Possible Relationship between Adiponectin and Renal Tubular Injury in Diabetic Nephropathy

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Abstract. Adiponectin is an adipose-derived protein which has anti-inflammatory and anti-atherogenic properties in addition to insulin-sensitizing effects. To date, the role of adiponectin in the pathogenesis of diabetic nephropathy remains unclear. The aim of the present study was to explore the relationship between adiponectin and renal tubular injury in diabetic nephropathy. We determined serum and urinary adiponectin levels in type 2 diabetic patients with normoalbuminuria (n = 19), microalbuminuria (n = 18), and overt diabetic nephropathy (n = 16), and then analyzed the correlations between serum and urinary adiponectin, urinary N-acetylglucosaminidase (NAG) as a clinical marker of renal tubular injury, urinary monocyte chemoattractant protein-1 (MCP-1) as an inflammatory marker in renal tubulointerstitium, and clinical markers of renal disease. Notably, serum and urinary adiponectin levels were significantly increased in patients with overt diabetic nephropathy compared to those with normoalbuminuria and microalbuminuria. In univariate linear regression analysis, serum adiponectin levels were positively correlated with serum creatinine (r = 0.648, P<0.0001), urinary albumin (r = 0.583, P<0.0001), urinary NAG (r = 0.406, P<0.01), urinary MCP-1 (r = 0.514, P<0.0001), and urinary adiponectin (r = 0.691, P<0.0001) levels in all diabetic patients. Urinary adiponectin levels were also positively correlated with serum creatinine (r = 0.729, P<0.0001), urinary albumin (r = 0.799, P<0.0001), urinary NAG (r = 0.701, P<0.0001), and urinary MCP-1 (r = 0.801, P<0.0001) levels in all diabetic patients. Multiple linear regression analysis showed that serum creatinine and urinary adiponectin levels were independently associated with serum adiponectin levels (r² = 0.522), and that serum creatinine, urinary NAG, urinary MCP-1, and serum adiponectin levels were independent determinants of urinary adiponectin levels (r² = 0.851). These results collectively indicate that renal insufficiency and tubular injury possibly play a contributory role in increases in serum and urinary adiponectin levels in overt diabetic nephropathy. We presume that an increase in circulating adiponectin in overt diabetic nephropathy might be a physiological response to mitigate renal tubular injury and to prevent the further progression of diabetic nephropathy through its anti-inflammatory and anti-atherogenic effects.

Key words: Adiponectin, Diabetic nephropathy, Renal tubular injury, N-acetylglucosaminidase, Monocyte chemoattractant protein-1

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ADIPONECTIN is a novel adipocytokine that has an insulin-sensitizing property. It is exclusively expressed in adipose tissue [1] and abundantly released into circulating blood [2]. Experimental studies have demonstrated that administration of recombinant adiponectin to mice decreases the expression of hepatic gluconeogenic enzymes such as phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), and reduces hepatic glucose production, leading to an increase in insulin sensitivity in liver which is a main insulin target organ [3, 4]. Decreased plasma adiponectin levels have been found to be strongly implicated in the development of insulin resistance in humans [5, 6] and mice [7]. Thus, adiponectin appears to work as an important modulator of insulin action.
and glucose metabolism.

In addition to the insulin-sensitizing effects, adiponectin has been known to have potential anti-inflammatory and anti-atherogenic properties, including inhibition of monocyte adhesion to endothelial cells [8], suppression of oxidized low density lipoprotein uptake of macrophage through scavenger receptors [9], suppression of macrophage-to-f0am cell transformation [9], and inhibition of vascular smooth muscle cell proliferation and migration [10]. Recent clinical studies have reported that plasma adiponectin levels are lower in type 2 diabetic patients with coronary artery disease (CAD) than in those without CAD [11, 12], and also indicated that patients with ischemic cerebrovascular disease (CVD) have decreased plasma adiponectin levels relative to subjects without CVD [13]. These findings suggest that hypoadiponectinemia is a risk factor for macrovascular diseases. Taken together, adiponectin may play a protective role against the development of macrovascular diseases through its anti-inflammatory and anti-atherogenic effects.

In contrast to the studies examining patients with macrovascular diseases such as CAD and CVD, we have recently found that serum and urinary adiponectin levels are increased in type 2 diabetic patients with overt nephropathy as a microvascular disease compared to those without nephropathy [14]. There is a similar report showing that plasma adiponectin levels are markedly increased in patients with nephrotic syndrome relative to patients with chronic nephropathies but without nephrotic syndrome and healthy subjects [15]. We presume that the synthesis of adiponectin in adipose tissue and its secretion into circulating blood might be enhanced in parallel with the progression of diabetic nephropathy to mitigate renal microvascular damage and inflammatory changes through its anti-inflammatory and anti-atherogenic effects. To date, however, the role of adiponectin in the pathogenesis of diabetic nephropathy remains unclear.

