Serum visfatin concentration and endothelial dysfunction in chronic kidney disease

Mahmut Ilker Yilmaz1,2, Mutlu Saglam3, Juan Jesus Carrero2, Abdul Rashid Qureshi2, Kayser Caglar1, Tayfun Eylie1, Alper Sonmez4, Erdinc Cakir5, Mujdat Yenicesu1, Bengt Lindholm2, Peter Stenvinkel2 and Jonas Axelsson2

1Departments of Nephrology, Gülhane School of Medicine, 06018 Etilk-Ankara, Turkey, 2Divisions of Renal Medicine and Baxter Novum, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, K 56 Karolinska University Hospital at Huddinge, Stockholm, Sweden, 3Departments of Radiology, 4Departments of Internal Medicine and 5Departments of Biochemistry, Gülhane School of Medicine, 06018 Etilk-Ankara, Turkey

Abstract

Background. Endothelial dysfunction (ED) is common in patients with moderate to advanced chronic kidney disease (CKD). Recently, visfatin, a protein with insulin-mimetic properties, was shown to be associated with sVCAM-1. Thus, we hypothesised that visfatin may be a marker of ED in CKD.

Methods. We studied 406 patients with different stages of non-diabetic CKD (50% males, 46 ± 12 years), testing the relationship between flow-mediated dilatation (FMD), assessed by high resolution brachial ultrasonography, and plasma adiponectin and visfatin concentrations. Eighty healthy volunteers (50% males, 46 ± 11 years) served as matched controls.

Results. Compared to healthy controls, ED was observed in all stages of CKD (Stages 1–5) and correlated strongly with the reduction in estimated glomerular filtration rate (eGFR). Whereas visfatin concentrations were found to be increased in all but CKD stages 1 and 2, adiponectin levels were found to be increased in all patients but CKD stage 1. Visfatin and adiponectin levels were strongly correlated with eGFR (rho = −0.62 and rho = −0.72, respectively, P < 0.001 for both). FMD levels were negatively correlated with both visfatin and adiponectin levels (rho = −0.53 and, rho = −0.57, respectively, P < 0.001 for both). In a multiple regression model, eGFR levels (Beta = 0.74, P < 0.001), visfatin (Beta = −0.15, P < 0.001), age (Beta = 0.06, P < 0.01), adiponectin (Beta = 0.09, P < 0.05), HOMA-IR (Beta = 0.07, P < 0.05) and hsCRP (Beta = −0.08, P < 0.05) were all found to be significantly related to FMD.

Conclusions. We conclude that the circulating levels of visfatin and adiponectin are associated with ED in all stages of CKD, independently of inflammation and insulin resistance.

Keywords: adiponectin; flow-mediated dilation; insulin resistance; visfatin

Introduction

Endothelial dysfunction (ED) has been implicated in the pathophysiology of different forms of cardiovascular disease, including chronic heart failure, diabetes mellitus, hypertension, coronary heart disease and chronic kidney disease (CKD) [1,2]. The etiology of ED is complex and involves dysregulation of multiple pathways [1]. Interestingly, adipose tissue has recently been shown to be stimulated by hormonal, neural and nutrient stimuli to secrete a number of small protein peptides, or adipokines [3]. These adipokines have been implicated in the metabolic syndrome [4], specifically the development of insulin resistance and hypertension. Adipose tissue also appears to be a modulator of vascular injury [5] and systemic inflammation [3]. Thus, it may be that adipokines link insulin resistance to ED, a prominent feature in both type 2 diabetes mellitus, obesity and CKD [6–8].

In CKD an association between adiponectin and ED has been demonstrated [9], while CD 146, a novel cell adhesion molecule localised at the endothelial junctions, is related to elevated adiponectin levels that may be the expression of a counter regulatory response aimed at mitigating the consequences of vascular inflammation in CKD. Furthermore, visfatin has recently been suggested to induce insulin resistance by binding to the insulin receptor [10,11]. In a recent report, we found no correlation with insulin resistance in CKD, but showed that circulating visfatin levels are influenced by renal function in patients with CKD and independently associated with levels of circulating...
soluble vascular adhesion molecule 1 (sVCAM-1), a marker of ED [12].

