ≥ 40 kg/m²) were recruited prospectively in the University Hospital of Patras (Greece) and were enrolled in the study. All participants underwent a complete clinical (including anthropometric measurements), biochemical and endocrine assessment before surgery, which included an oral glucose tolerance test with 75 g of glucose and measurement of the fasting insulin and C-peptide, baseline adrenocorticotropic (ACTH), thyrotrophin (TSH), tri-iodothyronine (T3), thyroxine (T4) and urinary free cortisol (UFC) (24-h urine collection). Moreover, serum and salivary cortisol levels were measured at scheduled times (0800 h, 1400 h, 1800 h, and 2300 h).

The patients were divided into two groups, MetS— and MetS+, according to the presence of MetS as defined by the IDF criteria (5). The MetS— group consisted of 18 patients (mean age 34.7 ± 8.3 years, BMI 53.7 ± 7.7 kg/m², five (28%) males, 13 (72%) females), whereas the MetS+ group consisted of 19 patients (mean age 44.1 ± 9.6 years, BMI 52.4 ± 5.8 kg/m², nine males (47%), ten females (53%)). All patients underwent bariatric surgery. None of these patients received any medication known to interfere with the regulation of HSD11B1 or the HPA axis.

IR was evaluated by the homeostatic model assessment (HOMA) using the following equation: fasting insulin (µIU/ml) × fasting plasma glucose (mg/dl)/405. Insulin sensitivity was also assessed by the quantitative insulin sensitivity check index (QUICKI) using the formula: 1/(log (fastingglucose mg/dl)).

Body composition was estimated by bioelectrical impedance (BC-418 Segmental Body Composition Analyzer; Tanita Europe B.V., Amsterdam, The Netherlands).

A signed consent was obtained from all patients and the protocol was approved by the Ethical Committee of the University Hospital of Patras (Greece).

Biochemical measurements/hormone assays

Glucose, urea, creatinine, electrolytes, cholesterol, triglycerides (TG), and liver chemistry, which included serum transaminases (alanine aminotransferase, aspartate aminotransferase), serum alkaline phosphatase, γ-glutamyl

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transpeptidase, bilirubin and albumin, were measured by an enzymatic colorimetric assay using an automated clinical chemistry analyser. Serum, salivary and urinary cortisol, ACTH, T₃, T₄, insulin and C-peptide levels were measured by chemiluminescence immunoassays (Modular Analytics E170; Roche Diagnostics). More specifically for urinary cortisol, 600 µl of urine and 3.0 ml of dichloromethane were mixed in a glass tube for 7 min. Then it was centrifuged for 5 min at 2500 g to separate the phases. The aqueous phase and possible residues at the phase interface were removed and discarded. The dichloromethane phase (1.5 ml) was transferred into a clean glass tube and, under a hood, it was reduced until dry by exposing it to a gentle nitrogen flow. The dry residue was reconstituted with 300 µl of diluent universal and incubated for 30 min at 15–25 °C while occasionally mixing it four times for 1 min in a rotating shaker. Finally, the reconstituted sample was analysed by chemiluminescence immunoassays (Modular Analytics E170; Roche Diagnostics). Saliva was collected into a commercially available device, a Salivette (Sarstedt, Numbrecht, Germany), using a cotton swab chewed on for 2–3 min, which was then inserted into a double-chamber plastic test tube. The intra-assay co-efficient of variation was 1.9–2% for insulin, 1.3–4.6% for C-peptide, 2–2.9% for ACTH, 1.8–8.6% for TSH, 2.5–4.7% for T₄ and 3.6–5.3% for T₃. Moreover, the intra-assay co-efficient of variation was 1.1–1.3% for serum cortisol, 1.5–6.1% for salivary cortisol and 2.2–2.9% for urinary cortisol. The inter-assay co-efficient of variation was 2.5–2.8% for insulin, 1.8–5% for C-peptide, 2.4–5.4% for ACTH, 3.3–8.7% for TSH, 3–6.9% for T₄, 4.7–5.4% for T₃, 1.5–1.6% for serum cortisol, 4.1–33.4% for salivary cortisol and 1.8–3.2% for urinary cortisol.

