Endothelial dysfunction in type-2 diabetics with early diabetic nephropathy is associated with low circulating adiponectin

Mahmut Ilker Yilmaz, Mutlu Saglam, Abdul Rashid Qureshi, Abdul Rashid Juan Jesus Carrero, Kayser Caglar, Tayfun Eyileten, Alper Sonmez, Erdinc Cakir, Yusuf Oguz, Abdulgaffar Vural, Mujdat Yenicesu, Peter Stenvinkel, Bengt Lindholm and Jonas Axelsson

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

Abstract

Background. Type-2 diabetes and diabetic kidney disease have additive effects on cardiovascular risk. Furthermore, the degree of proteinuria is an independent predictor of mortality in this patient group. We hypothesized that altered kidney clearance and/or metabolism of vasoactive peptides occurring during proteinuria could link early diabetic nephropathy to cardiovascular disease (CVD).

Methods. We performed a cross-sectional study of 85 incident patients (51 ± 5 years, 49 males) with type-2 diabetes and 38 age- and sex-matched controls. We further divided patients by the presence of minor (<500 mg/day; n = 40) or severe (≥500 mg/day; n = 45) proteinuria. Clinical and anthropometric data, along with ultrasonographic flow-mediated dilatation (FMD) of the brachial artery and carotid intima-media thicknesses (CIMT), were recorded in each group. Circulating NAMPT/visfatin, adiponectin (normalized to BMI), AHSG/fetuin-A and hsCRP levels were also measured using commercial ELISA.

Results. Plasma NAMPT/visfatin, CIMT, HOMA index and hsCRP levels were all significantly higher in diabetics than in control subjects, and all but CIMT correlated with proteinuria (ρ = 0.46; P < 0.001, ρ = 0.54; P > 0.05, ρ = 0.32; P = 0.003, ρ = 0.76; P < 0.001, respectively). FMD, adiponectin and AHSG/fetuin-A levels were significantly lower, and negatively correlated with proteinuria (ρ = −0.54; P < 0.001, ρ = −0.56; P < 0.001, ρ = −0.48; P < 0.001, respectively). In a multivariate regression analysis, the degrees of proteinuria (r² = −0.32, P = 0.04) and plasma levels of NAMPT/visfatin (r² = −0.33, P = 0.006) were independently related to FMD.

Conclusions. The present study suggests that the presence of proteinuria, regardless of the degree of renal function impairment, is an important predictor of endothelial dysfunction in early diabetic nephropathy and that it is associated with altered circulating levels of NAMPT/visfatin and adiponectin.

Keywords: albuminuria; CVD; fetuin-A; NAMPT/visfatin; PBEF-1

Introduction

While diabetes mellitus is well known to lead to the development of endothelial dysfunction [1, 2], the advent of diabetic nephropathy confers an additional and additive risk for cardiovascular disease (CVD) [3–5]. Indeed, chronic kidney disease [CKD; defined as a glomerular filtration rate (GFR) <90 ml/min for more than 3 months] from any cause is a well-recognized risk factor for endothelial dysfunction [6] and CVD [7] even without the presence of diabetes mellitus. However, it is so far unclear if the increased prevalence of endothelial dysfunction and CVD in patients with diabetic nephropathy is due to the loss of GFR, or if other mechanisms are also involved.

Proteinuria is a characteristic aspect of diabetic nephropathy, while it has recently been reported that protein loss in the urine is independently associated with elevated circulating markers of systemic inflammatory markers such as hsCRP [8, 9] and tumour necrosis factor-α (TNF-α) [9], as well as with endothelial adhesion molecules [8]. The putative link between proteinuria and endothelial damage is not known, and may indeed not exist should proteinuria simply be a marker of generalized vascular damage, but it may be that the inadvertent loss of vasoprotective peptides such as adiponectin through the urine may be involved.
Supporting such a hypothesis, two studies by Koshimura et al. [10] and Shimotomai et al. [11] show increased urinary adiponectin in proteinuric patients.

Based on this hypothesis, we studied endothelial function using high-resolution ultrasonography to measure flow-mediated dilatation (FMD) and nitroglycerine-mediated dilatation (NMD) of the brachial artery in 85 patients with early diabetic nephropathy, defined as manifest low-grade proteinuria but without an impaired GFR, and in 38 healthy control subjects who were matched for age, sex and body mass index (BMI).

