The Effect of Sodium Restricted Diet on Plasma Visfatin Levels in Hypertensive Patients with Visceral Obesity

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Key Words
Visfatin • Renin-angiotensin-aldosterone system • Hypertension

Abstract
Aim/Background: Experimental and clinical studies revealed contradictory data concerning the influence of renin-angiotensin-aldosterone (RAA) system activation on visfatin release. The aim of the present study was the assessment of the effect of dietary sodium restriction with RAA system activation on visfatin level in hypertensive and normotensive patients with visceral obesity. Methods: The study included 24 hypertensive patients with visceral obesity (12 women) and 22 normotensive subjects with visceral obesity (11 women) constituting the control group. Plasma renin activity, plasma insulin, aldosterone and visfatin levels were determined twice, on normal-salt diet after 6-8 h in recumbent position and the second time after 3 days of dietary sodium restriction and upright position for 2 h. Dietary compliance was controlled by 24 h natriuresis measurement. Results: Hypertensive patients had significantly higher plasma visfatin level than the control group [11.0 (8.5-13.5) vs. 6.8 (6.0-7.6) ng/ml, p=0.003]. Dietary sodium restriction and upright position caused significant increase in PRA and plasma aldosterone level in both groups. While, plasma visfatin level remained unaffected. In the combined group plasma visfatin levels correlated with BMI (r=0.398), waist circumference (r=0.391), glucose (r=0.328), insulin (r=0.663), HOMA-IR (r=0.698), triglycerides (r=0.500) and CRP (r=0.546) but not with percentage of fat mass, percentage of trunk fat, and blood pressure values. Conclusions: 1) Increased plasma visfatin concentration may play a significant role in the pathogenesis of hypertension in patients with visceral obesity. 2) RAA system activation by dietary sodium restriction and upright position has no effect on plasma visfatin levels in subjects with visceral obesity.
Introduction

The results of numerous epidemiological studies revealed that hypertension frequently coexist with obesity. The pathophysiological links between obesity and hypertension include dysregulation of adipokines release, systemic microinflammation, insulin resistance, endothelial dysfunction, increases activity of sympathetic nervous system and renin-angiotensin-aldosterone (RAA) system, impaired secretion of natriuretic peptides and obesity related glomerulopathy development [1].

Increased visceral fat accumulation is associated with suppression of adiponectin and omentin secretion but stimulation of numerous adipokines and cytokines release, including: leptin, resistin, visfatin, tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), retinol binding protein 4 (RBP4) [2]. This dysregulation in obese results in development of systemic microinflammation, insulin resistance and endothelial dysfunction with decreased NO synthesis [3].

Previously published studies revealed decreased circulating levels of adiponectin but increased levels of leptin and visfatin in hypertensive subjects [4, 5]. The role of low adiponectin and increased leptin levels in the development of hypertension has already been established, including impaired vasorelaxation, increased adhesion molecules production, migration and proliferation of vascular smooth muscle cells as well as activation of macrophages and lymphocytes [5, 6]. While, the role of visfatin in the pathogenesis of hypertension remains unknown. It is suggested that visfatin may cause endothelial dysfunction and favor atherosclerosis development by stimulation of TNF-α release [7] and deterioration of insulin resistance. It has also been shown that pharmacological blockade of RAA system influences visfatin release. However, the results of clinical studies are contradictory. Storka et al [8] observed that both ACE-Is and ARBs in diabetic patients increase circulating visfatin levels. Ferrari et al. [9] showed that treatment of hypertensive patients with ARBs but not ACE-Is increase visfatin level. Additionally, Eyileten et al [10] observed decrease of circulating visfatin levels during ACE-Is therapy in patients with diabetic nephropathy. Therefore, we hypothesized that there is a link between RAA system activity and visfatin release. To verify this hypothesis we have applied a non-invasive model of dietary sodium restriction to study the effect RAA system activation.

The aim of the present study was the assessment of the effect of dietary sodium restriction with RAA system activation on visfatin level in hypertensive and normotensive patients with visceral obesity.