Fat and liver biopsies

During surgery, biopsies of the VATs and SATs as well as the liver biopsies were obtained from all patients during the first 30–60 min after the skin incision. The samples were immediately placed in sample containers containing RNAlater RNA Stabilisation Reagent (Qiagen) and stored at −80 °C until further analysis.

RNA extraction and RT

Total RNA was extracted from 100 mg of VAT and 100 mg of SAT (RNeasy Lipid Tissue kit; Qiagen). Total RNA was also extracted from 100 mg of liver (RNeasy mini kit; Qiagen). The samples were incubated with DNase (Ambion, Austin, TX, USA) and then quantified using a Nanodrop-1000 spectrophotometer (NanoDrop, Fisher Thermo, Wilmington, DE, USA) and an MX3000p real-time (RT) PCR system (Stratagene, La Jolla, CA, USA) according to the instructions of the manufacturers. The RNA was checked by electrophoresis on 1% (w/v) agarose gels and then it was stored at −80 °C. A total of 1.8 µg of RNA was reverse transcribed into cDNA using 100u of Superscript III Reverse Transcriptase (Life Technologies), 300 ng of random primers (Foundation for Research and Technology-Hellas, Crete, Greece) and 5 nM dNTPs (Stratagene) in a final volume of 50 µl. A no enzyme control was used to ensure that the RNA was DNA-free. cDNA was stored at −20 °C until PCR was performed.

PCR for HSD11B1 and NR3C1α/β

The levels of expression of HSD11B1, NR3C1α, NR3C1β and TATA box-binding protein (TBP) were quantified using the intercalating dye SYBR Green I in a Kapa Sybr Fast MasterMix (KAPA Biosystems, Woburn, MA, USA) and the following gene-specific primers: NR3C1α forward: ACCACCCAGCCTCCAAAG, NR3C1α reverse: TTTCACTCCAGCCAACGTGTG; NR3C1β forward: CAGCGGTTTTATCAACTGAC, NR3C1β reverse: TGTGAGATGTGCTTTCTGG; HSD11B1 forward: AGCATGTGTGTCGTCCTCTC, HSD11B1 reverse: GAACCCTATCCCCAGAAC; TBP forward: AAAGCAATGTCACCTCCT; TBP reverse: GGTTCGTGGCTCTCCTTATCC). All primers were synthesised by Metabion ( Martinsried, Germany). The reactions contained 5-carboxy-x-rhodamine as a passive reference dye and cDNA equivalent to 75 ng of total RNA. The PCR was performed in triplicate using an MX3000p RT-PCR system (Stratagene). The results were analysed using the LinReg PCR analysis programme. The mRNA levels of NR3C1α, NR3C1β and HSD11B1 were normalised to the level of TBP, a transcription-related gene that has been shown to be a suitable reference gene (because of the lowest RNA transcription range over all tissues) and has been used in many studies (25, 26).

The mRNA levels of HSD11B1, NR3C1α, NR3C1β and TBP were expressed in arbitrary units (AU) as an output of LinReg programme.

