Expression of Neuropeptide Y, Omentin and Visfatin in Visceral and Subcutaneous Adipose Tissues in Humans: Relation to Endocrine and Clinical Parameters

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Summary
Objective: We aimed at exploring the expression of neuropeptide Y (NPY), omentin and visfatin in adipose tissues of adults along with clinical parameters and hormones. Methods: We included 168 adult patients (31 surgical obese patients and 31 surgical controls, 76 non-surgical obese patients, 30 non-surgical controls). We measured plasma NPY (by radioimmunoassay), cortisol (with an electrochemiluminescence immunoassay) and urinary cortisol metabolites (by gas chromatography/mass spectrometry). Expression of NPY, omentin and visfatin in subcutaneous and visceral adipose tissue specimens of the surgical patients was quantified using real-time PCR. Results: NPY was detectable in adipose tissue specimens and, like plasma NPY concentrations, comparable between groups. Omentin gene expression was higher in visceral than in subcutaneous adipose tissues (p < 0.0001). Visfatin expression was lower in the subcutaneous tissue of obese patients compared with controls (p < 0.05). Cortisol was lower in obese adults compared with controls (136.5 ± 74.1 vs. 162.2 ± 56.1 ng/ml; p < 0.05), cortisol metabolites were comparable between groups. Conclusion: In our obese adults, plasma NPY levels and the glucocorticoid measures were not elevated. Even though the expression of NPY, omentin and visfatin was comparable between obese individuals and controls, we have to consider differences in the total production rate of adipose tissue-derived factors.

Introduction
Obesity is associated with an increased risk for co-morbidities, principally cardiovascular diseases, type 2 diabetes, degenerative joint disease and also certain cancers [1, 2]. The prevalence of obesity is increasing worldwide and affecting ever younger age groups [3–5]. Basically, treatment options include diet therapy, physical exercise, lifestyle changes and, if indicated, other strategies such as drug therapy and surgical treatment. Laparoscopic gastric banding (LAGB) is a surgical option for extreme obesity in adults with a BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² together with co-morbidities. With bariatric surgery, weight loss of up to 70% of mean excess weight can be achieved in extremely obese patients [6]. In a former study, we have described the metabolic and endocrine profiles of obese patients treated with LAGB compared with normal-weight controls, focusing on leptin and ghrelin plasma concentration and gene expression in their adipose tissues. We could demonstrate in adipose tissue that the expression of anorexigenic leptin is weight-course dependent, but the expression of orexigenic ghrelin is not [7].

In this study we focus on adipose tissue-derived neuropeptide Y (NPY), omentin and visfatin along with the hormonal profiles of NPY, glucocorticoid measures and clinical parameters in obese patients and controls.

NPY consists of 36 amino acids and is one of the most widely distributed neuropeptides in the central nervous system and peripheral sympathetic nervous system [8, 9]. High concentrations of NPY are found in the brain, especially in the hypothalamus, the nucleus accumbens and the amygdala [10, 11]. It is of interest to know that NPY is also expressed and secreted by adipocytes [12]. NPY is an important orexi-
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Obesity is accompanied by increased levels of corticosterone [26], a glucocorticoid particularly active in rodents. 11β HSD1 overexpression in murine adipose tissue results in visceral obesity, insulin-resistant diabetes mellitus, hyperlipidemia and hyperphagia despite hyperleptinemia [26]. A tissue-specific deregulation of cortisol metabolism, such as an increased adipocyte 11β HSD1 activity, may therefore be involved in the etiology of visceral obesity and the metabolic syndrome.

This study was designed to investigate the expression of NPY, omentin and visfatin in visceral and subcutaneous adipose tissues of obese adults and controls and their possible correlation with clinical parameters such as BMI and blood pressure (BP). We also aim to determine the correlation between these adipose tissue-derived parameters on the one hand and blood NPY, cortisol and the glucocorticoid bioavailability, as reflected in the urinary (allo-THF + THF)/THE ratio, on the other hand.

