Adiponectin in chronic kidney disease is related more to metabolic disturbances than to decline in renal function

Fitsum Guebre-Egziabher1, Jacques Bernhard1,2, Tohru Funahashi3, Aoumeur Hadj-Aissa2 and Denis Fouque1

1Département de Néphrologie, Hôpital E. Herriot and Research Unit JE 2411, University Claude Bernard, Lyon, France, 2Laboratoire d’Explorations Fonctionnelles Rénales et Métaboliques, Lyon, France and 3Department of Internal Medicine and Molecular Science, Osaka Medical School, Osaka, Japan

Abstract

Background. Adiponectin, a newly discovered collagen-like protein of the collectin family exclusively produced by adipocytes, possesses anti-inflammatory properties. Plasma adiponectin is associated with a decreased cardiovascular risk in non-renal patients, and is reduced in obesity and insulin-resistant states. Although reports show an increase in the adiponectin level in maintenance haemodialysis, peritoneal dialysis and end-stage renal disease, there is no documentation of adiponectin levels and regulation in the early stages of chronic kidney disease (CKD).

Methods. We prospectively measured glomerular filtration rate (GFR) in 48 patients with CKD using inulin clearance. Fasting blood was drawn to determine insulin, leptin, adiponectin and C-reactive protein (CRP) levels. Body fat mass was calculated using skinfold thickness measurements.

Results. The patients’ mean GFR was 53.5±24.9 (SD) ml/min/1.73 m2. Adiponectin was in the normal range in men (9.8±2.9 mg/l) and women (16.6±5.0 mg/l) with CKD, being significantly higher in women than men (P<0.001). Serum leptin was above normal (10.4±10.7 mg/l), whereas serum insulin and CRP were within their normal ranges (3.5±3.3 mU/ml and 2.6±5.0 mg/l, respectively). In linear regression analysis, adiponectin was negatively correlated with GFR (P=0.02), fat mass (P=0.03) and body mass index (P=0.002), and strongly positively correlated with serum leptin (P=0.003). A positive relationship was also found between plasma adiponectin and the urinary albumin/creatinine ratio (P=0.007). No relationship was found between adiponectin and insulin or adiponectin and CRP. In multiple regression analysis, adiponectin was significantly positively correlated with leptin (P<0.0001), negatively with body mass index (P<0.0001) and only weakly with GFR (P=0.04).

Conclusions. Despite an adverse metabolic environment in chronic renal insufficiency, serum adiponectin increases in non-obese patients when renal function deteriorates. Adiponectin is only weakly affected by renal function per se, but appears influenced by proteinuria, and more significantly by body mass index and the change in serum leptin that accompanies decline in renal function.

Keywords: hormones; inflammation; insulin; kidney; patients

Introduction

Evidence is accumulating that adipose tissue releases a number of active metabolic compounds, including pro-inflammatory cytokines [1]. These compounds include leptin, adipin, resistin, angiotensinogen, tumour necrosis factor-α (TNF-α), plasminogen activator inhibitor type-1 and interleukin (IL)-6. Therefore, obese patients or patients whose clearance of cytokines is impaired, as in chronic renal failure (CRF), may be prone to insulin resistance and accelerated atherosclerosis [2, 3].

Adiponectin, the product of the transcript-1 (apM1) gene, which is exclusively and most abundantly expressed in adipose tissue, is a 244 amino-acid protein structurally homologous to collagen VIII, collagen X and complement fraction C1q [4]. Adiponectin is reported to be abundant in human blood, with its plasma levels in the mg/ml range and, thus, accounting for ~0.01% of total plasma protein. Plasma adiponectin is decreased in obesity, suggesting that the dysregulation of adiponectin may be relevant to obesity-linked disorders [5]; it is also reduced in type 2 diabetes,
coronary artery disease (CAD) and dyslipidaemia [6]. Several in vitro studies indicate that adiponectin plays an important role in the inhibition of the inflammatory response and possesses anti-atherogenic properties [7].

