Original Research Article for *Journal of Diabetes and Its Complication*

Title

Urinary adiponectin excretion is an early predictive marker of the decline of the renal function in patients with diabetes mellitus.

Masashi Ishizu[1], Hiroyasu Mori[1], Mami Ohishi[1], Akio Kuroda[1], Yuko Akehi[1], Sumiko Yoshida[2], Ken-ichi Aihara[3], Motohiro Aiba[4], Tomoharu Kawano[5], Seiichi Hashida[6], Munehide Matsuhisa[1]

1. Diabetes Therapeutics and Research Center, Institute of Advanced Medical Sciences, Tokushima University, Tokushima, Japan
2. Department of Hematology, Endocrinology and Metabolism, Institute of Biomedical Sciences, Tokushima University Graduate School, Tokushima, Japan
3. Department of Community Medicine and Medical Science, Tokushima University Graduate School of Biomedical Sciences, Tokushima, Japan
4. Life Style Diseases, Institute for Health Sciences, Tokushima Bunri University, Tokushima, Japan
5. Human Life Science, Tokushima Bunri University, Tokushima, Japan
6. Department of Diabetes and Molecular Genetics, Ehime University Graduate School of Medicine, Ehime, Japan

Corresponding Author

Munehide Matsuhisa, M.D., Ph.D.

Diabetes Therapeutics and Research Center, Institute of Advanced Medical Sciences,

Tokushima University,
Address 3-18-15, Kuramoto-cho, Tokushima, 770-8503, Japan
Phone +81-088-633-7587
E-mail matuhisa@tokushima-u.ac.jp

Running title: Urinary adiponectin in DKD

Word counts: Abstract: 200 /200 maximum, Main body: 4,570/5,000 maximum

References: 36

Figures: 1

Tables: 4

Supplemental Figures: 1

Supplemental Tables: 7

Abstract

Aims: Since diabetes-associated kidney complication changes from diabetic nephropathy to diabetic kidney disease (DKD), more suitable biomarkers than urinary albumin are required. It has been hypothesized that urinary adiponectin (u-ADPN) is associated with the progression of DKD. We therefore evaluated the effectiveness of u-ADPN in predicting the decline of the renal function in patients with diabetes prior to end-stage renal disease.

Methods: An ultrasensitive immune complex transfer enzyme immunoassay (ICT-EIA) was used to measure total and high molecular weight (HMW) adiponectin separately. We evaluated the relationships between the creatinine-adjusted urinary total-ADPN and HMW-ADPN, albumin (UACR) and liver-type fatty acid binding protein (L-FABP) at baseline and the 2-year change of the estimated glomerular filtration rate (ΔeGFR).

Results: This 2-year prospective observational study included 201 patients with diabetes. These patients were divided into three groups according to their ΔeGFR: ≤-10 ml/min/1.73m^2, >-10 and ≤0 ml/min/1.73m^2, and >0 ml/min/1.73m^2. Jonckheere-Terpstra test showed that lower ΔeGFR was associated with higher u-HMW-ADPN ($p = 0.045$). In logistic regression analysis, u-HMW-ADPN was associated with ΔeGFR after adjusted age, sex, and basal eGFR.

Conclusion: Urinary HMW-ADPN could predict a declining renal function in patients with diabetes.

Keywords: diabetes kidney disease, urinary adiponectin, estimated glomerular filtration rate

1. Introduction

Diabetes mellitus is a major causative disease of chronic kidney disease (CKD) and end-stage renal disease (ESRD) [1]. In the traditional disease concept of kidney injury in diabetes, patients develop diabetic nephropathy (DN), which shifts in the order of glomerular hyperfiltration, appearance of microalbuminuria, overt proteinuria, and a decline in the glomerular filtration rate (GFR), which finally leads to end-stage renal failure [2]. However, recent studies have reported that some patients with diabetes have an impaired renal function in the absence of microalbuminuria, macroalbuminuria or proteinuria [1,3,4,5]. In addition, among patients with a preserved estimated GFR (eGFR) and normoalbuminuria (urinary albumin excretion ratio <30 mg/gCr), 60–70% of patients already showed pathological changes, such as mesangial expansion, interstitial fibrosis and/or tubular atrophy in the kidney [6]. Thus, the disease concept of kidney injury in diabetes is shifting from DN to diabetic kidney disease (DKD). DKD involves conventional DN and diabetes-related renal diseases in which the renal function declines without albuminuria [4,7,8,9,10]. It has also been reported that a decline in the eGFR of ≥5 mL/min/1.73m^2 per year is a risk factor for subsequent ESRD and all-cause of mortality [11,12]. Thus, Kidney Disease Improving Global Outcomes (KDIGO) defines a reduction in eGFR of >5 mL/min/1.73m^2 as "rapid progression" [13]. Furthermore, a previous study showed that a >5 mL/min/1.73m^2 or 5% reduction of the eGFR per year was associated with an increased risk of heart failure, renal failure and all-cause mortality in hypertensive patients with diabetes in comparison to those without diabetes [14].

Several studies intended to establish biomarkers as predictors of DKD [15] or “rapid progression” in eGFR decline[16]; however, no biomarkers are available in the clinical setting at the present time. Since rapid progression was frequently observed, even in patients whose with an eGFR of >60 mL/min/1.73m^2 [17], there is a need for new comprehensive surrogate markers that can replace the conventional surrogate marker for kidney injury in diabetes and predict “rapid

progression" of eGFR.

Adiponectin is involved in the maintenance of renal glomerular homeostasis [18], and it has been reported that adiponectin is present in glomeruli by immunohistochemical analysis in non-diabetic kidney[19]. On the other hand, glomerular adiponectin was found to be markedly decreased and urinary adiponectin excretion was increased in patients with diabetes [19]. In addition, the urinary adiponectin level has been reported to be correlated with the urinary N-acetylglucosaminidase (NAG) and urinary monocyte chemoattractant protein-1 (MCP-1) levels in patients with renal tubular disorders [20]. Therefore, urinary adiponectin excretion may be elevated in both glomerular and tubular disorders and may be a comprehensive marker of DKD. In fact, several clinical studies have reported that the development of DN or DKD is associated with urinary adiponectin excretion in type 1 and type 2 diabetes [21,22,23,24] . However, these studies are cross-sectional or longitudinal studies with a short follow-up period.

We have recently developed an ultrasensitive immune complex transfer enzyme immunoassay (ICT-EIA) for measuring total and high molecular weight adiponectin with high (zeptomole) sensitivity [25,26,27] . Thus, the aim of this study was to evaluate the relationship between the progression of renal injury and the urinary adiponectin level, as measured by an ultrasensitive immunoassay, in a cross-sectional and longitudinal manner.