Urinary N-acetylglucosaminidase (NAG) is widely used as a clinical marker of renal tubular damage. Monocyte chemoattractant protein-1 (MCP-1) is a chemokine that mediates renal tubulointerstitial inflammation, tubular atrophy, and interstitial fibrosis [16, 17]. Recently, our laboratory has reported that urinary MCP-1 level represents the degree of renal tubular damage in diabetic nephropathy [18]. To explore a possible relationship between adiponectin and renal tubular injury in diabetic nephropathy, we analyzed correlations between serum and urinary adiponectin, urinary NAG, and urinary MCP-1 levels in type 2 diabetic patients with different stages of nephropathy.

Subjects and Methods

Subject

Fifty-three type 2 diabetic patients and 20 healthy normoglycemic control subjects were recruited for this study. Normoalbuminuria was defined as urinary albumin to creatinine ratio (ACR) <30 mg/g creatinine, microalbuminuria as ACR 30–299 mg/g creatinine, and macroalbuminuria as ACR ≥300 mg/g creatinine. Diabetic nephropathy was diagnosed clinically if the following criteria were fulfilled: persistent macroalbuminuria in two out of three consecutive determinations, presence of diabetic retinopathy, and no clinical or laboratory evidence of other kidney or renal tract diseases. Diabetic patients were divided into three groups of normoalbuminuria (n = 19), microalbuminuria (n = 18), and diabetic nephropathy (n = 16). Patients taking thiazolidinediones (TZDs) were not enrolled in this study, since TZDs are known to enhance serum adiponectin level [19, 20]. Written informed consent was obtained from all study participants. Fasting serum and overnight urine samples were collected from all the subjects and stored at −85°C before testing.

Measurement of adiponectin, NAG, and MCP-1 levels

Serum and urinary adiponectin levels were measured using a commercially available radioimmunoassay kit (Linco Research, St. Louis, MO, USA). Urinary NAG levels were determined by the m-cresol purple method (Shionogi, Osaka, Japan). Urinary MCP-1 levels were measured by an immunoradiometric assay that we developed recently [18]. Urinary excretion levels of adiponectin or MCP-1 were expressed as the ratio of urinary concentrations of adiponectin or MCP-1 to grams of urinary creatinine.

Statistical analysis

Statistical analyses were performed with GraphPad Prism software system (GraphPad, San Diego, CA, USA) and SPSS software system (SPSS, Chicago, IL,
USA). Data are presented as means ± SD for normally distributed values and median (range) for non-normally distributed values. Differences among groups were determined by ANOVA followed by Bonferroni’s multiple comparison test for normally distributed values and by Kruskal-Wallis analysis followed by Dunn’s multiple comparison test for nonparametric values. After non-normally distributed values were log-transformed to better approximate normal distributions, correlations were calculated by Pearson’s correlation analysis. Multiple linear regression analysis with serum or urinary adiponectin as a dependent variable was used to evaluate independent relationships. Frequencies were compared by chi-squared test. A $P$ value of less than 0.05 was considered statistically significant.

**Results**

General characteristics and clinical parameters of type 2 diabetic patients and age-matched healthy control subjects are summarized in Table 1. Three diabetic patient groups of normoalbuminuria, microalbuminuria, and diabetic nephropathy were well matched regarding age, body mass index, and HbA1c. Diabetic patients were treated with sulfonylureas alone, insulin alone, or no medicine. It is well known that hypertension is a frequent phenomenon in diabetic nephropathy [21–23]. Expectedly, diabetic nephropathy group showed longer duration of diabetes and higher frequency of hypertension. Serum creatinine, urinary NAG, and urinary MCP-1 levels were significantly higher in diabetic nephropathy group than each of normoalbuminuria and microalbuminuria groups. Therefore, patients of diabetic nephropathy group were regarded as having impaired renal function and renal tubular injury.

Serum adiponectin levels in control subjects and type 2 diabetic patient groups of normoalbuminuria, microalbuminuria, and diabetic nephropathy were 10.1 (5.7–15.9), 6.4 (3.0–10.5), 7.0 (3.2–11.2), and 11.6 (6.4–28.1) µg/ml, median (range), respectively (Fig. 1A). Patient groups of normoalbuminuria and microalbuminuria exhibited significantly reduced serum adiponectin levels relative to control subjects, while there was no statistically significant difference in serum adiponectin level between control subjects and patient group of diabetic nephropathy. Among the three diabetic patient groups, serum adiponectin level was significantly higher in the diabetic nephropathy group than each of the normoalbuminuria and microalbuminuria groups. No significant difference was observed in serum adiponectin level between the normoalbuminuria and microalbuminuria groups. Urinary adiponectin levels in control subjects and type 2 diabetic patient groups of normoalbuminuria, microalbuminuria, and diabetic nephropathy were 3.9 (1.5–6.0), 2.9 (1.5–5.7), 3.4 (1.0–5.5), and 24.3 (6.6–258.2) µg/g creatinine, median (range), respectively (Fig. 1B). Thus, the diabetic nephropathy group showed markedly increased urinary excretion of adiponectin. In contrast, control