Materials and methods

Patients

The outpatient clinic of the renal unit at Gülhane School of Medicine (Ankara, Turkey) is an established tertiary referral centre with about 800 patients/year being evaluated for the presence of diabetic renal disease, mostly before commencing antihypertensive treatment and in accordance with current guidelines [12]. For the present study, we screened 411 diagnosed type-2 diabetics with evidence of renal dysfunction (i.e. hyperfiltration and microalbuminuria, isolated microalbuminuria, proteinuria and/or abnormal urine microscopy, or reduced GFR) seen by one of the participating physicians between 2003 and 2006. Subjects were evaluated by standard physical examination, chest X-ray, baseline electrocardiogram, two-dimensional echocardiography and routine clinical laboratory tests, including liver and kidney function tests and 24-h urinary protein measurements. Of the evaluated patients, 173 had proteinuria (24-h protein excretion >200 mg/day) and thus fulfilled the study inclusion criteria of diagnosed type-2 diabetes mellitus for at least 6 months and micro- or macroalbuminuria. Study exclusion criteria were hypertension (systolic blood pressures ≥ 140 mmHg and/or diastolic blood pressures ≥ 90 mmHg), BMI ≥30 kg/m², manifest clinical coronary heart disease (defined as ischemic ST-T alterations and/or voltage criteria for left ventricular hypertrophy on the electrocardiogram, and/or a history of cardiac revascularization intervention), elevated liver enzymes (AST or ALT levels ≥ 40 U/l), manifest chronic kidney disease (CKD; serum creatinine levels ≥1.3 mg/dl), dyslipidemia (patients with total cholesterol levels >200 mg/dl and/or triglyceride levels >150 mg/dl), current smoking habit (>1 cigarette/week) or any other serious chronic or acute comorbidity requiring continuous pharmacological therapy or hospital treatment.

Subjects were also excluded if they were prescribed one or more of the following medications at the time of evaluation: exogenous insulin, angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARBs), statins, thiazolidinedione, estrogens or gestagens, glucocorticoids, α- or β-adrenergic receptor agonists or vitamins.

In the end, 85 patients were thus enrolled in the study (49 M, 36 F; mean age 51 ± 5 years, all Caucasians). Among these patients, 40 were defined as having minor proteinuria (<500 mg/24 h) and 45 had severe proteinuria (>500 mg/24 h) [13]. All patients were on dietary diabetes treatment and/or oral sulfonylurea, but were taking no other medications.

Using in-hospital advertisements, we also recruited a control group consisting of 38 apparently healthy subjects who were matched to the patients with respect to age, sex and BMI (20 M, 18 F, mean age 48 ± 6 years). They underwent comprehensive physical and laboratory evaluation to ascertain that they had no hypertension, metabolic, hepatic or renal diseases. The control subjects declared no family history of hypertension or DM, and all underwent an oral glucose tolerance test (OGTT) showing normal glucose tolerance and normal fasting blood lipid profiles. All study subjects gave informed consent for participating in the study, which was carried out in accordance with the Helsinki declaration. The local ethical committee of the Gülhane School of Medicine approved the study protocol prior to initiation of the study.

Study design and measurements

We performed a cross-sectional comparison between patients with diabetic nephropathy and healthy control subjects. Arterial blood pressure (the average value of three consecutive measurements) was measured and venous blood sampling performed in the morning after an 8-h fast, always after a 10- to 15-min resting period and mean values were calculated for systolic and diastolic pressures for all subjects. Mean arterial pressure (MAP) was calculated as DBP+[(SBP – DBP)/3]. In addition to routine biochemical tests carried out in the hospital laboratory, we stored plasma at −70 °C for biochemical analysis as described below. Estimated glomerular filtration rate (eGFR) was calculated based on serum creatinine by applying the simplified Modification of Diet in Renal Disease (MDRD) formula. Insulin sensitivity was also estimated using the homeostasis model assessment [HOMA; calculated as FPG (mg/dl) × (µU/ml)/405] [14]. To increase the precision of proteinuria estimates, 24-h urine collection was performed three times during the same week, and the average of the three 24-h proteinuria measurements was taken as representative of each participant’s 24-h protein excretion rate.