2. Materials and Methods

2.1. Study design

This observational prospective single center study was approved by the ethics committee of Tokushima University Hospital (#2894). We recruited consecutive patients with

type 1 and type 2 diabetes who were managed, as outpatients, at Tokushima University Hospital from August 2017 to December 2018. Adult patients with diabetes, without any of the exclusion criteria were eligible for inclusion in the present study. The exclusion criteria were as follows: 1) patient with cancer; 2) patient with secondary diabetes, such as steroid induced diabetes or pancreatic diabetes; 3) patient with kidney disease other than diabetes; and 4) patient with end-stage renal disease. We obtained written informed consent from all patients. The study design is shown in Fig A.1. We collected clinical data and urine samples at baseline and followed the estimated GFR (eGFR) for 2 years. Cross-sectional analyses were performed at baseline to evaluate whether urinary adiponectin was associated with DKD (n=239), and a longitudinal analysis was performed to evaluate the relationship between the change of the eGFR (ΔeGFR) and urinary parameters at baseline (n=201). In addition, blood and urine samples were collected from the patients 1 year later, with informed consent for additional blood draws to evaluate the influence of serum adiponectin levels on urinary adiponectin levels (n=140).

2.2. Data collection

We obtained clinical background data, including age, sex, type and duration of diabetes, smoking status, diabetes complications, history of hypertension and/or dyslipidemia, the drugs in use, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), visceral fat area, glycated hemoglobin (HbA1c), eGFR, urinary parameters at baseline. Diabetic neuropathy was defined as peripheral neuropathy with ≥2 of the following 3 criteria: 1) subjective symptoms, probably due to diabetic neuropathy, 2) impairment or loss of the bilateral Achilles tendon reflex or 3) impaired vibration sensation at the inner ankles according to the simplified diagnostic criteria for diabetic polyneuropathy proposed by the consensus of the Japanese study group of diabetic neuropathy. The visceral fat area was measured by a medical visceral fat

147 measuring device using a multi-frequency BIA (HDS-2000 DUALSCAN; OMRON, Japan). We
148 obtained urinary parameters at baseline and 1 year after baseline and the serum adiponectin level
149 at 1 year after baseline. The eGFR was measured at all visits for two years. BMI was calculated
150 by the formula of weight (kg) divided by height squared (m^2). Urinary samples were collected
151 early in the morning, and the albumin and liver-type fatty acid-binding protein (L-FABP) levels
152 were measured and corrected by the urinary creatinine concentration, as biomarkers of glomerular
153 injury and tubular injury, respectively. A chemiluminescence enzyme immunoassay was used to
154 measure the L-FABP level. The eGFR was calculated according to the formula of the Japanese
155 Society of Nephrology, as follows: [eGFR (mL/ min/ 1.73m^2) = 194 × serum and creatinine level$^{-1.094}$ × age$^{-0.287}$ (× 0.739 if female)] [28]. The 2-year change of the eGFR (ΔeGFR) was determined
157 from the amount of change of the eGFR. The change of the eGFR was calculated by linear
158 approximation using the eGFR values at all visits. A ΔeGFR of ≤-10 mL/min/1.73m^2 was defined
159 as rapidly progressive renal injury.
160
161 **2.3. Measurement of adiponectin**
162
163 The newly developed ICT-EIA was used to measure the serum and urinary adiponectin
164 levels [25,26,27]. The ICT- EIA achieves zeptomole sensitivity by transferring the complex of analytes
165 and labeled reactants from solid phase to solid phase with minimal dissociation of the complex.
166 This method is able to detect two isoforms of adiponectin, total (total-ADPN) and high molecular
167 weight adiponectin (HMW-ADPN), using different antibodies. Monoclonal mouse anti-human
168 Adiponectin/Acrp30 antibody (Product code: MAB10651, Clone: 166126, Antibody Registry:
169 AB_2221612) and monoclonal mouse anti-human Adiponectin/Acrp30 antibody (Product code:
170 MAB1065, Clone: 166128, Antibody Registry: AB_2273512) were chosen as capture and

detection antibodies, respectively, for the total-ADPN assay. Monoclonal mouse anti-human Adiponectin/Acrp30 antibody (Clone: 38, Sysmex, Hyogo, Japan) was used as both capture and detection antibodies for the HMW-ADPN assay. Recombinant Human Adiponectin (Oriental yeast, Tokyo, Japan) was used for calibrators. Details of this method are written in previous reports [25,26,27] The urinary adiponectin level was corrected with division by urinary creatinine. The fractional excretion of adiponectin (FE-ADPN) according was determined, in order to evaluate influence of serum adiponectin on the urinary level, using the following formula: (urinary adiponectin level/ serum adiponectin level) / (urinary creatinine level / serum creatinine level).

2.4. Statistical analysis

The Shapiro-Wilk test was performed to assess the normality of continuous variables. Continuous variables that showed normal distribution were described as the mean ± standard deviation (SD) and that showed non-normal distribution were described as the median (Q1, Q3). Categorical variables were described as n (%). The Mann–Whitney U test, Kruskal–Wallis test and Bonferroni correction were used to assess the difference in continuous variables. Differences between categorical variables were evaluated by Fisher's exact test. Spearman's rank correlation coefficient was calculated to evaluate the correlation of adiponectin levels between serum and urinary samples, the correlation between urinary parameters and eGFR at baseline, and the correlation between u-ADPN, and u-ACR or u-L-FABP. To evaluate the significance of urinary adiponectin level as an early surrogate marker, we evaluated correlation between eGFR and urinary makers in patients with a urinary creatinine level of <30 mg/g Cr, or an eGFR of >60 mL/min/1.73m^2 in a correlation analysis. To investigate the relationship between eGFR at baseline and urine parameters, logistic regression analysis was performed using the following models;

Model 1, unadjusted; Model 2, adjusted by sex and age; and Model 3, adjusted by Model 2 + BMI, HbA1c and SBP. In logistic regression analysis, eGFR of >60 mL/min/1.73m^2 was defined as an event. In addition, considering the effects of the type of diabetes, we also performed these cross-sectional analyzes by type of diabetes. A Jonckheere-Terpstra test was performed to assess whether the baseline urinary adiponectin level, albumin level and L-FABP level were associated with the ΔeGFR. In order to evaluate the relationship between u-HMW-ADPN and ΔeGFR in more detail, logistic regression analysis was performed with ΔeGFR ≥-10 mL/min/1.73m^2 as an event. In logistic regression analysis, all continuous variables were bisected by median and were entered into the model with reference to the lower group. In the longitudinal analysis by type of diabetes, there were few cases of rapidly progressive renal injury in type 1 diabetes, so only type 2 diabetes was analyzed. All statistical analyses were performed using the SPSS 27 software program (IBM Japan, Tokyo, Japan). Statistical tests were two-sided and p-values of <0.05 were considered to indicate statistical significance.