Statistical analyses

All values were expressed as the mean ± S.D., and statistical significance was set at P<0.05. The normality of variables was examined by Shapiro–Wilk and one-sample Kolmogorov–Smirnov tests. Mean values were compared among the groups by one-way ANOVA and post hoc analysis using the least significant difference (LSD) test, and by independent-samples t-test or Mann–Whitney U test for variables.
Table 1  Baseline characteristics of patients. Data presented as number of cases (n) or as mean ± s.d.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>MetS +</th>
<th>MetS −</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>37</td>
<td>19</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39.5 ± 10</td>
<td>44.1 ± 9.6</td>
<td>34.7 ± 8.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Gender (Males/females)</td>
<td>14/23</td>
<td>9/10</td>
<td>5/13</td>
<td>NS</td>
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<td>BMI (kg/m²)</td>
<td>53 ± 6.7</td>
<td>52.4 ± 5.8</td>
<td>53.7 ± 7.7</td>
<td>NS</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>142.7 ± 15.7</td>
<td>146.1 ± 17.5</td>
<td>139.2 ± 13.2</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>114.5 ± 41.1</td>
<td>129.8 ± 52.6</td>
<td>98.2 ± 10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>147.5 ± 100.1</td>
<td>195.1 ± 118.9</td>
<td>97.3 ± 32</td>
<td>&lt;0.01</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>44 ± 8.1</td>
<td>41.5 ± 6.8</td>
<td>46.7 ± 8.7</td>
<td>&lt;0.05</td>
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<tr>
<td>SBP (mmHg)</td>
<td>125.7 ± 14.9</td>
<td>133.7 ± 14.2</td>
<td>117.2 ± 10.5</td>
<td>&lt;0.01</td>
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<td>DBP (mmHg)</td>
<td>80.1 ± 7.1</td>
<td>83.2 ± 6.5</td>
<td>76.9 ± 6.5</td>
<td>&lt;0.01</td>
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<td>BSCORT (µg/ml)</td>
<td>13.74 ± 6.55</td>
<td>16.6 ± 7.2</td>
<td>10.7 ± 4.1</td>
<td>&lt;0.01</td>
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<td>BSLVCORT (µg/ml)</td>
<td>0.67 ± 0.38</td>
<td>0.87 ± 0.4</td>
<td>0.46 ± 0.21</td>
<td>&lt;0.01</td>
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<td>UFC (µg/24h)</td>
<td>95.46 ± 90.4</td>
<td>116.8 ± 106.6</td>
<td>71.3 ± 62.7</td>
<td>&lt;0.05</td>
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<td>Baseline ACTH (µg/ml)</td>
<td>35.98 ± 30</td>
<td>33.04 ± 17.61</td>
<td>39.08 ± 39.47</td>
<td>NS</td>
</tr>
<tr>
<td>AUCS CORT (µg/ml h)</td>
<td>159.18 ± 56.14</td>
<td>177.5 ± 61.5</td>
<td>139.8 ± 43.5</td>
<td>&lt;0.05</td>
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<tr>
<td>AUCSLVCORT (µg/ml h)</td>
<td>7.83 ± 3.73</td>
<td>9.26 ± 4.03</td>
<td>6.32 ± 2.75</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>TSH (µU/ml)</td>
<td>2.5 ± 1.69</td>
<td>1.93 ± 1.75</td>
<td>3.1 ± 1.43</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>T₄ (µg/dl)</td>
<td>11.77 ± 14.53</td>
<td>9.90 ± 2.03</td>
<td>13.75 ± 20.85</td>
<td>NS</td>
</tr>
<tr>
<td>T₃ (ng/ml)</td>
<td>3.94 ± 15.73</td>
<td>1.28 ± 0.33</td>
<td>6.74 ± 22.53</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Insulin (µIU/ml)</td>
<td>27.82 ± 18.34</td>
<td>31.73 ± 22.12</td>
<td>23.7 ± 12.61</td>
<td>NS</td>
</tr>
<tr>
<td>C-peptide (ng/ml)</td>
<td>5.75 ± 5.4</td>
<td>7.1 ± 7.24</td>
<td>4.33 ± 1.42</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>7.516 ± 5.508</td>
<td>7.902 ± 5.72</td>
<td>7.108 ± 5.41</td>
<td>NS</td>
</tr>
<tr>
<td>QUICKI</td>
<td>0.298 ± 0.026</td>
<td>0.294 ± 0.025</td>
<td>0.302 ± 0.028</td>
<td>NS</td>
</tr>
</tbody>
</table>