Laboratory procedures

Plasma NAMPT/visfatin, adiponectin, serum AHSG/fetuin-A, hsCRP and plasma insulin were all measured using commercial ELISA. Plasma NAMPT/visfatin levels were determined by the human NAMPT/visfatin ELISA kit (Phoenix Pharmaceuticals, Belmont, CA, USA) [sensitivity: (minimum detectable concentration) = 0.5–1 ng/ml, IntraCV: 4% and InterCV: 10.5%]. Plasma adiponectin concentrations were measured in duplicate by the RIA method (Human adiponectin RIA kit, Linco research Inc., St Charles, MO, USA) [sensitivity: (minimum detectable concentration) = 1 ng/ml, IntraCV: 3.33% and InterCV: 8.5%]. Adiponectin was also expressed normalized to BMI (adiponectin/BMI = adip/BMI) in an attempt to correct for differences in adipose tissue production between patients.

Serum concentrations of AHSG/fetuin-A were measured using a Human AHSG/fetuin-A ELISA kit (BioVendor Laboratory Medicine Inc., Brno, Czech Republic) in an
ELISA plate reader (Synergy HT, Multidetection Multi-Plate Reader, Bio-tek Instruments Inc., Highland Park, VT, USA). Interassay and intra-assay coefficients of variations were below 9%.

Plasma glucose, blood urea, serum creatinine, total protein, serum albumin, total cholesterol, HDL cholesterol and triglycerides were determined by enzymatic colorimetric method with Olympus AU 600 autoanalyzer using reagents from Olympus Diagnostics, GmbH (Hamburg, Germany). LDL cholesterol was calculated by Friedewald’s formula [15]. HbA1c was measured by inhibition of latex agglutination, using a DCA 2000 analyser (Bayer, Elkhart, IN, USA). Proteinuria was determined by a turbidimetric test with trichloroacetic acid (TCA). The serum basal insulin value was determined by the coated tube method (DPC-USA, USA).

**Brachial artery endothelial function**

Endothelium-dependent vasodilatation (FMD) and endothelium-independent vasodilatation (NMD) of the brachial artery were assessed non-invasively, using high-resolution ultrasound as described by Celermajer et al. [16]. All measurements were made by the same trained observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories Inc., Bothell, WA, USA) with a 12-MHz probe. Three adjacent measurements of end-diastolic brachial artery diameter were made from single 2D frames. Flow measurements were made 60 s post-deflation of the tourniquet. After a further 15 min, measurements were repeated and again 3 min after administration of sublingual glyceryl trinitrate 400 µg po. All ultrasound images were recorded on S-VHS videotape for subsequent blinded analysis. The maximum flow-mediated (FMD) and nitroglycerine-mediated (NMD) dilatation diameters were calculated as the average of the three consecutive maximum diameter measurements after hyperemia and nitroglycerin, respectively. The FMD and NMD were expressed as the percent change in diameter compared with baseline resting diameters. The intra-observer coefficient of variation was measured at 5.1%.

**Common carotid ultrasound**

In all subjects, a high-resolution B-mode ultrasound of the common carotid arteries was performed using an ATL 5000 ultrasound system with a middle frequency of 12 MHz (Advanced Technology Laboratories Inc., Bothell, WA, USA). For each carotid artery, two longitudinal measurements were obtained by rotating (180° increments) the vessels along the axis. All patients were blindly examined by the same experienced operator, and carotid intima-media thickness (CIMT) measured 1 cm proximally to the carotid bifurcation. CIMT diameters were calculated as the average of the both sides’ diameter measurements. Carotid atherosclerosis was defined as a CIMT greater than 0.8 mm and/or the presence of a carotid plaque protruding >1.3 mm into the vascular lumen [17]. The intra-observer coefficient of variation was measured at 3.8%.

**Statistical analysis**

Non-normally distributed variables were expressed as median (range) and normally distributed variables as mean ± SD. A P-value <0.05 was considered to be statistically significant. Between-group comparisons were performed for nominal variables using the chi-square test and for normally distributed variables using ANOVA followed by a post hoc adjusted Tukey–Kramer test for multiple comparisons. Spearman’s rank correlation was used to determine correlations between continuous variables. Stepwise multivariate regression analysis was used to assess the relative independence of predictors for flow-mediated dilatation levels. All statistical analyses were performed with SAS statistical software (Version 9.1; SAS Institute, Inc., Cary, NC, USA).