In the present study, we hypothesised that the circulating adipokines adiponectin and visfatin would be related also to functional measurements of ED. Thus, we measured flow-mediated vasodilatation (FMD) and nitroglycerin-mediated dilation (NMD) in patients with different stages of CKD and related the results to circulating adipokine levels.

**Subjects and methods**

**Patients**

Subjects were prevalent patients referred to the Renal Unit of the Gulhane School of Medicine Medical Center, Ankara, Turkey for the first time because of suspected or manifest renal failure. All patients were diagnosed as having CKD according to their estimated glomerular filtration rate (eGFR) and the presence of kidney injury as defined by National Kidney Foundation K/DOQI Guidelines [13]. In order to minimise any confounding effects of conditions that may influence ED, patients with cardiogenic shock, overt congestive heart failure, valvular heart disease, acute coronary syndrome or atrial fibrillation, smokers, nephrotic syndrome and patients taking angiotensin converting enzyme inhibitors (ACEIs; \( n = 102 \)), angiotensin receptor blockers (ARBs; \( n = 82 \)), statins (\( n = 166 \)) or supplemental vitamin pills (\( n = 43 \)) were excluded. In addition, patients with a prior diagnosis of diabetes, current use of oral antidiabetic medication or insulin, or with a fasting glucose level greater than 126 mg/dl were also excluded. Otherwise, other exclusion criteria than unwillingness to participate in the study were applied.

After the first evaluation, 406 CKD patients with a mean age of 46 ± 12 years were included in the study. The diagnoses of the CKD are given as Table 1. Hyper-tension was defined as systolic blood pressure (SBP) ≥140 mm Hg or diastolic blood pressure (DBP) ≥90 mm Hg on repeated measurements, or the use of antihypertensive drugs. Sixty-four of the patients were on antihypertensive therapy (45 patients were treated with calcium channel antagonists, 11 with beta-blocker agents and 8 with loop diuretics), CKD 5 patients were required to be under regular haemodialysis for at least 6 months prior to inclusion in the study.

 Patients were classified with respect to eGFR levels from stage 1 to 5 as determined by K/DOQI (Table 1), which was calculated according to the simplified version of the Modification of Diet in Renal Disease (MDRD) formula as defined by Levey et al. [14] [GFR = \( 186 \times \frac{Pcr^{−1.154}}{\text{age}^{−0.203}} \times \text{height} \times 0.742 \) (if female)].

The control group consisted of 80 age (46 ± 11 years) and gender-matched (50% males) healthy subjects recruited from among the relatives of the patients. Routine analyses showed that controls had normal renal function and no disorders of lipid metabolism. Subjects with a history of metabolic or other serious concomitant disease were excluded. All patients and controls were non-smokers. All procedures in the present study were carried out in accordance with institutional and national ethical guidelines for human studies. The ethical committee of Gulhane School of Medicine approved the study. Informed consent was obtained from each subject.

**Laboratory measurements**

After subjects fasted overnight, arterial blood pressure was measured by a physician three times after a 15-min resting period in the morning and mean values were calculated for systolic and diastolic pressures for all subjects. Mean arterial pressure (MAP) was calculated as DBP + ((SBP − DBP)/3).

All samples were obtained from patients and controls in the morning after 12 h of fasting (non-dialysis in haemodialysis (HD) patients), for measurement of fasting plasma glucose (FPG), serum albumin, total serum cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol. Total plasma cholesterol, TG and HDL cholesterol were measured by enzymatic colorimetric method with Olympus AU 600 auto analyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). LDL cholesterol was calculated by Friedewald’s formula [15].