Participants and Methods

Participants
Our study was approved by the Ethics Committee of the Friedrich-Alexander-University Erlangen-Nuremberg, Germany. The patients gave informed consent prior to the study. Generally, 168 adult patients were recruited and divided into 4 subgroups (surgical obese patients and surgical controls, non-surgical obese patients and non-surgical controls). In detail, we included 31 obese adults (16 women and 15 men, group A; table I), who underwent LAGB, and 31 age-matched controls (7 women and 24 men, group B), who underwent laparoscopic fundoplication. In addition to these operative subgroups, we included 2 non-operative subgroups consisting of 76 obese patients (52 women and 24 men, group C) and 30 normal-weight (8 women and 22 men, group D) non-surgical adults of whom we could study merely blood and urine samples.

None of the patients suffered from tumors, infectious or psychiatric diseases. Nine obese patients in group A had type 2 diabetes treated with insulin, metformin or glipizidamide. All subjects with type 2 diabetes had fasting glucose concentrations < 6.9 mmol/l whilst on treatment. All obese subjects had severe obesity (morbid obesity, obesity III) with a BMI > 40 kg/m² or a BMI > 35 kg/m² (obesity II) and concomitant type 2 diabetes.

Clinical Parameters
Clinical data were compiled during routine outpatient visits or inpatient treatment. In every patient arterial BP was taken at rest using the Dinamap device (Vital Daten Monitor 1846SX, Critikon, Norderstedt, Germany); weight and height were measured, and BMI calculated as body weight (kg) divided by height (m) squared.

Blood and Urine Samples, Adipose Tissue Specimens
Blood was collected in the fasted state at the same time with early morning urine samples. Samples for NPY analysis were transported on ice. After centrifugation, plasma samples were kept frozen at −20 °C till further analysis, as were urine and serum samples. Adipose tissue specimens of 62 surgical adult patients were investigated (group A, B). 31 obese patients had LAGB (Adjustable Gastric Banding System; BioEnterics Corp., Carpinteria, CA, USA), 31 control subjects received laparoscopic fundoplication because of gastroesophageal reflux disease, chronic gastritis and Barrett’s esophagus, similar to LAGB regarding duration of the anesthetic and operative procedure. Visceral adipose tissue specimens

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were taken from the region of the perigastric fat tissue, and subcutaneous fat tissue samples were taken from the incision site at the trunk. In our non-surgical cohort (group C, D) we could study blood and urine samples only.

**NPY Radioimmunoassay**

NPY was measured by a radioimmunoassay which was previously established by our group [27, 28]. Briefly, plasma specimens were extracted after dilution with 1% trifluoroacetic acid using octadecylsilica cartridges (Sep-Pak, Millipore, Eschborn, Germany), and extracts were prepared and measured by radioimmunoassay as described earlier in detail. This assay has a detection limit of 1 pmol/l and reaches 50% binding at 8 pmol/l NPY. Radioactive NPY was labeled with Iodine 125 (GE Healthcare, Munich, Germany); polyclonal rabbit antiserum was used in a final dilution of 1:100,000 as reported elsewhere [27]. The radioactivity in each tube (Sartstedt, Nurnbrecht, Germany) was assessed in a multi-crystal gamma-counter (Berthold, Bad Wildbad, Germany).

**Analysis of Serum Cortisol and Urinary Steroid Profile**

Morning cortisol levels in human serum were measured using a cortisol reagent kit in conjunction with a Roche Cobas e 411 analyzer (Roche Diagnostics, Mannheim, Germany). Briefly, this assay is a competitive electrochemiluminescence immunoassay with a within-run and between-run imprecision of 1.6–2.4% [30]. For the detection of urinary steroid profiles, we performed gas chromatography/mass spectrometry [31]. In detail, we extracted THE, THF and allo-THF in urine samples by the use of C18 SPE columns (Machery-Nagel, Düren, Germany) and elution with methanol [31]. The eluates were dried and hydrolyzed with β-glucuronidase/arylsulfatase (Roche, Penzberg, Germany) in sodium acetate buffer. Following addition of the internal standards androstadiol, coprostanol and cortisol-d₄, methylxylene-trimethylsilyl ether derivatives were produced by the use of 2% methoxyamine hydrochloride in pyridine and N-methyl-N-trimethylsilyltrifluoroacamide, 1-trimethylsilylimidazole and trimethylchlorosilane. The derivatives were analyzed on a Shimadzu QP5050 gas chromatograph (Shimadzu, Kyoto, Japan) with an integrated mass selective detector and a ZB-5ms column (Phenomenex, Aschaffenburg, Germany). For single ion monitoring we chose the following masses (qualifier ions): m/z 398.5 (408.7, 578.7) THE, m/z 382.5 (652.7, 472.7, 562.7) THF and allo-THF. The interassay coefficients of variation of quality control samples were 10% for THE (mean concentration 2.86 µg/ml), 11% for THF (mean concentration 1.92 µg/ml) and allo-THF (mean concentration 2.11 µg/ml). We here assessed the overall activity of 11β-HSD using the ratio of (allo-THF + THF)/THE.