3. Results

3.1. Clinical characteristics of study patients

We recruited 239 patients at baseline; 201 of these patients were followed for 2 years. The clinical characteristics at baseline are shown in Table 1. The median age was 63 years and 116 (48.5%) patients were male. The median eGFR was 68 mL/min/1.73m^2, the median urinary albumin-to-creatinine ratio (u-ACR) was 12 mg/g Cr and the urinary L-FABP (u-L-FABP) level was 1.5 μg/g Cr. The median urinary total adiponectin-to-creatinine ratio (u-total-ADPN) was 0.92 μg/g Cr and the median urinary HMW adiponectin-to-creatinine ratio (u-HMW-ADPN) was 0.12 μg/g Cr. The clinical characteristics at baseline by type of diabetes are shown in Table A.1.

Patients with type 1 diabetes were younger, thinner, and more female than those with type 2 diabetes. U-ACR was statistically higher in patients with type 1 diabetes than those with type 2 diabetes, but other urinary parameters were not significantly different depending on the type of diabetes.

3.2. Associations between the eGFR and urinary parameters

The following parameters were significantly correlated with the eGFR at baseline in all patients: u-total-ADPN ($r=-0.410$, $p<0.001$); u-HMW-ADPN ($r=-0.371$, $p<0.001$); u-ACR ($r=-0.306$, $p<0.001$); and u-L-FABP ($r=-0.247$, $p<0.001$) (Table 2). When we divided the patients into the two groups according to the u-ACR (cut-off value: 30 mg/g Cr), we observed that the eGFR was significantly inversely correlated with these urinary parameters in patients with a u-ACR ≥30 mg/g Cr. When we investigated the patients with a u-ACR <30 mg/g Cr, the eGFR was also found to be significantly correlated with u-total-ADPN ($r=-0.195$, $p=0.013$) and u-HMW-ADPN ($r=-0.161$, $p=0.041$); however, we did not observe any significant correlations between the eGFR and u-ACR or u-L-FABP (Table 2). Furthermore, when the patients were divided into two groups according to their eGFR (cut-off value: 60 mL/min/1.73m^2), the eGFR was found to be significantly inversely correlated with all urine parameters in patients with eGFR <60 mL/min/1.73m^2 (u-total-ADPN: $r=-0.481$, $p<0.001$; u-HMW-ADPN: $r=-0.483$, $p<0.001$; u-ACR: $r=-0.506$, $p<0.001$; and u-L-FABP: $r=-0.546$, $p<0.001$). However, in patients with eGFR ≥60 mL/min/1.73m^2, the only significant correlation was between eGFR and u-total-ADPN ($r=-0.182$, $p=0.021$); no other urine parameters were significantly correlated with the eGFR in this group.

The most of results were similar in the analysis by type of diabetes as shown in Table B.1.. However, significant correlation between u-ADPN and eGFR at baseline was observed in patients with type 1 diabetes prior to developing DKD (u-ACR ≥30 mg/gCr or eGFR <60 mL/min/1.73

m^2).

Table 3. shows logistic regression analysis between eGFR and urinary parameters at baseline. U-total-ADPN were significantly associated with eGFR in each model (Model 1: OR=4.0, p<0.001; Model 2: OR=3.4, p<0.001; Model 3: OR=3.4, p<0.001). U-HMW-ADPN were also significantly associated with eGFR in each model (Model 1: OR=3.7, p<0.001; Model 2: OR=3.5, p<0.001; Model 3: OR=3.5, p<0.001). Similar results were obtained by the type of diabetes (Table C.1.).

3.3. Correlations between u-ADPN, and u-ACR or u-L-FABP

U-total-ADPN and u-HMW-ADPN were highly correlated with u-ACR (u-total-ADPN: r = 0.623, p<0.001; u-HMV-ADPN: r = 0.732, P<0.001). U-total-ADPN and u-HMW-ADPN were also correlated with u-L-FABP (u-total-ADPN: r = 0.473, p<0.001; u-HMV-ADPN: r = 0.457, P<0.001). Similar results were also observed by the type of diabetes (data not shown).

3.4. The association of the adiponectin levels in urine and serum

Since u-ADPN and s-ADPN did not distribute normally, the logarithmic transformation was applied in these data. Fig 1. shows scatter plots of log (u-ADPN) and log (s-ADPN) of A) total-ADPN and B) HMW-ADPN at 1 year after baseline. u-total-ADPN and u-HMW-ADPN were found to be significantly correlated with the serum adiponectin (s-ADPN) levels using a nonparametric test (Fig 1.). A similar correlation was obtained by type of diabetes. We compared the urine, serum and fractional excretion (FE-ADPN) levels of total- or HMW-ADPN between patients grouped according to the u-ACR (cut-off value: 30 mg/g Cr) or eGFR (cut-off value: 60 mL/min/1.73m^2) (Table D.1). u-ADPN and s-ADPN of both total- and HMW-ADPN were significantly higher in patients with u-ACR ≥30 mg/ g Cr than in patients with u-ACR <30 mg/ g

Cr. Furthermore, the FE-ADPN levels of both total- and HMW-ADPN in the patients with u-ACR ≥30 mg/ g Cr were significantly higher in comparison to patients with u-ACR <30 mg/ g Cr. Similarly, u-ADPN and FE-ADPN of both total- and HMW-ADPN in the patients with eGFR <60 mL/min/1.73m^2 were significantly higher in comparison to patients with eGFR≥60 mL/min/1.73m^2. However, the s-ADPN levels of both the total- and HMW-ADPN in patients with eGFR <60 mL/min/1.73m^2 were not significantly higher in comparison to those in patients with eGFR ≥60 mL/min/1.73m^2 (Table D.1).

3.5. Relationship between ΔeGFR and urinary parameters

The patients were divided into three groups based on the ΔeGFR value as follows: ΔeGFR <0 mL/min/1.73m^2 (n=58), ΔeGFR >-10 to ≤0 mL/min/1.73m^2 (n=105), and ΔeGFR ≥-10 mL/min/1.73m^2 (n=38). The clinical characteristics of these 3 groups are shown in Table E.1. Table 4. shows the baseline urinary parameters in these 3 groups. u-HMW-ADPN was significantly correlated with ΔeGFR (p for trend = 0.045); however, u-total-ADPN, u-ACR, and u-L-FABP were not significantly correlated with ΔeGFR (p for trend = 0.493, 0.463 and 0.630, respectively). To better clarify the association between u-HMW-ADPN and ΔeGFR, which was significantly associated with the Jonckheere-Terpstra test, the logistic regression analysis was performed on a model adjusted for age, sex and eGFR at baseline. As a result, u-HMW-ADPN showed a significant association with ΔeGFR (OR=2.3, p=0.046). The clinical characteristics of these 3 groups of type 2 diabetes are shown in Table F.1. Similar results were obtained in Jonckheere-Terpstra test, conducted only patients with type 2 diabetes (Table G.1.).