Abnormal glucose, insulin and lipoprotein metabolism are common in patients with CRF. Also well known are increased cardiovascular morbidity and inflammation in these patients [8]. Plasma adiponectin has been reported to be increased in end-stage renal disease (ESRD) before dialysis and in patients on maintenance dialysis (haemodialysis and peritoneal dialysis) [9–11], and to be inversely related to incident cardiovascular events [9]. Whether or not this increase reflects impaired adiponectin clearance by the kidney or whether it is a compensatory mechanism aimed at counteracting increased cardiovascular risk factors is not elucidated yet. In a more recent paper, Zoccali et al. [12] report that adiponectin is increased in patients with nephrotic syndrome and that proteinuria is strongly related to circulating adiponectin.

The purpose of this study was to prospectively assess the relation of plasma adiponectin to proteinuria (as measured by urinary albumin/creatinine ratio), body composition, insulin metabolism and biological markers of inflammation in patients with a large range of renal insufficiencies.

Subjects and methods

Patients

We studied 48 patients with known kidney diseases, using as controls 20 volunteers matched for age and weight, with no known kidney disease and negative urinary dipstick analyses. The protocol was approved by the local ethics committee (CCPPRB Lyon A) and informed consent was obtained from each participant.

Kidney function measurement

We used inulin clearance to determine glomerular filtration rates (GFRs). Briefly, inulin [polyfructosan (inutest); Laevosan, Linz, Austria] was infused continuously for 3h after a priming dose and urine was collected every 30 min by spontaneous voiding. Inulin was measured by a standard colorimetric assay on a Technicon AutoAnalyzer (model AAI Technicon Corporation, Tarryton, NY, USA).

Blood measurements

To determine adiponectin, leptin, insulin and C-reactive protein (CRP), blood was drawn from all subjects at 07.45, before breakfast after an overnight fast. Samples were chilled immediately on ice and centrifuged at 3000 g, then serum or plasma was separated and samples were kept at −70 °C until laboratory analysis.

Plasma adiponectin was measured in the laboratory of the Department of Internal Medicine and Molecular Science of the Medical School of Osaka (Osaka, Japan) using a sensitive enzyme-linked immunosorbent assay [5] and recombinant adiponectin as standard. Serum leptin was measured in duplicate by radioimmunoassay (Mediagnost GmbH, Tübingen, Germany) using standards and a 125I-tracer prepared from recombinant leptin and a high-affinity polyclonal antibody that recognized specifically and quantitatively human leptin. Plasma insulin was measured by radioimmunometric assay using a mouse monoclonal antibody (ERIA Diagnostics Pasteur, Marnes la Coquette, France). Ultra-sensitive serum CRP was measured by an automated immunonephelometric method, whose detection limit was 0.2 mg/l. The albumin/creatinine ratio was calculated using measurements made on the first urine sample collected after an overnight fast.

Anthropometry

Anthropometric data were obtained once for each subject, at enrolment into the study. They included measured body weight and body fat mass calculated from the Durnin and Womersley tables [13], and were based on skinfold thickness measurements performed in triplicate at four different sites (bicipital, tricipital, subscapular and suprailiac).

Statistics

Data analysis was performed using a statistical software program (Statview, Abacus Concepts, Berkeley, CA, USA). Quantitative results are reported as means±SEM unless indicated otherwise. Correlation between two variables was searched by simple regression analysis. Multiple linear regression analysis was used to define the variables most predictive of circulating adiponectin concentrations, after selecting the parameters that were found to be associated with adiponectin by simple regression analysis or those known to be of importance in the physiology of adiponectin: body mass index (BMI), leptin, insulin, age and GFR. Adiponectin, leptin and the urinary albumin/creatinine ratio, which did not demonstrate a Gaussian distribution, were logarithmically transformed. A P-value of <0.05 was considered significant.

Results

Patients’ characteristics

We recruited 48 patients with chronic kidney disease (CKD) (36 males), aged 45±13 years, and 20 volunteers (13 males). The main clinical and anthropometric characteristics of the cohort are summarized in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Clinical and anthropometric characteristics of patients</th>
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<tbody>
<tr>
<td><strong>Age (years)</strong></td>
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<tr>
<td><strong>Gender (male/female)</strong></td>
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<tr>
<td><strong>Arterial pressure</strong></td>
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<tr>
<td><strong>Systolic (mmHg)</strong></td>
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<tr>
<td><strong>Diastolic (mmHg)</strong></td>
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<td><strong>BMI (kg/m²)</strong></td>
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<tr>
<td><strong>Body weight (kg)</strong></td>
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<td><strong>Fat mass (kg)</strong></td>
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<td><strong>Fat-free mass (kg)</strong></td>
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</table>