Material and Methods

Study subjects

Study included 24 hypertensive patients (12 women) with visceral obesity and 22 normotensive subjects with visceral obesity (11 women) as the control group in similar age, matched for gender. Patients characteristics is presented in Table 1. In all hypertensive subjects the antihypertensive drugs were discontinued 7 days before the beginning of study. Only in patients with blood pressure > 160/100 mm Hg the calcium channel antagonist (Nitrendipine) was used.

The exclusion criteria were as follows: chronic kidney disease (eGFR-MDRD < 60 ml/min/1.73m²) or liver disease, thyroid disease, diabetes, neoplasm, infections, heart failure (NYHA III or IV), RR≥ 180/110 mm Hg, pregnancy and age < 18 years old. Secondary forms of hypertension were excluded based on careful clinical and laboratory workup. Hypertension was defined according WHO criteria (RR ≥ 140/90 mm Hg or using hypertensive drugs). Visceral obesity was scored according EGIR 1999 criteria (waist circumference in men ≥ 94 cm and in women ≥ 80 cm).

Study protocol

Study protocol was accepted by Bioethics Committee of Medical University of Silesia (NN-013-319/02/03). All participants gave the written consent.
In all subjects blood samples for assessment of plasma renin activity (PRA), plasma insulin, aldosterone, adiponectin and visfatin levels were withdrawn twice during the morning: on normal-salt diet and overnight 6-8 hours recumbence and after 3 days of sodium restriction (to 30 mmol/day) in the diet and upright position for 2 hours. Additionally, 24 hours urine collection and 24 hours arterial blood pressure measurement (ABPM) were performed twice in patients on normal-salt diet (before restriction) and 3 days after dietary sodium restriction.

Before salt restriction anthropometric measurements were performed for calculation of body mass index (BMI), waist to hip ratio (WHR) and venous blood was withdrawn for the assessment of serum creatinine, lipids profile (total cholesterol, HDL, LDL and triglycerides), glucose and C-reactive protein (CRP). Additionally, densitometry (DEXA) of total body for assessment of fat mass was performed in each study subject.

**Biochemical examination**

Plasma samples in aliquots for estimation of PRA, insulin, aldosterone and visfatin levels were frozen and stored in -40°C. RIA method was used for estimation of PRA (Immunotech, Prague, Czech Republic), plasma aldosterone (Zen Tech, Angleur, Belgium). Plasma levels of visfatin (Phoenix Pharmaceuticals, Burlingame, USA) were assessed by ELISA method. Plasma insulin level was assessed by electrochemiluminescence method using kits from ROCHE Diagnostics (Mannheim, Germany).

Homeostasis model assessment insulin resistance index (HOMA-IR) was calculated according to the formula: fasting plasma glucose concentration [mmol/l] x fasting plasma insulin concentration

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**Table 1. Characteristics of hypertensive and normotensive subjects with visceral obesity (mean values and 95% confidence intervals)**