Plasma visfatin levels were determined by ELISA method (Human visfatin ELISA kit, Phoenix Pharmaceuticals, Belmont, CA, USA) [sensitivity: (minimum detectable...
HOMA-IR was computed by the following formula [16]:

\[
\text{HOMA-IR} = \frac{\text{serum insulin (mU/L)} \times \text{glucose (mg/dL)}}{405}
\]

Coated tube method (DPC-USA). An insulin resistance concentration = 0.5–1 ng/mL, IntraCV: 5% and InterCV: 12%. Plasma adiponectin concentrations were measured in duplicate by RIA method (Human adiponectin RIA kit, Linco research, Inc., St. Charles, MO, USA) [sensitivity: (minimum detectable concentration) = 1 ng/mL, IntraCV: 3.59% and InterCV: 9.25%].

The laboratory and vascular assessments according to groups

<table>
<thead>
<tr>
<th>Group</th>
<th>eGFR, ml/min/1.73 m²</th>
<th>Stage 1 (≥90) (n = 80)</th>
<th>Stage 2 (60–89) (n = 83)</th>
<th>Stage 3 (30–59) (n = 81)</th>
<th>Stage 4 (15–29) (n = 81)</th>
<th>Stage 5 (&lt;15) (HD) (n = 80)</th>
<th>P *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>115 (98–124)</td>
<td>94 (90–106)†</td>
<td>69 (60–89)†</td>
<td>44 (30–58)†</td>
<td>22 (15–29)†</td>
<td>9.5 (4–14)†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MAR mm Hg</td>
<td>99 (90–103)</td>
<td>99 (91–108)</td>
<td>100 (91–106)</td>
<td>100 (89–107)</td>
<td>100 (87–111)</td>
<td>100 (84–106)</td>
<td>0.155</td>
</tr>
<tr>
<td>Serum Albumin, g/dl</td>
<td>4.2 (3.5–5.1)</td>
<td>4.0 (3.6–4.6)</td>
<td>3.8 (3.5–4.6)</td>
<td>3.8 (3.5–4.6)</td>
<td>4.0 (3.3–4.6)</td>
<td>3.8 (3.2–4.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma Insulin, μIU/ml</td>
<td>7.0 (4.2–11.3)</td>
<td>6.7 (5.0–11.3)</td>
<td>6.7 (4.9–9.8)</td>
<td>7.0 (4.9–13)</td>
<td>6.8 (5.1–15.2)</td>
<td>7.4 (5.5–13.5)</td>
<td>0.066</td>
</tr>
<tr>
<td>Plasma glucose, mg/dl</td>
<td>83.5 (64–110)</td>
<td>82 (70–108)</td>
<td>82 (67–104)</td>
<td>84 (68–105)</td>
<td>90 (70–106)</td>
<td>89.5 (68–109)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.4 (0.9–2.3)</td>
<td>1.4 (1.0–2.4)</td>
<td>1.4 (1.1–2.3)</td>
<td>1.4 (1.1–3.1)</td>
<td>1.4 (1.1–3.1)</td>
<td>1.6 (1.2–3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total serum cholesterol, mg/dl</td>
<td>193 (159–235)</td>
<td>201 (160–239)</td>
<td>194 (149–235)</td>
<td>198 (171–237)</td>
<td>192 (159–236)</td>
<td>194 (149–237)</td>
<td>0.444</td>
</tr>
<tr>
<td>Serum triglycerides, mg/dl</td>
<td>138 ± 13</td>
<td>138 ± 14</td>
<td>136 ± 12</td>
<td>142 ± 14</td>
<td>138 ± 12</td>
<td>135 ± 20</td>
<td>0.085</td>
</tr>
<tr>
<td>Serum LDL cholesterol, mg/dl</td>
<td>121 ± 11</td>
<td>126 ± 15</td>
<td>127 ± 17</td>
<td>125 ± 16</td>
<td>127 ± 16</td>
<td>122 ± 20</td>
<td>0.061</td>
</tr>
<tr>
<td>Serum HDL cholesterol, mg/dl</td>
<td>44 (27–53)</td>
<td>47 (24–51)</td>
<td>46 (26–54)</td>
<td>43 (26–50)</td>
<td>43 (28–63)</td>
<td>43 (23–59)</td>
<td>0.103</td>
</tr>
<tr>
<td>hsCRP, mg/l</td>
<td>2.0 (1–4)</td>
<td>7.0 (3.2–10.0)</td>
<td>10.0 (5–15)</td>
<td>16.0 (6.5–22)</td>
<td>22.2 (8–28)</td>
<td>26.0 (11–37)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NMD, %</td>
<td>13.4 (11.8–13.9)</td>
<td>13.0 (11.8–13.8)</td>
<td>13.1 (12.0–13.8)</td>
<td>12.9 (12.0–13.9)</td>
<td>13.0 (11.6–13.7)</td>
<td>12.0 (10.0–13.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FMD, %</td>
<td>8.7 (7.5–12.6)</td>
<td>8.6 (7.2–10.1)</td>
<td>7.2 (6.7–8.3)</td>
<td>6.9 (6.2–8.2)</td>
<td>6.3 (5.2–8.2)</td>
<td>5.3 (3.5–7.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin, μg/ml</td>
<td>12.0 (6.5–14.2)</td>
<td>11.9 (9.2–13.6)</td>
<td>12.4 (9.8–14.5)</td>
<td>14.6 (10.8–17.8)</td>
<td>16.4 (11.6–19.6)</td>
<td>17.8 (11.0–19.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Visfatin, ng/ml</td>
<td>34.6 (25.2–42.9)</td>
<td>37.3 (21.0–46.6)</td>
<td>38.5 (28.1–48.0)</td>
<td>40.7 (30.8–55.0)</td>
<td>45.2 (23.0–81.2)</td>
<td>59.7 (39.0–78.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MAP, Mean arterial pressure; HOMA, homeostasis model assessment; eGFR, estimated glomerular filtration rate; FMD, Flow mediated dilatation; NMD, nitroglycerine mediated dilatation; HD, Hemodialysis.