Values are expressed as means±SD, n = 48.
Their renal diseases were glomerulonephritis \((n = 13)\), interstitial nephritis \((n = 6)\), nephrosclerosis \((n = 5)\), polycystic kidney disease \((n = 3)\), and were of other or unknown aetiologies in 21 patients. Diabetic patients and patients treated with steroids were excluded. Of the patients, 30 were on antihypertensive drugs [20 on single therapy with angiotensin-converting enzyme inhibitors (ACEI), calcium channel blockers, diuretics or \(\beta\)-blockers; 10 on double or triple therapy with various combinations of these drugs].

**Biochemical parameters**

The biochemical data are summarized in Table 2. The mean plasma adiponectin among the CKD patients \(11.5 \pm 4.0 \text{mg}/\text{l} \) (range: \(4.4–29.3 \text{mg}/\text{l}\)) was not significantly different from the average value for healthy subjects \((10.3 \pm 3.9 \text{mg}/\text{l}; P = 0.41)\). Plasma adiponectin was higher \((P < 0.0001)\) in female \((16.6 \pm 5.0 \text{mg}/\text{l})\) than in male CKD patients \((9.8 \pm 2.9 \text{mg}/\text{l})\). The between-genders difference in plasma adiponectin in the patients was similar to that observed in the healthy controls \((13.8 \pm 3.8 \text{mg}/\text{l} \text{in women} \text{ vs } 8.4 \pm 2.5 \text{mg}/\text{l} \text{in men}; P < 0.01)\). Plasma leptin levels among the CKD patients \((10.5 \pm 7.4 \text{ng}/\text{ml})\) were significantly higher than in healthy subjects \((5.49 \pm 4.0 \text{ng}/\text{ml}; P = 0.04)\). Women had higher circulating levels of leptin than men, both in CRF \((17.5 \pm 9.7 \text{ng}/\text{ml} \text{ vs } 8.2 \pm 5.7 \text{ng}/\text{ml}; P < 0.01)\) and in healthy subjects \((8.7 \pm 3.7 \text{ng}/\text{ml} \text{ vs } 3.7 \pm 2.9 \text{ng}/\text{ml}; P = 0.02)\). Plasma insulin and CRP levels were in their normal ranges \((3.5 \pm 2.4 \text{mU}/\text{l} \text{ and } 2.7 \pm 2.5 \text{mg}/\text{l}, \text{respectively})\). Microalbuminuria (as defined by a urine albumin/creatinine ratio between 2 and 20 mg/mmol) was found in 10 patients, whereas 27 patients presented creatinine ratio between 2 and 20 mg/mmol) was found in 10 patients, whereas 27 patients presented.

**Glomerular filtration rate**

The mean GFR was 53.5 \(\pm 24.9 \text{ml}/\text{min}/1.73 \text{m}^2\) (range: \(12–107 \text{ml}/\text{min}/1.73 \text{m}^2\); Table 2), corresponding to stage III of CKD. GFR was \(< 50 \text{ml}/\text{min}/1.73 \text{m}^2\) in 29 subjects.

**Table 2. Biochemical data in CKD patients and control subjects**

<table>
<thead>
<tr>
<th>Gender</th>
<th>CKD patients</th>
<th>Controls</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Male ((n = 36))</td>
<td>Female ((n = 12))</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m(^2))</td>
<td>55.9 (\pm 22.5)</td>
<td>51.2 (\pm 20.9)</td>
</tr>
<tr>
<td>Adiponectin (mg/l)</td>
<td>9.8 (\pm 2.9)</td>
<td>16.6 (\pm 5.0)</td>
</tr>
<tr>
<td>Leptin ((\mu)g/l)</td>
<td>8.2 (\pm 5.7)</td>
<td>17.5 (\pm 9.7)</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>3.8 (\pm 2.5)</td>
<td>2.5 (\pm 1.8)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>2.6 (\pm 5.5)</td>
<td>2.5 (\pm 3.3)</td>
</tr>
</tbody>
</table>

Values are expressed as means \(\pm\) SD. \(n = 48\).

\(^a P < 0.01,\) female vs male (CKD).