MetS, metabolic syndrome as defined by IDF criteria; MetS +, severely obese patients with MetS; MetS-, severely obese patients without MetS; SBP, systolic blood pressure; DBP, diastolic blood pressure; BSCORT, baseline serum cortisol; BSLVCORT, baseline salivary cortisol; UFC, urinary free cortisol; ACTH, adrenocorticotropic hormone; AUCS CORT, area under the curve of serum cortisol; AUCSLVCORT, area under the curve of salivary cortisol; TSH, thyroid-stimulating hormone; HOMA-IR, homeostatic model assessment-insulin resistance; QUICKI, quantitative insulin sensitivity check index; NS, not significant.

without normal distribution. Correlation coefficients (Spearman) were performed to quantify associations. The data were analysed using SPSS version 21 (SPSS, Inc.). The area under the curve (AUC) was calculated for the serum and salivary cortisol profiles over a 24-h period by the trapezoid rule.

Results

Subject characteristics

Table 1 gives the baseline clinical and biochemical characteristics of the subjects regarding the presence or absence of MetS as defined by the IDF criteria (5).

The baseline serum cortisol (BSCORT, MetS+ 16.6 ± 7.2 µg/ml, MetS− 10.7 ± 4.1 µg/ml; P<0.01), baseline salivary cortisol (BSLVCORT, MetS+ 0.87 ± 0.4 µg/ml, MetS− 0.46 ± 0.21 µg/ml; P<0.01) and UFC (MetS+ 116.8 ± 106.6 µg/24 h, MetS− 71.3 ± 62.7 µg/24 h; P<0.05) were significantly higher in the MetS+ group compared with the MetS− group. In the non-obese healthy controls, the normal values for BSCORT are 6.2–19.4 µg/dl, for BSLVCORT <0.69 µg/dl and for UFC 36–137 µg/24 h. The AUC of serum cortisol (AUCS CORT, MetS+ 177.5 ± 61.5 µg/ml h, MetS− 139.8 ± 43.5 µg/ml h; P<0.05) and the AUC of salivary cortisol (AUCSLVCORT, MetS+ 9.26 ± 4.03 µg/ml h, MetS− 6.32 ± 2.75 µg/ml h; P<0.05) were also significantly higher in the MetS+ group than in the MetS− group.

Serum TSH (Mann–Whitney U test; MetS+ 1.93 ± 1.75 µIU/ml, MetS− 3.1 ± 1.43 µIU/ml; P=0.01) and T₄ (Mann–Whitney U test; MetS+ 1.28 ± 0.33 ng/ml, MetS− 6.74 ± 22.53 ng/ml; P=0.005) levels were significantly lower in the MetS+ group compared with MetS− group. T₃ levels did not differ between groups. ACTH, insulin and C-peptide levels, as well as HOMA-IR, were similar between groups.

Correlations of the HPA axis parameters with MetS components

BSCORT and BSLVCORT were positively correlated with the TG (TG-BSCORT, r=0.433; P=0.007 and TG-BSLVCORT, r=0.316; P=0.057), waist circumference (WCirc) (WCirc-BSCORT, r=0.330; P=0.046 and WCirc-BSLVCORT, r=0.500; P=0.002) and the systolic blood pressure (SBP) (SBP-BSCORT, r=0.349; P=0.034 and SBP-BSLVCORT, r=0.476; P=0.003) (Fig. 1).

The WCirc was positively correlated with ACTH (r=0.408; P=0.012) and AUCS CORT (r=0.319; P=0.054). The TG were also positively correlated with
between groups. The mean \( HSD11B1 \) mRNA levels, independently of MetS designation, were significantly lower in the liver (2.186 ± 0.481 AU) than in SAT (2.976 ± 1.122 AU) and VAT (3.234 ± 2.381 AU) (one-way ANOVA, \( post \ hoc \) analysis by LSD test; \( P<0.05 \)) (Fig. 4).