* One Way ANOVA test † Tukey-Kramer test, statistically significant (p < 0.05) compared with control group.

Vascular assessment

**Endothelial dysfunction** Endothelium-dependent vasodilatation (FMD) and endothelium-independent vasodilatation (NMD) of the brachial artery were assessed non-invasively, using high resolution ultrasound as described by Celermayer et al. [17]. Measurements were made by a single observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories Inc., Bothell, WA, USA) with a 12-MHz probe. The non-fistula arm was used in the dialysis group. All vasoactive medications were withheld for 24 h before the procedure. The subjects remained at rest in the supine position for at least 15 min before the examination started. Subject’s arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were made from single 2D frames. All ultrasound images were recorded on S-VHS videotape for subsequent blinded analysis. A pneumatic tourniquet was inflated to 200 mm Hg with obliteration of the radial pulse. After 5 min the cuff was deflated. Flow measurements were made 60 s post-deflation. After a further 15 min, measurements were repeated and again 3 min after administration of sublingual glycerol trinitrate 400 µg po. The maximum FMD and NMD dilatation diameters were calculated as the average of the three consecutive maximum diameter measurements. The FMD and NMD were then calculated as the percent change in diameter compared with baseline resting diameters.

**Statistical analysis**

Non-normally distributed variables were expressed as median (range) and normally distributed variables were as mean ± SD as appropriate. A P-value <0.05 was considered to be statistically significant. Between-group comparisons were assessed for nominal variables with the χ²-square test. Difference among the groups was analyzed by ANOVA using Kruskal–Wallis test (ANOVA), followed by Post-hoc Dunns test for non-parametric comparisons. Spearman’s rank correlation was used to determine correlations between two variables. Multivariate regression analysis was used to assess the predictors for flow-mediated dilatation (FMD) levels. All statistical analyses were performed with SAS statistical software (Version 9.1; SAS Institute, Inc., Cary, NC, USA).
**Results**

**Clinical characteristics**

The characteristics of the patients and the controls are given in Tables 1 and 2. There were no significant differences between patients and the controls according to the age, BMI, MAP, total cholesterol, triglyceride, LDL- and HDL-cholesterol levels. These parameters were also similar within the different CKD groups.