\(^b P < 0.01\) and \(^c P < 0.05,\) female vs male (controls).

ND: not determined.

**Fig. 1.** The inverse relationship between GFR (as measured by inulin clearance) and fasting plasma adiponectin (mg/l, log transformed); \(n = 48, r = 0.31, P = 0.02\).
altered metabolic profile (e.g. serum leptin) that occurs with the impairment of renal function. Zoccali et al. [12] showed a positive linear relationship between proteinuria and plasma adiponectin in patients with nephrotic syndrome (mean proteinuria: 6.8±2.6 g/24 h) and in non-nephrotic proteinuric patients (mean proteinuria: 0.98±1.3 g/24 h). It is interesting to note here that we confirm this finding and also show that it occurs earlier during the course of renal disease, in patients with a less significant proteinuria (Figure 4; mean urinary albumin/creatinine: 80±13 mg/mmol; range: 0.3–310 mg/mmol). However, this relationship stops being significant when adjustments are made for other factors (GFR, BMI and serum leptin). Thus, it is possible that, in patients with nephrotic syndrome, massive proteinuria may impact adiponectin more strongly by triggering other yet unidentified metabolic disorders. Another point to clarify is the effect of ACE inhibition on adiponectin levels. Indeed, Furuhashi et al. [14] recently showed that ACE inhibition was associated with an increase in plasma adiponectin in patients with essential hypertension. Although we did not show differences in plasma adiponectin between patients who were taking ACE inhibitors and those who were not, our study was not designed to examine this question. This interesting point requires further research, since ACE inhibitors are the first line of therapy in CKD patients.

Adiponectin and insulin metabolism
Adiponectin is the most abundant gene product in adipose tissue. Plasma adiponectin is decreased in obesity and diabetes [6]. Hotta et al. [15] reported simultaneous reductions in insulin action and plasma adiponectin levels with the progression of obesity in rhesus monkeys. The administration of recombinant adiponectin improved hyperglycaemia and hyperinsulinaemia and it was able to reverse insulin resistance in obese mice. In a recent study of 148 women undergoing a glucose-clamp study, adiponectin was positively associated with improved glucose oxidation [16]. Altogether, these findings suggest that adiponectin actively influences glucose and insulin metabolism; however, the link between adiponectin and insulin sensitivity remains unclear. In this study, we were not able to identify a relationship between plasma adiponectin and fasting insulin, perhaps because of one of two reasons. First, a type 2 error due to the limited size of the study, which, however, did not prevent us from observing a rather strong relationship between adiponectin and leptin (the latter would have been strengthened by increasing the number of patients). Second, and more likely, our patients were not obese and had plasma insulin levels within its normal range, which might have limited the insulin-related regulation of adiponectin and allowed leptin to exert the predominant effect on adiponectin. Unfortunately, since we did not collect the necessary data (e.g. fasting blood glucose or HOMA index), we could not get a better insight into the relationship between insulin metabolism and adiponectin in renal failure. Dynamic interventions, such as prolonged fasting or insulin...
infusion studies, appear to be warranted to highlight these interactions in patients with CKD.

Adiponectin and body composition

Like many other metabolic hormones, body composition is a strong determinant of plasma adiponectin in other non-renal diseases as well as in renal patients. Plasma adiponectin is mainly inversely correlated with body fat mass, BMI or total body weight. Two recent studies have shown increases in plasma adiponectin after substantial weight loss following either gastroplasty or hypocaloric diet [17,18]. By contrast, exercise training had no effect on plasma adiponectin, although it significantly improved insulin sensitivity [19] (but it may not have changed body composition enough to alter plasma adiponectin). Another major determinant of plasma adiponectin is serum leptin [9,16,20,21]. Since serum leptin and plasma adiponectin are strongly linked to fat mass in opposite ways, the relationship between serum leptin and plasma adiponectin still remains unclear. An inverse relationship between leptin and adiponectin has been reported in healthy women, in obese patients [20] and in patients on MHD [9], whereas a positive correlation has been shown in patients with lipodystrophy [21]. In the present study, we found a strong positive relationship between serum leptin and plasma adiponectin (Figure 3), which remains highly significant (P < 0.0001) after adjusting for body composition and GFR. It is the most important relationship accounting for the increased plasma adiponectin in CKD patients.