**Results**

**Baseline characteristics**

The demographic and clinical characteristics of the enrolled patients and healthy control subjects are shown in Table 1. The estimated duration of clinically overt DM in patients enrolled into the study was 52 ±10 months. There were no significant differences between patients and controls regarding age, sex, BMI, eGFR, blood pressures, NMD or serum lipids. CIMT, HOMA, plasma NAMPT/visfatin, insulin and hsCRP levels were all significantly higher (P < 0.001 for all) in diabetics than in control subjects, while FMD, plasma adiponectin, adiponectin/BMI ratio, serum albumin and serum AHSG/fetuin-A levels were significantly lower (P < 0.001 for all; Figure 1).

**Differences between patients with minor and severe proteinuria**

Clinical and laboratory values of patients grouped according to minor (<500 mg/24 h) or severe proteinuria (>500 mg/24 h) are also presented in Table 1. Average 24-h urinary protein excretion was 368 (200–500) mg/day in the first group and 1250 (550–3450) mg/day in the second group. While we found no significant differences between these subgroups in age, sex, BMI, blood pressures, duration of diabetes, NMD, CIMT, plasma insulin, plasma glucose or blood lipids, the patients with severe proteinuria had significantly higher plasma NAMPT/visfatin, HbA1c, hsCRP and HOMA index than patients with minor proteinuria. Meanwhile, severe proteinuria was also associated with decreased plasma adiponectin, adiponectin/BMI ratio, FMD and serum AHSG/fetuin-A levels (Figure 1).

**Univariate correlations with degree of proteinuria and vasoactive peptides**

Univariate correlations between the degree of proteinuria and selected biomarkers are shown in Table 2. The same general relationships were also seen in a separate analysis of only the patients with severe proteinuria, but not in patients with mild proteinuria or controls.
Table 1. Baseline clinical and biochemical characteristics of the patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 38)</th>
<th>Patients with 24-h urinary protein excretion</th>
<th>ANOVA(P)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤500 mg/day (MP; n = 40)</td>
<td>&gt;500 mg/day (SP; n = 45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>48 ± 6</td>
<td>50 ± 5</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>20/18</td>
<td>26/14</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.7 ± 2.5</td>
<td>29.7 ± 2.2</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73 m²)</td>
<td>115 ± 5</td>
<td>115 ± 8</td>
<td>&lt;0.01</td>
<td>C versus P, MP versus SP</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>131 (112–139)</td>
<td>133 (112–142)</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>83 (78–93)</td>
<td>81 (80–91)</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>194 (174–210)</td>
<td>195 (152–242)</td>
<td>&lt;0.05</td>
<td>MP versus SP</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>133.5 ± 11.2</td>
<td>130.9 ± 21.5</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>117.9 ± 15.7</td>
<td>118.3 ± 13.4</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>41 (34–52)</td>
<td>41 (24–50)</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Blood urea (mg/dl)</td>
<td>34.7 ± 10.4</td>
<td>34.9 ± 10.1</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.9 ± 0.2</td>
<td>0.7 ± 0.2</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.7 ± 0.5</td>
<td>6.9 ± 0.4</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>4.1 ± 0.3</td>
<td>3.8 ± 0.3</td>
<td>&lt;0.001</td>
<td>C versus P, MP versus SP</td>
</tr>
<tr>
<td>Duration of diabetes (month)</td>
<td>50 ± 9</td>
<td>54 ± 12</td>
<td>ns</td>
<td>–</td>
</tr>
<tr>
<td>Plasma insulin (µIU/ml)</td>
<td>7.8 (6.0–10.7)</td>
<td>11.4 (8.9–20.0)</td>
<td>&lt;0.001</td>
<td>C versus P</td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td>85.3 ± 10.9</td>
<td>138.4 ± 23.7</td>
<td>&lt;0.001</td>
<td>C versus P</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.7 ± 0.4</td>
<td>4.2 ± 1.1</td>
<td>&lt;0.001</td>
<td>C versus P, MP versus SP</td>
</tr>
<tr>
<td>Proteinuria (mg/day)</td>
<td>65 (20–140)</td>
<td>368 (200–500)</td>
<td>&lt;0.001</td>
<td>C versus P, MP versus SP</td>
</tr>
<tr>
<td>hsCRP (mg/l)</td>
<td>2.0 (1.0–3.3)</td>
<td>7.5 (5–10)</td>
<td>&lt;0.001</td>
<td>C versus P, MP versus SP</td>
</tr>
<tr>
<td>AHSG/fetuin-A (g/l)</td>
<td>0.41 (0.35–0.49)</td>
<td>0.39 (0.33–0.47)</td>
<td>&lt;0.001</td>
<td>C versus P, MP versus SP</td>
</tr>
<tr>
<td>CIMT (mm)</td>
<td>0.55 ± 0.05</td>
<td>0.59 ± 0.05</td>
<td>0.60 ± 0.05</td>
<td>C versus P</td>
</tr>
<tr>
<td>FMD (%)</td>
<td>9.8 ± 0.9</td>
<td>8.6 ± 0.6</td>
<td>7.9 ± 0.8</td>
<td>C versus P, MP versus SP</td>
</tr>
<tr>
<td>NMD (%)</td>
<td>13.1 ± 0.7</td>
<td>12.9 ± 0.4</td>
<td>13.0 ± 0.4</td>
<td>0.577</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>24.1 ± 6.1</td>
<td>16.8 ± 2.7</td>
<td>13.3 ± 3.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Adiponectin/BMI ratio</td>
<td>0.82 ± 0.21</td>
<td>0.57 ± 0.10</td>
<td>0.44 ± 0.11</td>
<td>0.001</td>
</tr>
<tr>
<td>NAMPT/visfatin (ng/ml)</td>
<td>35.6 ± 4.2</td>
<td>40.1 ± 4.2</td>
<td>43.5 ± 6.1</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ns, not significant; P, patients; C, controls; MP, mild proteinuria; SP, severe proteinuria; BP, blood pressure; BMI, body mass index; eGFR, estimated glomerular filtration rate; hsCRP, high-sensitive C-reactive protein; HOMA, homeostasis model assessment; FMD, flow-mediated dilatation; NMD, nitroglycerine-mediated dilatation; CIMT, carotid intima-media thickness; AHSG, alpha 2HS-glycoprotein; NAMPT, nicotinamide-phosphoribosyltransferase.