Plasma visfatin levels in CKD 1–2 groups and adiponectin levels in CKD stage 1 were similar to those of the controls, while the other CKD groups had significantly elevated values (P < 0.001, Table 2, Figure 1A, B).

As expected, the higher the stage of the CKD, the lower the degree of FMD (Figure 1C). Nitroglycerine mediated dilatation (NMD) levels were similar within patients with stage 1, 2, 3, 4 CKD, while NMD was significantly (P < 0.05) lower in CKD stage 5 patients (Table 2). Also, NMD calculations in CKD 1–4 groups were similar to those of the controls.

The hsCRP levels were higher and FMD and serum albumin levels were significantly (P < 0.001) lower in all stages of CKD than those of the controls (Table 2).

FMD levels were also negatively correlated with visfatin, adiponectin and hsCRP, levels (rho = −0.53, P < 0.001; rho = −0.57, P < 0.001; rho = −0.79, P < 0.001, respectively) (Figures 2A, B and Figure 3A). There were significant positive correlations between FMD and eGFR (Figure 3B) and albumin levels (rho = 0.84, P < 0.001; rho = 0.16, P < 0.001, respectively).

Both adiponectin and visfatin correlated with HOMA (rho = 0.25, P < 0.001 for adiponectin; rho = 0.11, P = 0.023 for visfatin) and hsCRP levels (rho = 0.67, P < 0.001 for adiponectin; rho = 0.56, P < 0.001 for visfatin). On the
Endothelial dysfunction and adipocytokines in CKD

In order to clarify independent predictors of ED in the patient material we performed a multiple regression model. Variables expected to influence FMD [sex, high age (>44 years), total cholesterol, presence of hypertension, antihypertensive therapy, eGFR, HOMA, high hs-CRP (>10 mg/l) and adiponectin] and as well as visfatin were included in the multivariate regression analysis. In this model, eGFR levels, visfatin, age, adiponectin, HOMA-IR and hs-CRP were significant associates to FMD levels (Table 3).

Discussion

In the present study, we investigated the marker of endothelial function (assessed using nitroglycerine and flow-mediated vasodilatation) in 406 CKD patients and 80 healthy controls. We confirm the previously reported associations between renal function, inflammation and signs of vascular damage, and show for the first time an independent relationship between adiponectin and visfatin levels and endothelial function.

While it is well known that CKD patients suffer a several fold higher risk of CVD and mortality [18], the reasons for this is unclear. Progression of CKD is associated with both decreased endothelial function, increased prevalence of atherosclerosis, and vascular media calcification [19], all of which have been associated with mortality [19,20]. The present study thus confirms earlier reports showing progressively increased prevalence of cardiovascular risk factors in patients with mild-moderate CKD. Specifically, in our data patients with CKD 5 FMD that was a median 40% smaller and a nitrogen-mediated dilation that was 10% lower than control values. Another common marker of cardiovascular risk, dyslipidemia, showed a statistically not significant trend towards more pro-atherogenic values with decreasing GFR.