Thus, the rise in plasma adiponectin during progressive renal failure and before ESRD may be the consequence of the well-described increase of serum leptin that occurs when GFR falls [22]. These results disagree with those obtained in MHD patients by Zoccali et al. [9], who reported an inverse relationship between serum leptin and plasma adiponectin. These authors also found a negative correlation between adiponectin and insulin, which we did not observe in our patients. It is possible that, in the present study in the absence of hyperinsulinaemia, hyperleptinaemia might have contributed to the increase of plasma adiponectin. There is presently no clear understanding why adiponectin is low in patients with increased fat mass when adiponectin is produced by adipose tissue. This may result from a negative feedback. Our hypothesis is that such a feedback is impaired in CKD. Alternatively, since serum leptin in obese adults seems to depend on subcutaneous fat while plasma adiponectin depends more on visceral fat [23], it is possible that an abnormal fat repartition in patients with CKD may cause the difference we report here. Indeed, a recent report by Stenvinkel et al. [11] in ESRD patients before dialysis (GFR: 6–7 ml/min/1.73 m²) found a negative correlation between adiponectin and truncal fat mass, although no correlation was found between adiponectin and arm and leg fat mass. A further possible explanation could be that in the present study we did not include obese patients [the mean BMI of our cohort was 25.1 kg/m² (range: 18–37 kg/m²) and only five patients had a BMI ≥30 kg/m²], whereas some of Zoccali’s patients had a BMI of 45 kg/m² [9]. The fact that obesity induces hyperinsulinaemia and alters plasma adiponectin might partly explain these discrepancies. In addition, we excluded diabetic patients in order to avoid confounding factors with possible impacts on adiponectin levels, a limitation not imposed in other studies of CKD [9–11]. Finally, there is virtually no information on the potential effects of dialysis membranes and biocompatibility on adiponectin metabolism. Thus, dialysis characteristics or differences among patients’ body composition and metabolic status may partly explain these observations.

Adiponectin and inflammation

Recent data have demonstrated that in obese patients there is an inverse relationship between, on the one hand, plasma adiponectin and, on the other hand, serum CRP and IL-6 [24], which could underlie a possible mechanism for the well-described increase in cardiovascular risk factors with excessive adiposity. It has been suggested that adiponectin may inhibit the detrimental effects of TNF-α on insulin signalling. Adiponectin may also be a direct marker of CAD, since low plasma adiponectin levels are associated with active CAD [7,11,17]. In vitro studies have shown an inhibitory effect of adiponectin on the TNF-α-induced expression of endothelial adhesion molecules [7] and the suppression of the transformation of macrophage to foam cell [25].

Recent data in CKD patients indicate that plasma adiponectin is associated with several risk factors, such as hypercholesterolaemia or hyperinsulinaemia [9,12]. Zoccali et al. [9] have reported that plasma adiponectin is an inverse predictor of incident cardiovascular events in MHD patients, despite their adiponectin values being higher than those of controls. It should be noted that cardiovascular risk was still higher in patients with lesser elevations of adiponectin. Thus, in maintenance dialysis, adiponectin seems to remain protective, although this protection may occur in the presence of higher plasma adiponectin levels [9]. We did not find a relationship between plasma adiponectin and CRP here, possibly because of the absence of any true inflammatory process (mean CRP: 2.7 ± 5.2 mg/l; Table 2). This observation is in accordance with the report by Zoccali et al. [9]. Two recent studies, however, showed an inverse relationship between adiponectin and CRP among peritoneal dialysis and ESRD patients before dialysis [10,11]. Nevertheless, this point seems important enough to strongly encourage longitudinal prospective studies that examine the relationship between adiponectin and inflammation in CRF patients.

In conclusion, we have shown that plasma adiponectin is elevated when renal function is impaired – as early as CKD stage III (Figure 1) – and particularly in proteinuric patients. This increase is associated mainly with body composition (e.g. BMI) and plasma leptin,
which is known to increase when renal function deteriorates. In our non-obese and non-diabetic patients, no relationship was found between adiponectin and serum insulin or CRP, these latter parameters being in their normal ranges. Thus, the plasma adiponectin rise that occurs when renal function deteriorates may represent an adaptive response to the altered metabolic profile associated with a high cardiovascular risk in CKD patients.

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