Fig. 1. Plasma NAMPT/visfatin (A), plasma adiponectin (B) levels, AHSG/fetuin-A (C) and FMD (D) in control subjects and type-2 diabetic patients with minor and severe proteinuria.
analysed separately. Furthermore, in the whole patient population, both NAMPT/visfatin and adiponectin were associated with hsCRP ($\rho = 0.33$, $P = 0.02$ and $r = -0.50$, $P < 0.001$ respectively), HbA1c ($\rho = 0.26$, $P = 0.02$ and $r = -0.28$, $P = 0.01$) and HOMA index ($\rho = 0.27$, $P = 0.01$ and $r = 0.24$, $P = 0.03$) as well as with eGFR ($\rho = -0.31$, $P = 0.004$ and $r = 0.33$, $P = 0.002$). The same was true for serum AHSG/fetuin-A levels, which correlated with hsCRP ($\rho = -0.41$, $P < 0.001$), HbA1c ($\rho = -0.29$, $P = 0.007$), CIMT ($\rho = -0.23$, $P = 0.03$) and eGFR ($\rho = 0.22$, $P = 0.04$), but not with circulating NAMPT/visfatin, adiponectin, FMD or HOMA. However, FMD did not correlate with CIMT in these patients.

Adiponectin/BMI ratios were positively correlated with FMD, serum albumin and eGFR ($\rho = 0.51$, $P < 0.001$, $\rho = 0.28$, $P = 0.01$ and $\rho = 0.29$, $P = 0.008$) and negatively correlated with proteinuria ($\rho = -0.55$, $P < 0.001$), NAMPT/visfatin ($\rho = -0.54$, $P < 0.001$), hsCRP ($\rho = -0.49$, $P < 0.001$), HbA1c ($\rho = -0.29$, $P = 0.007$) and HOMA ($\rho = -0.23$, $P = 0.031$).

