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

Stephanie Barth, Peter Klein, Thomas Horbach, Jörg Dötsch, Manfred Rau, Wolfgang Rascher, Ina Knerr

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Adipose tissue · Obesity · Omentin · Neuropeptide Y · Visfatin

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-
genic appetite regulator [12], favoring in particular the intake of carbohydrate-rich foods [13]. In addition to its effect on food intake, NPY displays numerous functions such as vasoconstriction [14] and angiogenesis [15], and exerts neuroendocrine effects on fertility [16]. It has been shown that NPY plasma levels are elevated in patients with primary hypertension [17]. A reduction of NPY labeling, indicating alterations in innervation, is detectable in the ureter tissues of patients with congenital ureteropelvic junction obstruction [18]. Moreover, there is evidence for a critical role for NPY in stress-related exaggeration of abdominal obesity, ‘limo-remodeling’ and the metabolic syndrome [13]. Basically, NPY release is induced by stress. Its expression and secretion is regulated by e.g. sympathetic stimuli and leptin; glucocorticoids also contribute to its actions [13, 16, 19, 20].

Omentin (or omentin-1) is a 313-amino acids peptide and primarily expressed in visceral adipose tissue [21]. In vitro studies have shown that omentin increases insulin signal transduction and enhances glucose transport in human adipocytes. Omentin increases insulin sensitivity [21]. A homologue of omentin-1 was designated as omentin-2. The two genes, omentin-1 and omentin-2, are localized adjacent to each other at 1q22-q23, a chromosomal region recently linked to type 2 diabetes. Omentin plasma levels are reduced in obese patients, together with a reduced expression of both omentin-1 and omentin-2 in visceral fat tissues, as described in a small cohort of 2 men and 18 women [22].

Like omentin, visfatin is an adipocytokine mainly expressed in visceral adipose tissue [23, 24]. Visfatin, a 52-kDa cytokine, is also known as pre-B-cell colony enhancing factor 1 (PBEF1) or nicotinamide phosphoribosyltransferase (Nampt). It enhances cell proliferation and the biosynthesis of nicotinamide mono- and dinucleotide and may also have hypoglycemic effects [24]. In obesity, plasma levels tend to be elevated [23, 24]. In a study with 101 male and female subjects, a significant positive correlation between the amount of visceral fat mass and visfatin plasma levels was found [23]. It has been demonstrated that visfatin is able to bind to the insulin receptor, but visfatin and insulin apparently do not compete for the binding of the insulin receptor, as they ligate to different binding domains [23]. Although visfatin/Nampt/PBEF may induce glucose uptake in skeletal muscle and adipose tissue under certain circumstances, these data remain controversial [23, 25]. Visfatin may exhibit robust nicotinamide adenine dinucleotide biosynthetic activity, which is essential for beta cell function [25].

It is well-known that glucocorticoids play a major role in determining adipose tissue metabolism and distribution. Cortisol is mainly metabolized by the 11β hydroxysteroid dehydrogenase system (11β HSD): Cortisol is activated by 11β HSD1 (from inactive cortisone) and inactivated by 11β HSD2 (by conversion to cortisol).

The overexpression of 11β HSD1 in murine adipose tissue (comparable to the activity found in fat tissue from obese humans) 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 glibenclamide. 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 1.0 pmol/l and reaches 50% binding at 8 pmol/l NPY. Radioactive NPY was labeled with Iodide 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 (Starstedt, 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 androstandiol, coprostanone and cortisol-d₄, methylxoxymethyltrimethylsilyl ether derivatives were produced by the use of 2% methoxamine hydrochloride in pyridine and N-methyl-N-trimethylsilyltrifluoroacetamide, 1-trimethylsilylimidazole and trimethylsilylsilane. 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 (488.7, 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.