<table>
<thead>
<tr>
<th></th>
<th>Hypertensive (N=24)</th>
<th>Normotensive (N=22)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>42 (32-52)</td>
<td>41 (22-59)</td>
<td>NS</td>
</tr>
<tr>
<td>Gender [m/f]</td>
<td>12/12</td>
<td>11/11</td>
<td>NS</td>
</tr>
<tr>
<td>Weight [kg]</td>
<td>84.7 (78.8-90.6)</td>
<td>83.8 (76.7-90.8)</td>
<td>NS</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>30.5 (28.4-32.5)</td>
<td>29.0 (27.4-30.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Waist circumference [cm]</td>
<td>100.5 (94.1-106.9)</td>
<td>96.8 (91.6-102.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Fat content [%]</td>
<td>37.2 (33.5-41.0)</td>
<td>36.2 (31.5-40.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Trunk fat content [%]</td>
<td>36.1 (32.7-39.3)</td>
<td>37.6 (33.1-42.2)</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of hypertension [years]</td>
<td>8.2 (4.2-12.2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypertensive retinopathy [grade 0/I/II]</td>
<td>6/1/7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Number of antihypertensive drugs [n]</td>
<td>2.1 (1.6-2.5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mean systolic blood pressure [mmHg]</td>
<td>127 (128-131)</td>
<td>120 (113-127)</td>
<td>0.05</td>
</tr>
<tr>
<td>Mean diastolic blood pressure [mmHg]</td>
<td>87 (74-82)</td>
<td>71 (68-75)</td>
<td>0.05</td>
</tr>
<tr>
<td>eGFR (MDRD) [ml/min/1.73m²]</td>
<td>88.2 (83.3-93.1)</td>
<td>88.0 (80.5-95.6)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum total cholesterol [mmol/l]</td>
<td>5.5 (5.0-6.0)</td>
<td>4.9 (4.6-5.3)</td>
<td>0.06</td>
</tr>
<tr>
<td>Serum LDL cholesterol [mmol/l]</td>
<td>3.3 (2.8-3.8)</td>
<td>2.9 (2.6-3.1)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum HDL cholesterol [mmol/l]</td>
<td>1.1 (1.0-1.2)</td>
<td>1.3 (1.2-1.5)</td>
<td>0.05</td>
</tr>
<tr>
<td>Serum triglycerides [mg/dl]</td>
<td>2.0 (1.5-2.5)</td>
<td>1.6 (1.3-1.9)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum glucose [mmol/l]</td>
<td>5.0 (4.8-5.3)</td>
<td>5.0 (4.6-5.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma insulin [µU/ml]</td>
<td>13.0 (10.5-15.4)</td>
<td>9.9 (8.2-11.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.9 (2.1-3.6)</td>
<td>2.3 (1.8-2.7)</td>
<td>NS</td>
</tr>
<tr>
<td>HOMA-IR&gt;2.29 [n/%]</td>
<td>14 (58%)</td>
<td>7 (32%)</td>
<td>NS</td>
</tr>
<tr>
<td>Serum CRP level [mg/l]</td>
<td>4.8 (1.7-7.8)</td>
<td>3.9 (2.5-5.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma renin activity (PRA) [ng/ml/h]</td>
<td>1.9 (1.1-2.8)</td>
<td>1.0 (0.6-1.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Serum aldosterone level (Ald) [ng/ml]</td>
<td>20.6 (14.1-27.0)</td>
<td>14.5 (10.4-18.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Ald/PRA</td>
<td>20.7 (10.9-30.4)</td>
<td>28.5 (11.5-45.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Plasma visfatin [ng/ml]</td>
<td>11.0 (8.5-13.5)</td>
<td>6.8 (6.0-7.6)</td>
<td>0.003</td>
</tr>
<tr>
<td>Urinary sodium excretion [mmol/24h]</td>
<td>124 (99-149)</td>
<td>147 (107-187)</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary sodium to creatinine excretion ratio (mmol/10mmol creatinine)</td>
<td>109 (86-132)</td>
<td>138 (105-171)</td>
<td>NS</td>
</tr>
</tbody>
</table>
Measurement of body composition by DEXA

Body composition was measured by dual-energy X-ray absorptiometry (DEXA) using Lunar DPX-L scanner (Lunar Radiation Corporation, USA). This method allows to assess the total fat mass (TFM) and fat mass of the trunk (TTM).

Statistical analysis

Statistical analysis was performed using Statistica 9.0 PL software. The data were presented as mean values and 95% confidence intervals. The χ² test (qualitative variables) and ANOVA, followed by Tukey’s test (quantitative variables) were used. Correlation coefficient was calculated according to Pearson. In all analyses statistical significance was considered achieved at a value of p<0.05.