| Table 1. Clinical parameters of healthy control subjects and type 2 diabetic patients |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                | Control subjects | Type 2 diabetic patients |                  |
|                                |                 | Normoalbuminuria | Microalbuminuria | Diabetic nephropathy |
| $n$                             | 20              | 19              | 18              | 16              |
| Age (years)                    | 57 ± 8          | 62 ± 5          | 61 ± 6          | 60 ± 8          |
| Gender (M/F)                   | 11/9            | 16/3            | 15/3            | 9/7             |
| Duration of diabetes (years)   | NA              | 10 ± 5          | 15 ± 9          | 20 ± 6†         |
| Treatment (Diet/Sulfonylureas/Insulin) | NA       | 4/11/4       | 2/8/8           | 1/5/10          |
| Retinopathy (N/S/P)            | NA              | 14/4/1          | 9/1/8           | 0/2/14          |
| Hypertension ($n$ [%])         | NA              | 12 (63)         | 17 (94)‡        | 16 (100)§       |
| Body mass index (kg/m$^2$)     | 22.9 ± 2.7      | 23.5 ± 2.1      | 24.9 ± 2.7      | 24.2 ± 3.3      |
| HbA1c (%)                      | 5.0 ± 0.2       | 7.1 ± 0.6*      | 7.4 ± 0.9*      | 7.0 ± 0.6*      |
| Serum creatinine (mg/dl)       | 0.80 (0.60–0.90)| 0.70 (0.60–1.00)| 0.75 (0.40–1.00)| 1.30 (0.50–4.40)†‡|
| Urinary albumin (mg/g creatinine) | 4.5 (0.8–14.2) | 5.0 (2.6–15.6) | 81.6 (30.4–197.8)*§ | 1904.0 (397.7–5477.0)*†‡|
| Urinary NAG (U/g creatinine)   | ND              | 4.5 (2.8–7.8)   | 5.2 (2.3–10.5)  | 10.5 (4.2–18.3)†‡|
| Urinary MCP-1 (ng/g creatinine) | ND              | 156 (90–313)    | 190 (102–566)   | 394 (212–1277)†‡|

Data are presented as means ± SD or median (range). Retinopathy: N, normal; S, simple; P, proliferative. NA, not applicable; ND, not determined. *$P<0.001$ vs. control subjects; †$P<0.001$ vs. normoalbuminuria; ‡$P<0.001$ vs. microalbuminuria; †‡$P<0.05$ vs. normoalbuminuria; §$P<0.01$ vs. normoalbuminuria.
subjects and the normoalbuminuria and microalbuminuria groups exhibited low levels of urinary adiponectin excretion, and there were no significant differences among the three groups. As shown in Fig. 2, serum adiponectin levels were positively correlated with urinary adiponectin levels in all diabetic patients \((r = 0.691, P < 0.0001)\).

We next analyzed correlations between serum or urinary adiponectin levels and clinical markers of renal disease in all diabetic patients (Fig. 3). In univariate linear regression analysis, serum adiponectin levels were positively correlated with serum creatinine \((r = 0.648, P < 0.0001)\), urinary albumin \((r = 0.583, P < 0.0001)\), urinary NAG \((r = 0.406, P < 0.01)\), and urinary MCP-1 \((r = 0.514, P < 0.0001)\) levels. Urinary adiponectin levels were also positively correlated with serum creatinine \((r = 0.729, P < 0.0001)\), urinary albumin \((r = 0.799, P < 0.0001)\), urinary NAG \((r = 0.701, P < 0.0001)\), and urinary MCP-1 \((r = 0.801, P < 0.0001)\) levels. In multiple linear regression analysis for factors significantly correlated with serum and urinary adiponectin, serum creatinine and urinary adiponectin levels were independently associated with serum adiponectin levels \((r^2 = 0.522)\) (Table 2). When urinary adiponectin was used as a dependent variable, serum
Fig. 3. Univariate analysis of relationships between serum (A, C, E, G) or urinary (B, D, F, H) adiponectin levels and clinical markers of renal disease in type 2 diabetic patients. Serum and urinary adiponectin, serum creatinine (A, B), urinary albumin (C, D), urinary NAG (E, F), and urinary MCP-1 (G, H) levels were log-transformed. NA, MA, and DN indicate type 2 diabetic patients with normoalbuminuria, microalbuminuria, and diabetic nephropathy, respectively.
creatinine, urinary NAG, urinary MCP-1, and serum adiponectin levels were independent determinants of urinary adiponectin levels ($r^2 = 0.851$) (Table 2).