### Discussion

In the present study, we demonstrate endothelium-dependent vascular dysfunction and altered levels of several vasoactive peptides in proteinuric type-2 diabetic patients with normal renal function, here termed early diabetic nephropathy. Compared to healthy matched controls, FMD was decreased by a mean of 12% in patients with minor proteinuria and with 30% in patients with severe proteinuria. Furthermore, the amount of protein in the urine correlated with FMD but not with NMD. Also, when proteinuria was completely removed from the second model, both adiponectin/BMI ratio ($r^2 = 0.12$, $P = 0.002$) and plasma NAMPT/visfatin levels ($r^2 = 0.10$, $P = 0.004$) were still independently related to FMD (adjusted $r^2 = 0.34$).
of the present study is thus the sharply delineated sample of untreated early diabetic nephropathy patients without a decreased GFR that we enrolled; these patients exhibited few comorbid conditions and had a low prevalence of other CVD risk factors. Somewhat unexpectedly, we did not find any correlation between FMD and CIMT in either patients or control subjects. While such a correlation has been reported in both atherosclerotic patients [18] and young healthy individuals [19], several other studies in apparently healthy populations [20] have also failed to show that these two risk factors are related. Thus, it may be that endothelial dysfunction (e.g. FMD) and atherosclerotic thickening of the arteries (e.g. cIMT) may represent different stages of the atherosclerotic process. Furthermore, in CKD patients, this relationship is further complicated by the ubiquitous presence of arterial medial calcification [21], which is thought to be reflected by CIMT and to influence FMD.

While the present study does not offer any clear explanation for the observed endothelial dysfunction, Krzyzanowska et al. [22] have suggested that serum levels of the NO-synthase inhibitor ADMA increase with increasing proteinuria, presumably due to an increased protein turnover. Proteinuria may also be associated with the loss of vasoprotective circulating proteins such as adiponectin and fetuin. Indeed, two studies by Koshimura [10] and Shimomotai et al. [11] recently demonstrated an increased urinary adiponectin in proteinuric patients, and earlier studies [23–25] have also suggested a link between low circulating adiponectin in type-2 diabetic patients and CVD. In the present study, we also found low circulating levels of adiponectin in proteinuric patients, as well as a negative association with urinary protein appearance.

As it has recently been reported that proteinuria is a marker of endothelial cell damage [26,33] and to act synergistically on the insulin receptor in vitro [26]. By finding an association with endothelial dysfunction (assessed as FMD) and NAMPT/visfatin, we confirm a previous report by Takebayashi et al. [2], but also extend this finding by showing an independent positive association between NAMPT/visfatin and proteinuria (Table 3).

Although few hypotheses exist to explain why a cytosolic protein catalyzing the rate-limiting step of nicotinamide adenine dinucleotide (NAD+ +) production [27] and reported to have insulinimetic effects [28] should be linked to proteinuria, a recent report in vitro showed that introducing the NAMPT gene into human vascular smooth muscle cells substantially lengthened the cell lifespan and increased their resistance to oxidative stress [29]. Thus, it may be that increased NAMPT/visfatin transcription is a physiological cell response to elevated oxidative or other stress. However, even though no correlation has so far been found between plasma NAMPT/visfatin levels and insulin sensitivity in humans [30–32], we cannot exclude that the reported association is simply a reflection of systemic glucose control. Clearly, the role of NAMPT/visfatin in diabetic nephropathy should be investigated in separate, mechanistic studies.

A number of weaknesses of the present study should be discussed. Foremost, the cross-sectional design of the study and the limited number of the patients preclude any assessment of causality. The highly selected group strengthens the reported associations, but limits the universality of our findings. Also, we did not measure the appearance of any of the studied peptides in the urine. Finally, we did not measure surrogate markers of oxidative stress or evaluate vascular morphology, two things that could be of great importance when interpreting our data.

In summary, in a well-defined group of type-2 diabetic patients, we show that already low-grade proteinuria with a normal GFR is associated with marked endothelial dysfunction and detrimentally altered circulating levels of vasocative peptides. We hypothesize that loss of these peptides in the urine may contribute to the increased risk of CVD seen in this patient group.

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Conflict of interest statement. B.L. is an employee of Baxter Healthcare Inc. P.S. is a member of the scientific advisory board of Gambro AB. The other authors have no conflicts of interest to declare.
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