Results

Both groups of subjects with visceral obesity were comparable in the aspect of parameters describing obesity and visceral obesity (waist circumference, BMI and body fat percentage, trunk fat percentage) - Table 1. Hypertensive patients were characterized by significantly higher insulin levels and PRA. Subjects with insulin resistance (HOMA-IR > 2.29 [11]) were more frequent among hypertensive patients, however the difference was not statistically significant.

Plasma visfatin level was significantly (p<0.05) increased in hypertensive group than in normotensives (Figure 1). Similar visfatin values were observed in males and females in both study groups [11.3 ng/ml (6.6-15.9) vs. 10.7 (7.9-13.5) in hypertensive group and 7.6 (6.2-8.9) ng/ml vs. 6.0 (5.1-6.9) ng/ml in normotensive group, respectively].

As expected, dietary sodium restriction verified by its urinary excretion measurement and upright position caused significant increase of PRA and plasma aldosterone concentration in both study groups (Table 2). Plasma visfatin levels remained unaffected by dietary sodium restriction and upright position in both study groups as well as in the combined group.

Univariate correlation analyses

In the combined group of patients with visceral obesity plasma visfatin levels correlated significantly with BMI (r=0.398, p=0.02), waist circumference (r=0.391, p=0.02), but not with total fat mass, percentage of fat mass, percentage of trunk fat, and blood pressure values. Additionally, there were positive correlations with serum concentration of glucose (r=0.328,
p<0.05), insulin (r=0.663, p<0.001), HOMA-IR (r=0.698, p<0.001), triglycerides (r=0.500, p=0.002) and CRP (r=0.546, p<0.001). There was no correlation between plasma visfatin concentration and both PRA and serum aldosteron levels.

**Discussion**

In the present study we have found the significantly increased plasma visfatin level in hypertensive patients regardless of nutritional status. Until now there are only few published studies, analyzing visfatin levels in hypertensive patients. In the study by Dogru et al [12] enrolling 33 young men with untreated hypertension without obesity, higher plasma visfatin concentration by 10% than in the control group was found. The authors did not show the correlation between plasma visfatin level and blood pressure values. Recently Gunes et al [13] have demonstrated significantly higher plasma visfatin levels even in prehypertensive subjects (120-130/80-89 mmHg) than in normotensive participants and further increase of serum visfatin levels in patients in stage 1 and in stage 2 of hypertension. They showed a significantly positive correlation between plasma visfatin concentration and both systolic and diastolic BP values. In our study the analysis of plasma visfatin levels in relation to the stages of hypertension was not possible, as the group was homogenous, consisting almost exclusively of patients in stage 1 of hypertension.

The results of our study suggest that visfatin may participate in the development of hypertension in patients with visceral obesity. Unfortunately, a potential link connecting the increased circulating visfatin levels with mechanisms involved in the pathogenesis of hypertension remains unclear. Moreover the association do not show the causality. Therefore, both hypotheses, assuming the role of visfatin in the pathogenesis of hypertension and stating that its increased level is the consequence of disturbed metabolic or endocrine pathways are possible. Increased visfatin level in hypertensive patients may be associated with insulin resistance, frequent in this clinical condition. The simple link between circulating level of visfatin and insulin resistance is difficult to be established. Increased circulating visfatin levels in the obese may be one of the compensatory mechanisms at the early stage of insulin resistance development. Visfatin also stimulates production and release of proinflammatory cytokines by monocytes and macrophages, such as tumor necrosis factor alpha (TNF-α) and interleukin-6 (Il-6). These cytokines are involved in the pathogenesis of insulin resistance [3].
In the present study we found the correlation between plasma visfatin concentration and nutritional status indicators, e.g. BMI and waist circumference in combined group of subjects with visceral obesity. First reports showing the correlation between serum visfatin level and visceral adipose tissue originated from Fukuhara study in vitro [14]. The majority of in vivo studies confirmed the existence of correlation between plasma visfatin concentration and BMI [15, 16]. Consistently with previous studies [17, 18], we have also found the correlation between plasma visfatin level and insulin resistance indicators, e.g. HOMA-IR, serum concentration of glucose and insulin.