**Discussion**

In the present study, we measured serum and urinary adiponectin levels in 53 type 2 diabetic patients with different stages of nephropathy and 20 healthy control subjects, and analyzed correlations between serum or urinary adiponectin levels and clinical markers of renal disease in all type 2 diabetic patients. In comparison between healthy control subjects and type 2 diabetic patients with normoalbuminuria which were matched regarding age, body mass index, and urinary albumin excretion, we found that serum adiponectin levels were reduced in patients with type 2 diabetes (Fig. 1A).

Similarly, recent studies have reported that plasma adiponectin levels are lower in obese patients with type 2 diabetes than in obese subjects with normal glucose tolerance [5, 20]. These findings collectively suggest that hypoadiponectinemia is associated with the development of type 2 diabetes.

In addition to a close link between serum adiponectin levels and glucose tolerance, there seems to be a possible relationship between serum adiponectin levels and diabetic nephropathy. We have recently reported that serum adiponectin levels are elevated in type 2 diabetic patients with overt nephropathy compared to those without nephropathy [14]. After our data were published, elevations in serum adiponectin levels in overt diabetic nephropathy have been reported also in type 1 diabetic patients [24, 25] and obese type 2 diabetic Pima Indians [26]. Also in the present study, comparison among three type 2 diabetic patient groups of different stages of nephropathy, which were well matched regarding age, body mass index, and HbA1c levels, expectedly revealed that serum adiponectin levels are elevated in patients with overt diabetic nephropathy but not in those with microalbuminuria, i.e., incipient diabetic nephropathy (Fig. 1A).

Elevations in serum adiponectin levels could result from either increased adiponectin synthesis or reduced adiponectin clearance or a combination of the two. We found that both serum and urinary adiponectin levels were markedly increased in diabetic patients with overt nephropathy (Fig. 1), and that serum adiponectin levels were positively correlated with urinary adiponectin excretion in all diabetic patients (Fig. 2). Therefore, it is plausible that the elevated serum adiponectin levels in overt diabetic nephropathy are due to enhanced adiponectin synthesis in adipose tissue and increased adiponectin secretion into circulating blood rather than reduced adiponectin clearance.

An important question is why adiponectin production is enhanced in patients with overt diabetic nephropathy. Renal tubular injury and infiltration of inflammatory cells into tubulointerstitial area are widely observed in overt diabetic nephropathy [27]. In the present study, patients with overt diabetic nephropathy exhibited increased urinary levels of NAG and MCP-1 (Table 1), implying that they had renal tubular injury and tubulointerstitial inflammation. On the other hand, patients with microalbuminuria did not have increased urinary levels of NAG and MCP-1 (Table 1), suggesting that renal tubular injury remains unremarkable clinically at the stage of incipient diabetic nephropathy. Given the evidence that adiponectin has potential anti-inflammatory and anti-atherogenic effects [8–10], we hypothesized that enhanced adiponectin production in overt diabetic nephropathy might be related to renal tubular injury and tubulointerstitial inflammation. To test this hypothesis, we analyzed correlations between serum or urinary adiponectin and clinical markers of renal disease including urinary NAG and MCP-1 levels in all diabetic patients. Both serum and urinary adiponectin levels were positively correlated with serum creatinine levels (Fig. 3). In addition, serum creatinine was a common independent determinant of serum and urinary adiponectin levels (Table 2). These results clearly indicate that renal insufficiency triggers increases in serum and urinary adiponectin levels. Furthermore, we found that both serum and urinary adiponectin levels were positively correlated with urinary NAG and MCP-1 levels (Fig. 3), and especially showed that urinary NAG and MCP-1 were independent determinants of urinary adiponectin levels (Table 2). Based on these findings, renal tubular injury appears to play a contributory role in increases in adiponectin production and urinary adiponectin excretion.

Diabetic patients with renal insufficiency have an increased risk of cardiovascular events [28], and hypoadiponectinemia has been reported to be associated with the development of cardiovascular events [11, 12]. Nevertheless, we found that diabetic patients with overt nephropathy clearly have elevated serum adiponectin levels. Notably, serum adiponectin levels appear to be
increased in proportion to the degree of renal tubular injury and tubulointerstitial inflammation. We presume that an increase in circulating adiponectin in overt diabetic nephropathy might be a physiological response to mitigate renal tubular injury and to prevent the further progression of diabetic nephropathy through its anti-inflammatory and anti-atherogenic effects.

References