| Table 3. Multivariate regression analysis of predictors of flow mediated dilatation levels in CKD patients |
|-------------------------------------------------------|-------------------|-----------------|-----------------|-------------------|
| Estimate | Standard error | Beta | Semi-partial r | p value |
| Intercept | 5.35 | 0.50 | 0.74 | 0.17 | <0.0001 |
| eGFR, ml/min | 0.028 | 0.002 | -0.09 | 0.003 | <0.0001 |
| Visfatin, ng/ml | -0.016 | 0.004 | -0.15 | 0.012 | <0.0001 |
| Age, >44 years | 0.09 | 0.04 | 0.06 | 0.004 | <0.05 |
| Adiponectin, µg/ml | 0.041 | 0.021 | 0.09 | 0.003 | <0.05 |
| HOMA—IR | 0.14 | 0.07 | 0.06 | 0.003 | <0.05 |
| hs-CRP, >10 mg/l | -0.22 | 0.11 | -0.08 | 0.003 | <0.05 |
| Presence of hypertension | 0.07 | 0.09 | 0.02 | 0.02 | >0.05 |
| Antihypertensive therapy | -0.08 | 0.09 | -0.02 | -0.02 | >0.05 |
| Total cholesterol, mg/dl | 0.002 | 0.002 | 0.03 | 0.05 | >0.05 |
| Sex | -0.02 | 0.07 | -0.006 | -0.006 | >0.05 |

Variables known to influence FMD levels [sex, high age (>44 years), total cholesterol, presence of hypertension, antihypertensive therapy, eGFR, HOMA-IR, high hs-CRP (>10 mg/l) and adiponectin] as well as visfatin were included in the models.

Whole-model adjusted $r^2 = 0.69$. 
The present study also related the observed increase in cardiovascular risk factors to several circulating cytokines and adipokines. We are thus able to show, for the first time in CKD patients, that adiponectin is independently related to FMD, along with hsCRP and visfatin. It is well known that, unlike other adipokines, the insulin-sensitizing and anti-atherogenic adipokine adiponectin is decreased in obesity and associated with the development of ED [21,22]. Although adiponectin levels are elevated in CKD compared to healthy controls [23,24], it is unclear if this elevated adiponectin levels are in the bioactive form. Nevertheless, our results imply that elevated circulating adiponectin cannot to completely normalize FMD in the inflamed milieu. Our findings extend the recent report of Malyszko et al. [9] showing elevated ED in uremic patents with high adiponectin. Moreover, our results extend the findings of Axelsson et al. [12], who showed an association between visfatin and a surrogate marker of ED, endothelial adhesion molecules. Taken together, these data may suggest that in CKD patients, a dysfunctional adipose tissue signaling—reflected here by high adiponectin and visfatin levels—may directly influence the vascular endothelium, causing ED independently of inflammation and insulin resistance, both known to be common occurrences in CKD patients [25].

Interestingly, while insulin resistance showed the expected and independent association with FMD also in this study (Table 3), both in the present study and in our previous study [12], we could not find any relationship between insulin resistance (assessed by HOMA-IR) and circulating visfatin levels. This conflicts with a previous study showing associations between visfatin and glucose levels in mice and in vitro [10]. However, several other human studies have also failed to find such a relationship [8,26], and it may be that glucose homeostasis is more complex in man, or that the effects of visfatin signaling require more accurate assessments of insulin signaling than have been applied previously. Indeed, visfatin was originally described as NAMPT, an intracellular enzyme catalyzing the rate limiting step of NADPH synthesis, and may thus be more of a cell damage marker [27].

A number of weaknesses of the present study should be mentioned. First, as diabetic patients were excluded, this may limit the value of the observed results. Second, we did not control for a number of medications that can affect vascular status, including statins and NSAIDs. Third, we found surprisingly strong associations between the investigated factors. One possible reason for this may be the selection bias in recruiting patients for the present study, meaning that the results may be hard to transfer to more heterogeneous populations. Finally, by assessing GFR indirectly we may have limited the power of the study to show the true extent of progression to CKD 5.

In summary, the present study confirms a dramatic increased prevalence of cardiovascular dysfunction in mild-moderate CKD. Moreover, our data imply that elevated circulating adiponectin levels are not able to normalize ED in the uremic milieu. Finally, we report an association between visfatin and functional changes in FMD in CKD patients, independent of inflammation and insulin resistance, suggesting that this ubiquitous enzyme may play a previously unknown role in ED in uremia.

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Conflicts of interest. B.L. is an employee of Baxter Healthcare Inc. P.S. is a member of the scientific advisory board of Gambio AB.


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