4. **Discussion**

The present study analyzed the relationship between the progression of renal injury and two

291 isoforms of urinary adiponectin, as measured by an ultrasensitive immunoassay, in patients with
292 diabetes in a cross-sectional and longitudinal manner. In this study, u-total-ADPN was cross-
293 sectionally associated with eGFR in patients without DKD (u-ACR < 30 mg/gCr and eGFR ≥60
294 mL/min/1.73m^2), and those with DKD (u-ACR ≥30 mg/gCr or eGFR <60 mL/min/1.73 m^2). u-
295 HMW-ADPN was also significantly associated with the eGFR in normoalbuminuric patients in
296 the cross-sectional analysis. In addition, we showed, by a longitudinal analysis, that only u-HMW-
297 ADPN was associated with the degree of decline in the renal function over 2 years.
298 Urinary albumin excretion has been known to be a common early biomarker of renal injury in
299 patients with diabetes; however, it is not sensitive for predicting the progression of DKD [29 30]. U-
300 total-ADPN and u-HMW-ADPN were correlated with the eGFR in patients with micro- and
301 macro-albuminuria, and in those with normoalbuminuria in the present study. In contrast, u-ACR
302 and u-L-FABP were only associated with the eGFR in patients with DKD. Thus, urinary
303 adiponectin excretion could be a more beneficial marker for predicting the progression of DKD
304 in comparison to urinary albumin excretion. In the present study, the significant correlation
305 between u-ADPN and eGFR at baseline was observed mainly in patients with type 1 diabetes
306 prior to developing DKD (u-ACR ≥30 mg/gCr or eGFR <60 mL/min/1.73 m^2). Since patients
307 with type 2 diabetes showed older, heavier, higher systolic blood pressure, higher UACR, and
308 higher prevalence of hypertension and dyslipidemia than those in patients with type 1 diabetes,
309 renal injury in type 2 diabetes might be complicated due to accumulation of the risks compared
310 with type 1 diabetes. Therefore, urinary adiponectin might be statistically associated with eGFR
311 prior to overt renal injury solely in patients with type 1 diabetes. A previous study reported that
312 adiponectin was strongly stained in the glomeruli of healthy subjects, and monomer and dimer
313 adiponectin were excreted in the urine in these subjects [19]. Thus, low molecular adiponectin exists
314 in the kidney, especially the glomeruli, and is released into the urine in healthy individuals. On

315 the other hand, immunohistochemical staining of adiponectin is markedly decreased in patients
316 with diabetes, and trimer adiponectin, which was not detected in the urine of heathy subjects, was
317 excreted into urine, even in the absence of albuminuria [19]. Thus, a diabetic condition might
318 increase urinary excretion of adiponectin molecules of higher molecular weight and this seemed
319 to be influenced by the severity of renal injury.

320 A previous study showed that urinary adiponectin becomes expressed in the renal tubules of
321 patients with diabetes who have overt renal injury [19]. Serum and urinary adiponectin levels have
322 been reported to be associated with markers of tubular injury, urinary NAG and MCP-1 in overt
323 diabetic nephropathy [20]. In the present study, u-total-ADPN and u-HMW-ADPN were also
324 correlated with u-L-FABP, which is an index of renal tubular injury [31], as well as u-ACR. Thus,
325 urinary adiponectin excretion could be a sensitive marker of DKD, because it can reflect renal
326 tubular injury as well as glomerular injury. A comparison between histological findings and u-
327 total-ADPN would be necessary to clarify the relationship between the pathology and u-total-
328 ADPN. In addition, only u-total-ADPN was associated with the eGFR in patients with eGFR ≥60
329 mL/min/1.73m^2. Watanabe et al. have also reported that u-total-ADPN may increase earlier [32].
330 Since total adiponectin involves all isoforms of adiponectin, it might be a more sensitive marker
331 of renal injury than u-HMW-ADPN.

332 Thirty-eight of 201 (18.9%) patients showed a >10 mL/min/1.73m^2 reduction in their eGFR
333 during the 2-year follow-up period, so-called “rapid progression”. Thirty-one of the 201 patients
334 had a baseline eGFR of >60 mL/min/1.73m^2. These patients accounted for 23.8% of the subjects
335 with an eGFR of >60 mL/min/1.73m^2 at baseline. This ratio of patients with "rapid progression"
336 of renal injury was more than 10% higher than reported in a previous study [17]. Thus, physicians
337 should take care in relation to the possible decline in the renal function, even in patients with an
338 eGFR >60 mL/min/1.73m^2, since u-HMW-ADPN, but not u-total-ADPN, u-ACR and u-LFABP,

was found to be significantly associated with a decreased renal function and u-HMW-ADPN could predict the rapid progression of the renal function (Table 4). Two similar studies have examined adiponectin and decreased renal function [24, 33]. One study has more longer observation period than our study and shows association between CKD progression and u-ADPN [33]. The other study with a cohort with a shorter observation period in comparison to the present study also showed that u-HMW-ADPN was a better predictor of the decline of the renal function than u-total-ADPN [24].

It was reported that adiponectin-deficient mice exhibited albuminuria and podocyte dysfunction, which were improved by the administration of adiponectin [18]. In addition, it was reported that the adiponectin receptor exists in the kidney, and adenosine monophosphate-activated protein kinase is activated by adiponectin during renal injury due to diabetes, and acts to protect the kidney by reducing oxidative stress and suppressing apoptosis [34]. Thus, it is suggested that adiponectin is involved in the maintenance of the renal function. Taken together, it is suggested that a part of urinary adiponectin was derived from renal damage, which in turn may be excreted.

Increased serum adiponectin is known to be a biomarker of renal injury [35]. In this study, significant positive correlations were observed between the u-ADPN and s-ADPN of total- or HMW-ADPN, respectively (Fig 1.), suggesting that s-ADPN might contribute to u-ADPN. However, when u-ADPN and s-ADPN of both total- and HMW-ADPN were compared between each of the two groups of patients categorized according to u-ACR (cut-off value: 30 mg/gCr) or eGFR (cut-off value: 60 mL/min/1.73m^2), the increase in u-HMW-ADPN was significantly higher than that of s-HMW-ADPN during renal injury. Furthermore, FE-ADPN increased with the decrease in the renal function (u-ACR ≥30 mg/gCr or eGFR <60 mL/min/1.73m^2). This suggests that the increase in u-ADPN in patients with decreased renal function (u-ACR ≥30 mg/gCr or

eGFR <60 mL/min/1.73m^2) is influenced by some factors other than the increase in s-ADPN. The production of adiponectin in the renal tubules has been considered as on possible factor. It has already been reported that adiponectin is produced in the renal tubules, and this production is increased by inflammatory stimuli [36].