The expression of \( NR3C1\alpha \) in all of the tissue samples was similar between the two groups. The mean \( NR3C1\alpha \) mRNA levels, independently of MetS designation, were significantly lower in the liver (2.186 ± 0.481 AU) than in SAT (2.976 ± 1.122 AU) and VAT (3.234 ± 2.381 AU) (one-way ANOVA, \( post \ hoc \) analysis by LSD test; \( P<0.05 \)) (Fig. 4).

The expression of \( NR3C1\beta \) was not detected either in the visceral, SAT or hepatic tissue in either group.

In the MetS− group, the expression of \( HSD11B1 \) in the liver (LHSD11B1) exhibited a negative correlation with the liver \( NR3C1\alpha \) (LNR3C1\alpha) (\( r = -0.494; \ P = 0.037 \)) (Fig. 5), whereas no association was found in the MetS+ group.

**Figure 1**
Correlations of BSCORT and BSLVCORT with MetS characteristics. Bivariate correlations were established by the Spearman’s \( p \)-test. BSCORT, baseline serum cortisol; BSLVCORT, baseline salivary cortisol; TG, triglycerides; SBP, systolic blood pressure.

**Figure 2**
Correlations between hypothalamic–pituitary–adrenal (HPA) axis parameters with MetS components. Bivariate correlations were established by the Spearman’s \( p \)-test. AUCSCORT, area under the curve of serum cortisol; AUCSLVCORT, area under the curve of salivary cortisol; UFC, urinary free cortisol; ACTH, adrenocorticotropic hormone; SBP, systolic blood pressure; WCirc, waist circumference; TG, triglycerides; MetS, metabolic syndrome.
Moreover, in SAT, the mRNA levels of \textit{HSD11B1} were positively correlated with \textit{NR3C1\textalpha} expression ($r=0.505; P=0.033$). In the VAT, an association between \textit{HSD11B1} and \textit{NR3C1\textalpha} was not detected.

Correlations of \textit{HSD11B1} and \textit{NR3C1\textalpha} mRNA levels with MetS components

In severely obese patients with or without MetS, visceral \textit{HSD11B1} mRNA levels (VAT \textit{HSD11B1}) were positively correlated with the TG ($r=0.388; P=0.018$) and WCirc ($r=0.346; P=0.036$) (Fig. 6), both major components of MetS.

\textit{LHSD11B1} mRNA levels were positively correlated with the fasting glucose ($r=0.341; P=0.039$), WCirc ($r=0.383; P=0.019$) and BMI ($r=0.443; P=0.006$); LNR3C1\textalpha\ mRNA levels were inversely correlated with BMI ($r=-0.391; P=0.017$) (Fig. 7).

Other correlations

Regardless of group (MetS+ or MetS−), IR, as it was estimated by the HOMA, was positively correlated with TG ($r=0.440; P=0.006$) and negatively correlated with HDL ($r=-0.376; P=0.022$). Insulin sensitivity, as it was determined by the QUICKI, was positively correlated with HDL ($r=0.404; P=0.013$), whilst exhibiting a negative correlation with TG ($r=-0.472; P=0.003$) (Fig. 8).

Moreover, the TG were positively correlated with insulin ($r=0.409, P=0.012$) and C-peptide ($r=0.500, P=0.002$) levels (Fig. 9).

Discussion

In this study, we evaluated the expression of the enzyme \textit{HSD11B1} and the \textit{NR3C1\textalpha}, \textit{NR3C1\textbeta} in the liver, VAT and SAT, as well as the activity of the HPA axis in severely obese patients with and without MetS.

We found that the HPA axis was activated in severely obese patients with MetS. Also, the expression of VAT \textit{HSD11B1} was higher in MetS+ group, whereas in MetS− group a negative correlation of the liver \textit{HSD11B1} mRNA levels with the LNR3C1\textalpha\ was observed.