**Table 1. Distribution of patients’ sex, age, BMI and blood pressure (BP) for the surgical and non-surgical cohorts**

<table>
<thead>
<tr>
<th></th>
<th>Group A operative obese patients</th>
<th>Group B operative controls</th>
<th>Group C non-operative obese patients</th>
<th>Group D non-operative controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>31</td>
<td>31</td>
<td>76</td>
<td>30</td>
</tr>
<tr>
<td>Sex female/male (number)</td>
<td>16/15</td>
<td>7/24</td>
<td>52/24</td>
<td>8/22</td>
</tr>
<tr>
<td>Age, years (mean ± SD)</td>
<td>40.8 ± 10.1</td>
<td>45.0 ± 14.3 (n.s.)</td>
<td>39.1 ± 10.4**</td>
<td>48.1 ± 15.4</td>
</tr>
<tr>
<td>BMI, kg/m² (mean ± SD)</td>
<td>48.2 ± 6.4**</td>
<td>27.7 ± 3.5</td>
<td>49.1 ± 8.8**</td>
<td>26.7 ± 4.1</td>
</tr>
<tr>
<td>Systolic BP, mm Hg (mean ± SD)</td>
<td>142.9 ± 15.3****</td>
<td>128.1 ± 16.8</td>
<td>138.9 ± 15.6**</td>
<td>128.7 ± 17.0</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg (mean ± SD)</td>
<td>88.9 ± 9.6****</td>
<td>80.0 ± 9.3</td>
<td>86.6 ± 9.3*</td>
<td>80.9 ± 8.3</td>
</tr>
<tr>
<td>Visceral adipose tissue samples (number)</td>
<td>31</td>
<td>29</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue samples (number)</td>
<td>31</td>
<td>30</td>
<td>N/A/ N/A</td>
<td>N/A/ N/A</td>
</tr>
</tbody>
</table>

N/A = Not available; n.s. = not significant.

*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 compared with controls.

**Neuropeptide Y, Omentin, Visfatin and Glucocorticoids in Obesity**

**Analysis of Gene Expression of NPY, Omentin and Visfatin in Adipose Tissues**

Adipose tissue specimens (mean weight 0.5 g) were transported in liquid nitrogen and frozen at –80 °C until RNA was isolated. Then we performed reverse transcription of 1 µg of total RNA. In order to monitor gene expression of NPY, omentin and visfatin together with the housekeeping gene β-actin, we used quantitative real-time reverse-transcription PCR [18]. Subsequently, we normalized the quantities of NPY, omentin and visfatin transcripts to the mRNA levels of β-actin. Primer sequences were:

- NPY (GenBank accession: K01911): 5'-CCG AGA ACA TGG CCA GAT ACT-3' (sense), 5'-TCC ATA TCT CTG CCT GGT GAT G3-3' (antisense), fluorogenic probe 5'(FAM)-CGG CGC TGC GAC ACT ACA TCA ACC-(TAMRA)-3' (sense); omentin (GenBank accession: AY549722): 5'-AAC GCC TCC TGT GGT CGA AT3-3' (sense), 5'-GTA TCC TCC ACC AAC GAT GCA-3' (antisense), fluorogenic probe 5'(FAM)-TCA CCG GAT GTA ACA CTG AGC ACC A-(TAMRA)-3' (sense); visfatin (GenBank accession: NM_021524): 5'-GCC GGT GGC ATT AAC GTC TTC TCC-3' (sense), 5'-AAAT CGG CCC TTT TTG GAC C3-3' (antisense), fluorogenic probe 5'(FAM)-AGG ACC CAG TGT CTG ATC CCA ACA AA-(TAMRA)-3' (sense); β-actin (GenBank accession: M10277): 5'-CCG CGA GAA GAT GAC CCA G3-3' (sense), 5'-CCA GTG GTA CGG CCA GAG G3-3' (antisense), fluorogenic probe 5'(FAM)-CCA GCC ATG TAC GTT GCT GTC ACG CAC-(TAMRA)-3' (sense). Each fluorogenic probe was marked with a reporter dye, FAM (6-carboxy-fluorescein), and a quenching dye, TAMRA (6-carboxy-tetramethyl-rhodamine). Further analytical details are reported elsewhere [29].