4.1. Limitations

The present study was associated with some limitations. First, the study population was relatively small. Only 38 patients showed rapid progression of renal injury, and it was difficult to conduct a detailed examination or an analysis with grouping according to the type of diabetes. However, a relationship between u-ADPN and the decline of the eGFR could be demonstrated. Second, the observation period was relatively short. We were able to follow the patients for 2 years and found rapidly progressing cases; however, the prognosis after 2 years was not evaluated. Third, this study was conducted in a single center. Fourth, this study did not consider the effects of drugs or therapeutic interventions. However, many of the patients showed good blood glucose control and were assumed to be less affected by the temporal use of medications during the 2-year study period. Fifth, this study did not verify the histology or pathology. According to these limitations, there is a need for further studies with a larger study population and a longer follow-up period. Finally, the results of this study were only observed in Japanese patients and may differ by race. Thus, further worldwide study is needed.

5. Conclusions

Adiponectin measured by an ultrasensitive immunoassay may be a comprehensive biomarker for DKD and may predict longitudinal deterioration of the renal function.

Author contributions

Masashi Ishizu: Conceptualization, Methodology, Formal analysis, Investigation, Data Curation, Writing – Original draft. **Hiroyasu Mori:** Conceptualization, Methodology, Investigation, Data Curation, Writing – Review & Editing. **Mami Ohishi:** Investigation, Data Curation. **Akio Kuroda:** Resources, Writing – Review & Editing. **Yuko Akehi:** Resources, Writing – Review & Editing. **Sumiko Yoshida:** Resources. **Ken-ichi Aihara:** Resources. **Motohiro Aiba:** Resources. **Tomoharu Kawano:** Resources. **Seiichi Hashida:** Resources. **Munehide Matsuhisa:** Conceptualization, Methodology, Resources, Writing – Review & Editing, Project administration, Funding acquisition.

Acknowledgements

We sincerely thank Prof. Makoto Funaki and Dr. Akiko Hata for their meaningful discussion on urinary adiponectin in healthy subjects. We also appreciate Ms. Reiko Suzuki and Ms. Misuzu Okamoto for their excellent support in the completion of this study.

Funding/ support

This study was funded by Sysmex Corporation (Hyogo, Japan).

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Figure captions

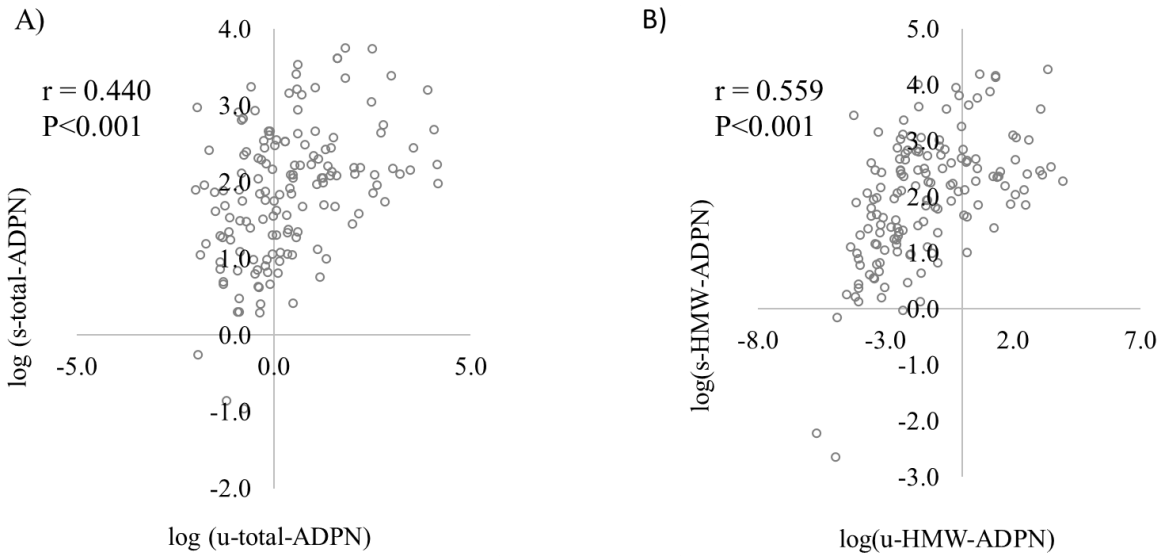
Fig 1. Correlations between u-ADPN and s-ADPN at 1 year after baseline

These scatter plots show the correlations between log (u-ADPN) and log (s-ADPN) of A) total-ADPN and B) HMW-ADPN at 1 year after baseline (n=140). u-ADPN and s-ADPN were significantly correlated.

u-total-ADPN, urinary total adiponectin-to-creatinine ratio; s-total-ADPN, serum total adiponectin-to-creatinine ratio; u-HMW-ADPN, urinary HMW adiponectin-to-creatinine ratio; s-HMW-ADPN, serum HMW adiponectin-to-creatinine ratio

Figures

Fig 1.



Tables

Table 1. Clinical characteristics at baseline

(n=239)	
Age (years)	63 (50, 71)
Sex (male, female)	116, 123 (48.5%, 51.5%)
Type of diabetes (type1, type2)	61, 178 (25.5%, 74.5%)
Duration of diabetes (years)	11 (5, 20)
HbA1c (%)	7.0 (6.5, 7.7)
BMI (kg/m^2)	24.5 (22.1, 28.8)
Systolic blood pressure (mmHg)	133 (119, 149)
Diastolic blood pressure (mmHg)	81 ± 13
eGFR ($mL/min/1.73m^2$)	68 (56, 85)
Smoking status (current, past, never, data missing)	36, 68, 129, 6 (15.1%, 28.5%, 54.0%, 2.5%)
Urinary albumin-to-creatinine rate (mg/g Cr)	12 (6, 51)
Urinary L-FABP-to-creatinine rate (μg/ g Cr)	1.5 (0.6, 2.8)
Urinary total adiponectin-to-creatinine rate (μg/ g Cr)	0.92 (0.49, 2.27)
Urinary HMW adiponectin-to-creatinine rate (μg/ g Cr)	0.12 (0.04, 0.48)
Diabetic Retinopathy (Non-DR, background DR, proliferative DR, data missing)	139, 32, 43, 25 (58.2%, 18.0%, 13.4%, 10.4%)
Diabetic Neuropathy	113 (47.3%)
Hypertension	167 (69.9%)
Dyslipidemia	168 (70.3%)
Insulin	138 (57.7%)
Glucagon-like peptide-1 receptor agonist	40 (16.7%)
Sodium glucose cotransporter 2 inhibitors	37 (15.5%)
other oral hypoglycemic agents	136 (56.9%)
Statins	103 (43.1%)
RAS inhibitors	75 (31.4%)
Calcium channel blockers	37 (15.5%)