Independently of the MetS presence, the \textit{HSD11B1} mRNA levels were significantly higher in the liver than in SAT and VAT, a finding consistent with another study (13). This dominant expression of \textit{HSD11B1} in the liver may serve the important role of glucocorticoids in the regulation of gluconeogenic enzymes, hepatic glucose production and glycogen stores (27, 28). Glucocorticoids, through classical glucocorticoid-induced promoters, stimulate the transcription of crucial hepatic gluconeogenic enzymes, particularly of phosphoenolpyruvate carboxykinase and glucose 6-phosphatase (G6Pase).
cortisol exerts intracellular actions by binding to this receptor. In addition, the LNR3C1α expression exhibited a negative correlation with the BMI. Taken together the downregulation of these receptors could represent a compensatory mechanism preventing the appearance of MetS in severely obese patients. In our study, the expression of NR3C1α in all tissue samples was similar between the two groups (MetS+ and MetS), whereas in another study (35) it was demonstrated that the expression of NR3C1α in the liver is higher in morbidly obese patients with MetS in comparison with those without. Furthermore, the mean NR3C1α mRNA levels, independent of the MetS designation, were significantly lower in the liver than in the SAT and VAT. In both groups NR3C1β expression was not detected in any of the tissue samples.

Also, the HSD11B1 mRNA levels in the VAT (VAT HSD11B1) were significantly higher in the MetS+ group when compared with the MetS− group, while there was no difference between groups in the expression of HSD11B1 in the SAT and the liver. The results are conflicting when comparing HSD11B1 mRNA levels in the adipose tissue and liver of obese patients with or without MetS. In contrast to our results, Baudrand et al. (13) did not find differences in HSD11B1 expression between obese subjects with and without MetS, while Torrecilla et al. (35) observed higher mRNA levels of HSD11B1 in the liver of patients with MetS. Nevertheless, this particular finding of ours is in line with previous studies, in animal models and more particularly in transgenic mice, where overexpression of the HSD11B1 selectively in adipose tissue led to full MetS, with visceral obesity, hypertension, dyslipidaemia and IR (14). These effects were probably induced by increased levels of FFA and cortisol delivered to the liver via the portal vein (14).

**Figure 5**
Correlation of LNR3C1α with LHSD11B1 in MetS− group. Bivariate correlation was established by the Spearman’s p-test. Statistical significance was set at P<0.05. LHSD11B1, liver hydroxysteroid (11-beta) dehydrogenase 1; LNR3C1α, liver nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) α; MetS, metabolic syndrome; AU, arbitrary units. (29, 30). Thus, as was expected, in this study, LHSD11B1 was found to be positively correlated with fasting glucose. G6Pase also shares its substrate (glucose-6-phosphate) with hexose-6-phosphate-dehydrogenase (H6PDH). H6PDH is an endoplasmic reticulum enzyme that generates cofactor NADPH and thus ameliorates NADPH-dependent HSD11B1 reductase activity (31, 32, 33). The significance of H6PDH was demonstrated in a study in which transgenic mice that were overexpressing H6PDH in the adipose tissue exhibited fasting hyperglycaemia, glucose intolerance with IR and elevated circulating free fatty acids (FFA) levels (34). Moreover, hepatic HSD11B1 mRNA levels were positively associated with the WCirc and BMI, suggesting that visceral obesity could induce an increase in the expression of LHSD11B1, which in turn contributes to the appearance of MetS, as is demonstrated in several animal studies (14). However, some obese subjects do not develop MetS, representing a distinguished phenotype of metabolically healthy obese subjects. Why this happens is a question we will try to answer hereinafter by suggesting some hypothetical mechanisms.