**Statistical Analysis**

To analyze our data, we used GraphPad Prism software 4.0 (San Diego, CA, USA). If not otherwise stated, values are given as mean ± standard deviation (SD). We calculated Spearman’s correlation coefficient and linear regression with a 95% confidence interval, if applicable. In order to assess differences or similarities, we used the Mann Whitney test (non-parametric t-test). A p value < 0.05 was considered significant.

**Results**

**Clinical Parameters**

Basically, in obese patients, not only BMI was significantly higher compared with controls (p < 0.0001) but also systolic (p < 0.0001) and diastolic (p < 0.05) BP (table 1). Moreover, we found a significant correlation between BMI and BP. In
Plasma NPY Concentrations

In the entire cohort, NPY plasma concentrations ranged from ≤1.0–13.3 pg/ml. In detail, mean NPY plasma concentrations (± SD) were 3.8 ± 2.3 pg/ml for obese adults (range ≤1.0–12.5 pg/ml) versus 3.9 ± 3.5 pg/ml for controls (range ≤1.0–13.3 pg/ml).

To our surprise, there was no significant correlation between plasma NPY concentrations and systolic or diastolic BP, serum cortisol levels, urinary cortisol metabolites or BMI in either group (data not shown).

Serum Cortisol Levels and Urinary Cortisol Metabolites

Circulating cortisol concentrations were somewhat lower in obese than in non-obese patients. In detail, in obese patients, morning serum cortisol levels were 137 ± 74 ng/ml (range 40–375 ng/ml) versus 162 ± 56 for controls (range 70–264 ng/ml), p < 0.05.

Urinary cortisol metabolites were not significantly different between groups. Next, we calculated the urinary (allo-THF + THF)/THE ratio, which reflects the activity of the enzyme 11β HSD.

For obese patients, the (allo-THF + THF)/THE ratio was 1.13 ± 0.58 (range 0.65–3.90) compared to 1.16 ± 0.35 for controls (range 0.60–2.00). This finding suggests that the bioavailability of cortisol within tissues (renal) was not increased in the group of obese patients compared with controls.

Gene Expression of NPY, Omentin and Visfatin in Visceral and Subcutaneous Adipose Tissues and Correlation with Other Parameters

NPY gene expression was found in visceral and subcutaneous adipose tissues of obese or normal-weight adults in comparable quantities (fig. 1). Moreover, it was similar for male and female patients (data not shown). NPY gene expression did not correlate with either systolic/diastolic BP or BMI in our patients. In this study NPY gene expression in adipose tissue did not correlate with NPY plasma concentrations.

Omentin gene expression was much higher in visceral than in subcutaneous adipose tissues of obese and normal-weight adults (p < 0.0001) (fig. 2). Its expression was slightly but not significantly decreased in visceral adipose tissues of obese patients compared with controls.

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Omentin gene expression was much higher in visceral than in subcutaneous adipose tissues of obese and normal-weight adults (p < 0.0001) (fig. 2). Its expression was slightly but not significantly decreased in visceral adipose tissues of obese patients compared with controls. Omentin gene expression did not correlate with either systolic/diastolic BP or BMI in our patients. In this study NPY gene expression in adipose tissue did not correlate with NPY plasma concentrations.
obese adults compared with controls. Omentin gene expression was slightly, but not significantly decreased in visceral adipose tissues of weight adults (p < 0.0001) (fig. 2). Its expression was slightly higher than in subcutaneous adipose tissues of obese and normal-weight controls. NPY and visfatin gene expression in visceral adipose tissue is related with systolic blood pressure (r = –0.7164, p < 0.01) in obese patients than in controls. The visfatin gene expression in visceral compared with subcutaneous adipose tissues. Omentin gene expression was much higher in visceral compared with subcutaneous adipose tissue specimens of adult patients (LAGB = gastric banding group (n = 31); Co = controls (n = 31)). Visfatin expression in adipose tissues was slightly increased in obese patients compared with controls (*p < 0.05).