**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′-CGG AGA ACA TGG CCA GAT ACT-3′ (sense), 5′-TCC ATA TCT CGT CCT GGT GAT G-3′ (antisense), fluorogenic probe 5′-(FAM)-CGG CGC TGC GAC ACT ACA TCA ACC-(TAMRA)-3′ (sense); omentin (GenBank accession: AY549722); 5′-AAC GCC TCT TGT GGT GCA AT-3′ (sense), 5′-GTA TCC TCC ACC AAT GCA G-3′ (antisense), fluorogenic probe 5′-(FAM)-TCA CCG GAT GTA ACA CTG AGC ACC A-(TAMRA)-3′ (sense); visfatin (GenBank accession: NM_021524); 5′-GGC CTT GGG ATT AAC GTC TTC TTG GAT CAC C-3′ (antisense), fluorogenic probe 5′-(FAM)-AGG ACC CAG TGG CTG ATC CCA ACA AA-(TAMRA)-3′ (sense); β-actin (GenBank accession: M10277); 5′-CGG CGA GAA GAT GAC CCA G-3′ (sense), 5′-CCA GTG GTA CGG CGC GAC G-3′ (antisense), fluorogenic probe 5′-(FAM)-CCA GCC ATG TAC GTT GCT ACG CAC G-(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

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**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>vN/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

N/A = Not available; n.s. = not significant. *Data are given as mean ± SD.

*p < 0.05, ** p < 0.01, ***p < 0.001, ****p < 0.0001 compared with controls.
detail, BMI was positively correlated with systolic BP \((r = 0.3296, p < 0.001)\) and diastolic BP \((r = 0.3183, p < 0.01)\) in the entire cohort.

**Plasma NPY Concentrations**

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

Visfatin expression in adipose tissues was slightly higher in obese than in non-obese patients (LAGB = gastric banding group \((n = 31)\); Co = controls \((n = 31)\)), \(* * * p < 0.0001\) for the omentin gene expression in visceral compared with subcutaneous adipose tissues. Omentin gene expression was slightly, but not significantly decreased in visceral adipose tissues of obese patients compared with controls.

**Fig. 1.** NPY gene expression normalized to β-actin for reference in visceral (vis) and subcutaneous (sc) adipose tissue specimens of adult patients (LAGB = gastric banding group \((n = 31)\); Co = controls \((n = 31)\)); no significant differences between groups.

**Fig. 2.** Omentin gene expression normalized to β-actin for reference in visceral (vis) and subcutaneous (sc) adipose tissue specimens of adult patients (LAGB = gastric banding group \((n = 31)\); Co = controls \((n = 31)\)).

\(* * * * p < 0.0001\) for the omentin gene expression in visceral compared with subcutaneous adipose tissues.
obese adults compared with controls. Omentin gene expres-

but not significantly decreased in visceral adipose tissues of

visceral (vis) and subcutaneous (sc) adipose tissue specimens of adult pa-

****p < 0.0001 for the omentin gene expression in visceral compared with

visceral omentin gene expression and BMI (r = –0.5516,

expression in adipose tissues was comparable in obese pa-

and male subjects (data not shown). Visfatin gene expression

visceral visfatin gene expression and BMI, indicative of an increased car-

similarly, visfatin is proposed to exert insulin-mimicking effects

visceral and subcutaneous adipose tissues. Omentin gene expression was slightly, but

expression in subcutaneous adipose tissues of obese patients compared with controls (*p < 0.05).

Fig. 3. Visfatin gene expression normalized to β-actin for reference in

visceral (vis) and subcutaneous (sc) adipose tissue specimens of adult pa-

therefore, is not a consistent finding in human obesity, but

One possible reason for this finding could be that our

groups. One possible reason for this finding could be that our

obese female (r = 0.6727, p < 0.05) and male patients (r =

basically, we found positive correlations between BMI

visfatin expression in subcutaneous fat tissue for female controls (r = 0.89, p < 0.05) and as a trend for male

controls (r = 0.4361, p = 0.055).

Discussion

The purpose of our study was to investigate the expression of

not significantly different from those of the controls. The

visfatin expression in visceral adipose tissues in humans and to analyze a possible correlation

with clinical parameters and hormonal profiles in obese pa-

patients and controls.

Basically, we found positive correlations between BMI

and systolic and diastolic BP, indicative of an increased car-

diovascular 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 sup-

posed 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 pa-

tients 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

obese patients and 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 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

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 demon-

strated 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 systemic 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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