First of all, in the MetS− group the liver mRNA levels of HSD11B1 (LHSD11B1) were negatively correlated with those of NR3C1α (LNR3C1α). This negative correlation could work as counter-regulatory mechanism in the appearance of MetS in severely obese patients, because cortisol exerts intracellular actions by binding to this receptor. In addition, the LNR3C1α expression exhibited a negative correlation with the BMI. Taken together the downregulation of these receptors could represent a compensatory mechanism preventing the appearance of MetS in severely obese patients. In our study, the expression of NR3C1α in all tissue samples was similar between the two groups (MetS+ and MetS), whereas in another study (35) it was demonstrated that the expression of NR3C1α in the liver is higher in morbidly obese patients with MetS in comparison with those without. Furthermore, the mean NR3C1α mRNA levels, independent of the MetS designation, were significantly lower in the liver than in the SAT and VAT. In both groups NR3C1β expression was not detected in any of the tissue samples.
Moreover, FFA are also known to cause providing a continuous source of energy (ATP) and hepatic IR. In addition, FFAs accelerate gluconeogenesis by overproduction of cortisol locally, which in turn increases visceral obesity, IR, diabetes mellitus, dyslipidaemia, hypertension and premature vascular mortality. We also found a strong correlation between the ACTH and baseline serum cortisol with the components of MetS, such as the WCirc, TG and SBP. This observation confirms the fact that there is an over activity of the HPA axis in patients with MetS as demonstrated in our previous study (45) and other studies (42).

A limitation of our study was that the MetS+ patients were ~10 years older than the patients without MetS. We cannot exclude the possibility that some of the MetS− patients could become MetS+ due to ageing or behavioural and environmental influences during the following years. However, in our study, we did not find any correlation between age and the studied parameters. Also, in other studies (46, 47), no correlation was found between human tissue HSD11B1 expression and age. Moreover, it is known that the hyperactivity of the HPA axis is reported mainly in the elderly people and there are no significant differences during adulthood (48, 49).

The overexpression of HSD11B1 in the VAT leads to the overproduction of cortisol locally, which in turn increases the levels of FFA because it is known that glucocorticoids enhance lipolysis by upregulating the expression of the lipoprotein lipase (36) and the hormone-sensitive lipase (37). Considerable evidence from other studies (38) suggests that in patients with MetS, increased FFA draining from the VAT into the portal circulation contributes to hepatic IR. In addition, FFAs accelerate gluconeogenesis by providing a continuous source of energy (ATP) and substrate (39). Moreover, FFA are also known to cause systematic IR by inhibiting insulin-stimulated glucose uptake and glycogen synthesis, mainly on the site of skeletal muscles (40). Thus as was expected, in this study we found that IR, estimated by the HOMA-IR, was positively correlated with TG. We also found that VAT HSD11B1 was positively correlated with the TG levels and WCirc, both strong components of MetS. Therefore, according to our findings, we can speculate that VAT HSD11B1 overexpression is associated with the appearance of MetS in severely obese patients.

The baseline serum, salivary cortisol and UFC were significantly higher in the MetS+ group compared with the MetS− group. Also, AUC of serum cortisol and AUC of salivary cortisol were significantly higher in the MetS+ group than in the MetS− group. These findings are in accordance with numerous of other studies (41, 42, 43, 44, 45). As can be seen in Cushing’s syndrome, glucocorticoid excess can cause visceral obesity, IR, diabetes mellitus, dyslipidaemia, hypertension and premature vascular mortality. We also found a strong correlation between the ACTH and baseline serum cortisol with the components of MetS, such as the WCirc, TG and SBP. This observation confirms the fact that there is an over activity of the HPA axis in patients with MetS as demonstrated in our previous study (45) and other studies (42).

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Figure 9
Correlations of TG with insulin and C-peptide. Bivariate correlations were established by the Spearman’s ρ-test.

According to our findings, it seems that in severely obese patients without MetS, the downregulation of NR3C1 in the liver as well as the lower expression of HSD11B1 in the VAT might protect them from the development of metabolic consequences of their obesity. Further studies are needed to investigate the exact role of cortisol production and action in tissue level, in order to clarify its contributing role in the development of MetS in severely obese patients.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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