Discussion

The purpose of our study was to investigate the expression of NPY, omentin and visfatin in visceral and subcutaneous adipose tissues in humans and to analyze a possible correlation with clinical parameters and hormonal profiles in obese patients and controls.

Basically, we found positive correlations between BMI and systolic and diastolic BP, indicative of an increased cardiovascular risk in obese patients [32, 33]. However, in our obese patients, peripheral plasma NPY concentrations were not significantly different from those of the controls. The central expression of NPY is inhibited by leptin; it is supposed that the suppression of NPY results in a reduction of food intake, an increase of energy expenditure and in a change of peripheral metabolic status [34]. However, we can only speculate about the NPY levels in the nervous system and whether our findings may be in line with central leptin resistance as this is beyond the scope of our study. NPY gene expression in adipose tissues was comparable in obese patients and controls and also in men and women. Considering the huge differences in total body fat, total NPY production and tissue concentrations may be higher in obese patients and associated with increased sympathetic nervous activity and increased total peripheral vascular resistance [35]. In our study, NPY plasma concentrations did not differ between groups. One possible reason for this finding could be that our obese patients were otherwise apparently healthy; only 9 obese patients had type 2 diabetes and were treated with antidiabetic drugs. In addition, we did not focus on NPY gene polymorphisms or catecholamine concentrations which could provide us with further information regarding, e.g., sympathetic nerve activity.

Omentin is mainly expressed in visceral adipose tissue [21, 36]. However, we were able to demonstrate omentin gene expression in subcutaneous adipose tissue, too, although at much lower amounts compared with visceral fat. De Souza Batista et al. [22] described a negative correlation between omentin gene expression and BMI. We also found a negative correlation between visceral omentin gene expression and BMI, particularly in obese males. As omentin plasma concentrations are reduced in obese patients [22], a down-regulation of omentin-1 may contribute to the insulin-resistant state in obese individuals, particularly in males with visceral obesity.

Next, we confirmed that visfatin/PBEF/Nampt is expressed in adipose tissue of obese and normal-weight patients. Basically, visfatin is proposed to exert insulin-mimicking effects and to attenuate insulin resistance. It is particularly important for the biosynthesis of nicotinamide adenine dinucleotide [37] and acts as pre-B cell colony enhancing factor [38], but there are contradictory data regarding the mechanisms underlying its putative beneficial effects on insulin sensitivity [25, 39–41]. Whereas Fukuhara et al. [23] reported in 2006 that visfatin had an insulin-mimetic effect, binding to the insulin receptor and leading to the reduction of plasma glucose concentrations, Revollo et al. [25] reported in 2007 that visfatin does not have insulin-mimetic effects neither in vitro nor in vivo, but regulates the biosynthesis of NAD. As a possible explanation for the diverse findings, a very recent study investigated the possibility that single nucleotide polymorphisms (SNPs) in the visfatin gene may be associated with either obesity or type 2 diabetes [42]. Blakemore et al. [42] described one rare SNP, rs10487818, located in intron 4 of the visfatin gene which was associated with severe obesity and had lower allele frequency in controls.

At last, we focused on cortisol metabolites. It has been demonstrated that an increased activity of 11β HSD1, which regenerates cortisol from cortisone, causes hyperphagia along with visceral obesity and metabolic complications in mice [26]. However, it is of interest to show that morning serum cortisol levels were significantly lower in our obese patients than in controls. A previous study has also demonstrated that serum cortisol concentrations may be lower in obese than in normal-weight adults [43]. Excess cortisol, therefore, is not a consistent finding in human obesity, but cortisol production rates may be altered in the course of the disorder. Along these lines a recent study demonstrates that increased 11β HSD-1 gene expression in subcutaneous fat is a consequence rather than cause of obesity, particularly in male patients [44].
Our study corroborates that visceral and subcutaneous adipose tissues produce NPY, omentin and visfatin with only subtle alterations in obese subjects.

As obesity in humans is a complex and multifactorial disease with a huge spectrum, parameters such as adipocytokines, NPY or cortisol must be considered within the frame of a large variety of other biomarkers [45]. Further investigations involving a systems biology approach, combining cell culture techniques (including primary adipocytes) with genomics, proteomics and metabolomics, will clarify links between adipose tissue and metabolic disease.

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Disclosure

The authors declare that there is no conflict of interests

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