538 Data are described as the mean ± standard deviation (SD), median (Q1, Q3) or n (%).
539 BMI, body mass index; eGFR, estimated glomerular filtration rate; L-FABP, L-type fatty acid
540 binding protein; HMW, high molecular weight; NDR, nondiabetic retinopathy; SDR, simple
541 diabetic retinopathy; PPDR, pre-proliferative diabetic retinopathy; PDR, proliferative
542 diabetic retinopathy; RAS, renin-angiotensin system
543

Table 2. Correlations between eGFR and urinary parameters at baseline

	All (n=239)		u-ACR				eGFR			
			<30 mg/g Cr (n=161)		≥30 mg/g Cr (n=78)		≥60 mL/min/1.73m^2 (n=160)		<60 mL/min/1.73m^2 (n=79)	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
u-total-ADPN (μg/ g Cr)	-0.410	<0.001	-0.195	0.013	-0.554	<0.001	-0.182	0.021	-0.481	<0.001
u-HMW-ADPN (μg/ g Cr)	-0.371	<0.001	-0.161	0.041	-0.429	<0.001	-0.096	0.228	-0.483	<0.001
u-ACR (mg/g Cr)	-0.306	<0.001	0.042	0.593	-0.544	<0.001	0.044	0.584	-0.506	<0.001
u-L-FABP (μg/ g Cr)	-0.247	<0.001	0.125	0.113	-0.622	<0.001	0.087	0.273	-0.546	<0.001

eGFR, estimated glomerular filtration rate; u-total-ADPN, urinary total adiponectin-to-creatinine ratio; u-HMW-ADPN, urinary HMW adiponectin-to-creatinine ratio; u-ACR, Urinary albumin-to-creatinine ratio; u-L-FABP, Urinary L-FABP-to-creatinine ratio.

Table 3. Logistic regression analysis between eGFR and urinary parameters at baseline

	Model 1		Model 2		Model 3	
(n =239)	OR	p-value	OR	p-value	OR	p-value
u-total-ADPN (μg/ g Cr)	4.0	<0.001	3.4	<0.001	3.4	<0.001
u-HMW-ADPN (μg/ g Cr)	3.7	<0.001	3.5	<0.001	3.5	<0.001
u-ACR (mg/g Cr)	2.8	<0.001	2.7	0.001	2.9	0.001
u-L-FABP (μg/ g Cr)	2.3	0.005	2.3	0.006	2.3	0.007

Model 1: unadjusted

Model 2: adjusted by sex and age

Model 3: adjusted by Model2 + BMI, HbA1c and SBP

eGFR, estimated glomerular filtration rate; u-total-ADPN, urinary total adiponectin-to-creatinine ratio; u-HMW-ADPN, urinary HMW adiponectin-to-creatinine ratio; u-ACR, Urinary albumin-to-creatinine ratio; u-L-FABP, Urinary L-FABP-to-creatinine ratio; SBP, Systolic blood pressure.

Table 4. Comparison of basal urinary parameters among three groups categorized according to ΔeGFR

ΔeGFR (n=201)	> 0 (n=58)	≦ 0, > -10 (n=105)	≦ -10 (n=38)	p for trend
u-total-ADPN (μg/ g Cr)	0.84 (0.41, 2.05)	1.01 (0.57, 2.31)	0.92 (0.35, 3.37)	0.493
u-HMW-ADPN (μg/ g Cr)	0.08 (0.03, 0.33)	0.13 (0.04, 0.62)	0.15 (0.06, 0.88)	0.045
u-ACR (mg/g Cr)	11 (6, 36)	8 (5, 69)	20 (6, 109)	0.463
u-L-FABP (μg/ g Cr)	1.53 (0.66, 2.44)	1.43 (0.40, 3.15)	1.77 (0.62, 3.23)	0.630

Data are described as the median (Q1, Q3).

eGFR, estimated glomerular filtration rate; u-total-ADPN, urinary total adiponectin-to-creatinine ratio; u-HMW-ADPN, urinary HMW adiponectin-to-creatinine ratio; u-ACR, urinary albumin-to-creatinine ratio; u-L-FABP, urinary L-FABP-to-creatinine ratio

Supplementary materials

Fig A.1. Study design

In this study, cross-sectional analyses were performed at baseline and one year later. In addition, the eGFR monitored for to 2 years to longitudinally evaluate the association with urinary adiponectin.