It’s known, that patients with hypertension are characterized by features of systemic inflammation like increase of serum concentration of CRP, s-ICAM and MCP. Moreover, the newest studies indicate, that the increase of serum CRP levels precedes the development of hypertension [19]. These observations suggest, that CRP action on blood vessel or tubular cells may lead to vessel contraction, sodium retention and development of hypertension [20]. In the present study, consistently with previous reports, we have also found the correlation between CRP and plasma visfatin concentration. These results indicate that circulating visfatin may reflect inflammation status.

Moreover, all the above mentioned mechanisms (insulin resistance, chronic inflammation status and increased plasma adipokine levels: leptin, resistin and visfatin) lead to endothelium dysfunction, which plays also important role in pathogenesis of hypertension. Harmful effect of circulating visfatin on endothelium function manifests itself by increase of NF-kB activity, production of proinflammatory cytokines (Il-6, Il-8 and TNF-α) and adhesive molecules (ICAM-1, VCAM-1 and E-selectin) [21].

Until now no study directly evaluating plasma visfatin concentration in relation to the activation of RAA system was published. In few studies the effect of pharmacological blockade of RAA system on plasma visfatin level was described [8-10]. In the first study both the angiotensin converting enzyme inhibitors (ACE-Is) and angiotensin II receptor AT1 inhibitors use was followed by the increase of visfatin secretion from endothelial cells, adipocytes and myocytes [8]. However, results obtained in further studies weren't so clear. Ferrari et al [9] showed the increase in plasma visfatin concentration after treatment with angiotensin II receptor AT, inhibitors, but not after using ACE-Is in 288 hypertensive patients. Contrary Eyileten et al [10] have showed the decrease of plasma visfatin concentration after treatment with ACE-Is in patients with diabetic nephropathy. The reason for the observed discrepancies is unclear. Regardless of the existing confusing data, our study suggests that differences in RAA activity in hypertension do not explain why visfatin level is increased in patients with hypertension and visceral obesity.

Dietary sodium restriction and upright position causes also activation of the sympathetic nervous system, that stimulates both RAA system and β-adrenergic receptors in adipose tissue [22, 23]. Yet the influence of sympathetic nervous system activation on visfatin release has not been studied. The obtained in this study data suggest that the activation of sympathetic nervous system has no effect on plasma visfatin level, or alternatively antagonistic to RAA system activation effect. As we did not assess its activity the verification of these hypotheses in not possible. It should be stressed that microneurography is the most accurate method of the sympathetic nervous system activity assessment [24]. However, this method is not easily accessible. Whereas, the evaluation of catecholamines levels or their metabolites in serum or in urine has significant limitations. First, circulating noradrenaline represents only a small fraction (5-10%) of the amount of neurotransmitter secreted from nerve terminals. Second, it is influenced, in addition to the level of sympathetic neural outflow, by prejuncional modulation of neurotransmitter release and uptake, as well as the clearance and metabolism. Moreover, plasma measurements do not allow for discrimination between central and peripheral mechanisms of increased levels of noradrenaline. Third, plasma measurements do not take into consideration profound regional differences in the activity and control of sympathetic function. Other methods for evaluation of sympathetic nervous system activity are still the heart rate variability (based on long-term ECG recordings) and the baroreflex sensitivity [25].
There are several limitations of our study. As mentioned above it is the lack of evaluation of sympathetic nervous system activity. Additionally, in some patients the discontinuation of hypertensive drugs wasn’t possible, and calcium channel antagonists were maintained. It could influence plasma adipokines concentrations, RAA system activity and especially blood pressure values. This may explain why we did not showed the correlation between plasma visfatin levels and BP values.

Conclusion

The results of present study suggest that 1) Increased plasma visfatin concentration may play a significant role in the pathogenesis of hypertension in patients with visceral obesity 2) RAA system activation by dietary sodium restriction and upright position has no effect on plasma visfatin levels in subjects with visceral obesity.

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Visfatin in Hypertension and Obesity