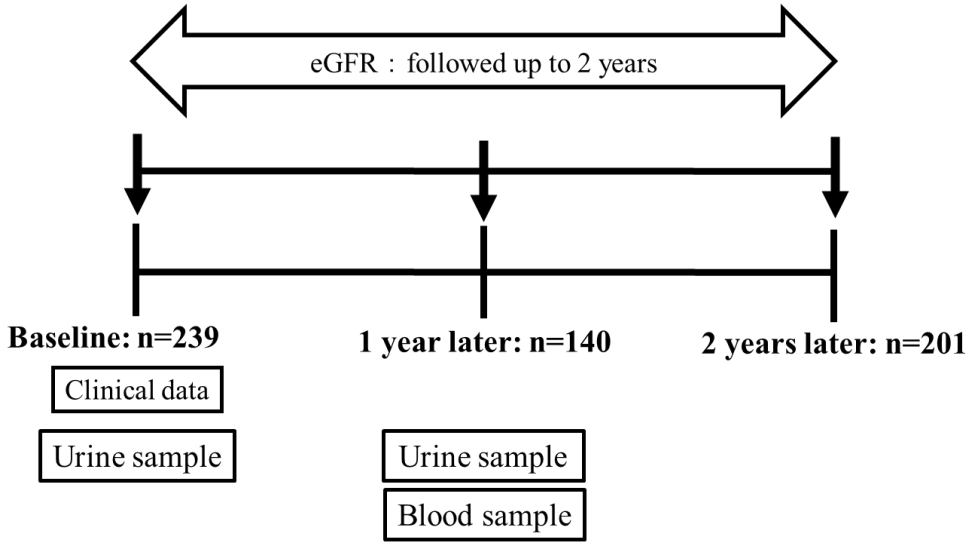


Table A.1. Clinical characteristics at baseline by type of diabetes

	Type 1 diabetes (n=61)	Type 2 diabetes (n=178)	p-value
Age (years)	52 (43, 67)	64 (53, 72)	<0.001
Sex (male, female)	22, 39 (36.1%, 63.9%)	94, 84 (52.8%, 47.2%)	0.027
Duration of diabetes (years)	15 (9, 26)	10 (4, 19)	0.006
HbA1c (%)	7.1 (6.4, 7.8)	6.9 (6.5,7.6)	0.276
BMI (kg/m^2)	22.1 (21.2, 24.1)	26.0 (23.2, 30.0)	<0.001
Systolic blood pressure (mmHg)	127 (112, 137)	134 (121, 153)	0.007
Diastolic blood pressure (mmHg)	78 (70, 86)	81 (72, 91)	0.149
eGFR ($mL/min/1.73m^2$)	74 (58, 88)	68 (54, 84)	0.229
Smoking status (current, past, never, data missing)	10, 13, 36, 2 (16.4%, 21.3%, 59.0%, 3.3%)	26, 55, 93, 4 (14.6%, 30.9%.52.2%, 2.2%)	0.377
Urinary albumin-to-creatinine rate (mg/g Cr)	8 (5, 18)	16 (6, 65)	0.006
Urinary L-FABP-to-creatinine rate (μg/ g Cr)	1.3 (0.4, 2.6)	1.6 (0.7, 3.1)	0.259
Urinary total adiponectin-to-creatinine rate (μg/ g Cr)	0.72 (0.40, 2.85)	0.96 (0.52, 2.17)	0.436
Urinary HMW adiponectin-to-creatinine rate (μg/ g Cr)	0.10 (0.03, 0.48)	0.13 (0.05, 0.49)	0.231
Diabetic Retinopathy (Non-DR,	32, 9, 9, 11 (52.5%, 14.8%,	107, 23, 34, 14 (60.1%, 12.9%,	0.152

background DR, proliferative DR, data missing)	14.8%, 18.0%)	19.1%, 7.9%)	
Diabetic Neuropathy	23 (37.7%)	90 (50.4%)	0.102
Hypertension	33 (54.1%)	134 (75.3%)	0.002
Dyslipidemia	32 (52.5%)	136 (76.4%)	0.001
Insulin	60 (98.4%)	78 (43.8%)	<0.001
Glucagon-like peptide-1 receptor agonist	1 (1.6%)	39 (21.9%)	<0.001
Sodium glucose cotransporter 2 inhibitors	0 (%)	37 (20.8%)	<0.001
other oral hypoglycemic agents	2 (3.2%)	134 (75.3%)	0.001
Statins	20 (32.8%)	83 (46.6%)	0.004
RAS inhibitors	10 (16.4%)	65 (36.5%)	0.004
Calcium channel blockers	7 (11.5%)	30 (16.9%)	0.413

Data are described as the mean ± standard deviation (SD), median (Q1, Q3) or n (%).

BMI, body mass index; eGFR, estimated glomerular filtration rate; L-FABP, L-type fatty acid binding protein; HMW, high molecular weight; NDR, nondiabetic retinopathy; SDR, simple diabetic retinopathy; PPDR, pre-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; RAS, renin-angiotensin system

Table B.1. Correlations between eGFR and urinary parameters at baseline by type of diabetes

Type 1 diabetes	All (n=61)		u-ACR <30 mg/g Cr (n=49)		u-ACR ≥30 mg/g Cr (n=12)		eGFR ≥60 mL/min/1.73m² (n=44)		eGFR <60 mL/min/1.73m² (n=17)	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
u-total-ADPN (μg/ g Cr)	-0.554	<0.001	-0.312	0.029	-0.658	0.020	-0.305	0.044	-0.770	<0.001
u-HMW-ADPN (μg/ g Cr)	-0.575	<0.001	-0.325	0.023	-0.666	0.018	-0.283	0.063	-0.778	<0.001
u-ACR (mg/g Cr)	-0.425	0.001	-0.051	0.730	-0.722	0.008	-0.181	0.239	-0.645	0.005
u-L-FABP (μg/ g Cr)	-0.200	0.122	0.201	0.148	-0.723	0.008	0.242	0.114	-0.737	0.001
Type 2 diabetes	All (n=178)		<30 mg/g Cr (n=112)		≥30 mg/g Cr (n=66)		≥60 mL/min/1.73m² (n=116)		<60 mL/min/1.73m² (n=62)	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
u-total-ADPN (μg/ g Cr)	-0.346	<0.001	-0.129	0.176	-0.482	<0.001	-0.115	0.219	-0.366	0.003
u-HMW-ADPN (μg/ g Cr)	-0.284	<0.001	-0.076	0.425	-0.351	0.004	0.008	0.935	-0.386	0.002
u-ACR (mg/g Cr)	-0.250	0.001	0.100	0.293	-0.504	<0.001	0.145	0.120	-0.439	<0.001
u-L-FABP (μg/ g Cr)	-0.241	0.001	0.107	0.260	-0.550	<0.001	0.045	0.635	-0.422	0.001

eGFR, estimated glomerular filtration rate; u-total-ADPN, urinary total adiponectin-to-creatinine ratio; u-HMW-ADPN, urinary HMW adiponectin-to-creatinine ratio; u-ACR, Urinary albumin-to-creatinine ratio; u-L-FABP, Urinary L-FABP-to-creatinine ratio.

Table C.1. Logistic regression analysis between eGFR and urinary parameters at baseline by type of diabetes

Type 1 diabetes	Model 1		Model 2		Model 3	
(n=61)	OR	p-value	OR	p-value	OR	p-value
u-total-ADPN (μg/ g Cr)	7.5	0.002	7.2	0.003	8.2	0.004
u-HMW-ADPN (μg/ g Cr)	12.1	0.001	12.2	0.001	11.8	0.001
u-ACR (mg/g Cr)	4.7	0.011	4.4	0.017	4.7	0.022
u-L-FABP (μg/ g Cr)	4.2	0.017	4.0	0.024	4.1	0.027
Type 2 diabetes	Model 1		Model 2		Model 3	
(n=178)	OR	p-value	OR	p-value	OR	p-value
u-total-ADPN (μg/ g Cr)	3.2	0.001	2.7	0.007	2.7	0.009
u-HMW-ADPN (μg/ g Cr)	2.6	0.004	2.4	0.012	2.4	0.015
u-ACR (mg/g Cr)	2.4	0.011	2.6	0.009	2.6	0.011
u-L-FABP (μg/ g Cr)	1.8	0.073	1.9	0.060	1.9	0.076

Model 1: unadjusted

Model 2: adjusted by sex and age

Model 3: adjusted by Model2 + BMI, HbA1c and SBP

eGFR, estimated glomerular filtration rate; u-total-ADPN, urinary total adiponectin-to-creatinine ratio; u-HMW-ADPN, urinary HMW adiponectin-to-creatinine ratio; u-ACR, Urinary albumin-to-creatinine ratio; u-L-FABP, Urinary L-FABP-to-creatinine ratio; SBP, Systolic blood pressure.

Table D.1. Comparison of the urine, serum and FE-ADPN levels of total- or HMW-ADPN in patients categorized according to u-ACR (cut-off value: 30 mg/g Cr) or eGFR (cut-off value: 60 mL/min/1.73m²)

	u-ACR			eGFR		
(n=140)	<30 mg/g Cr (n=89)	≥30 mg/g Cr (n=51)	p-value	≥60 mL/min/1.73m² (n=90)	<60 mL/min/1.73m² (n=50)	p-value
Total-ADPN						
Urinary level (μg/ g Cr)	0.76 (0.41, 1.51)	3.73 (1.33, 12.82)	<0.001	0.86 (0.43, 1.80)	2.98 (0.83, 11.91)	<0.001
Serum level (ng/ mL)	5.39 (2.86, 10.35)	8.66 (5.51, 14.10)	0.002	6.35 (2.96, 10.67)	8.04 (4.50, 13.76)	0.075
FE-ADPN	0.111 (0.041, 0.301)	0.357 (0.167, 1.810)	<0.001	0.109 (0.047, 0.279)	0.433 (0.155, 1.579)	<0.001
HMW-ADPN						
Urinary level (μg/ g Cr)	0.08 (0.03, 0.18)	1.74 (0.31, 7.38)	<0.001	0.11 (0.04, 0.39)	0.71 (0.08, 4.74)	<0.001
Serum level (ng/ mL)	6.18 (2.73, 12.35)	9.75 (6.09, 17.11)	0.006	6.80 (3.12, 14.15)	9.09 (4.71, 17.05)	0.123
FE-ADPN	0.010 (0.004, 0.019)	0.114 (0.046, 0.711)	<0.001	0.012 (0.005, 0.036)	0.046 (0.016, 0.621)	<0.001

Data are described as the median (Q1, Q3).

u-ACR, urinary albumin-to-creatinine ratio; eGFR, estimated glomerular filtration rate; ADPN, adiponectin; HMW, high molecular weight; FE-ADPN, fractional excretion of adiponectin

Table E.1. Clinical characteristics at baseline among three groups categorized according to ΔeGFR

ΔeGFR Median (Q1, Q3) (n=201)	> 0 2.8 (1.1, 5.3) (n=58)	≤ 0, > -10 -4.5 (-6.8, -2.6) (n=105)	≤ -10 -15.1 (-18.1, -12.0) (n=38)	p for trend
Age (years)	64.0 (48.3, 73.0)	64.0 (52.5, 70.0)	55.5 (42.5, 66.3)	0.029
Sex (male, female)	33, 25 (56.9%, 43.1%)	44, 61 (41.9%, 51.8%)	19, 19 (50.0%, 50.0%)	Not analyzed
Type of diabetes (type1, type2)	12, 46 (20.7%, 79.3%)	33, 72 (31.4%, 68.6%)	8, 30 (21.1%, 78.9%)	Not analyzed
Duration of diabetes (years)	12.0 (8.0, 19.5)	15.0 (7.0, 22.5)	9.5 (3.0, 20.5)	0.478
HbA1c (%)	7.1 (6.6, 7.8)	6.9 (6.5, 7.6)	7.0 (6.5, 7.7)	0.406
BMI (kg/m^2)	26.1 (23.3, 31.4)	23.6 (21.5, 26.5) *	26.8 (22.6, 28.9)	0.270
Systolic blood pressure (mmHg)	134 (120, 154)	130 (118, 142)	133 (124, 153)	0.895
Diastolic blood pressure (mmHg)	81 (70, 92)	79 (71, 86)	82 (75, 97)	0.377
eGFR ($mL/min/1.73m^2$)	62 (53, 80) *	66 (54, 83) *	85 (63, 100)	0.002
Smoking status (current, past, never, data missing)	12, 13, 31, 2 (20.7%, 22.4%, 53.4%, 3.4)	10, 35, 58, 2 (9.5%, 33.3%,55.2%, 1.9%)	5, 11, 22, 0 (13.2%, 28.9%, 57.9%, 0%)	Not analyzed
Diabetic retinopathy (Non-DR, background DR, proliferative DR, data missing)	35, 7, 9, 7 (60.3%, 12.1%, 14.5%, 12.1%)	62, 14, 17, 12 (59.0%, 13.3%,16.2%, 11.4%)	22, 7, 7, 2 (57.9%, 18.4%, 18.4%, 5.3%)	Not analyzed
Diabetic neuropathy	32 (55.2%)	52 (49.5%)	17 (44.7%)	Not analyzed
Hypertension	45 (77.6%) *	73 (69.5%)	22 (57.9%)	Not analyzed

Dyslipidemia	52 (89.7%) *	70 (66.7%)	22 (57.9%)	Not analyzed
Insulin	31 (53.4%)	68 (64.8%)	21 (55.3%)	Not analyzed
Glucagon-like peptide-1 receptor agonist	12 (20.7%)	15 (14.3%)	9 (23.7%)	Not analyzed
Sodium glucose cotransporter 2 inhibitors	15 (25.9%)	11 (10.5%)	6 (15.8%)	Not analyzed
other oral hypoglycemic agents	40 (69.0%)	49 (46.7%) *	24 (63.2%)	Not analyzed
Statins	28 (48.3%)	45 (42.9%)	13 (34.2)	Not analyzed
RAS inhibitors	15 (25.9%)	41 (39.0%)	10 (26.3%)	Not analyzed
Calcium channel blockers	11 (19.0%)	5 (13.2%)	15 (14.3%)	Not analyzed

Data are described as the mean ± standard deviation (SD), median (Q1, Q3) or n (%).

*: $p<0.05$; vs. -10 mL/min/1.73m^2

BMI, body mass index; eGFR, estimated glomerular filtration rate; L-FABP, L-type fatty acid binding protein; HMW, high molecular weight; NDR, nondiabetic retinopathy; SDR, simple diabetic retinopathy; PPDR, pre-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; RAS, renin-angiotensin system.

Table F.1. Clinical characteristics at baseline among three groups categorized according to ΔeGFR of type2 diabetes

ΔeGFR Median (Q1, Q3) (n=148)	> 0 2.8 (1.1, 5.3) (n=46)	≤ 0, > -10 -4.5 (-6.8, -2.6) (n=72)	≤ -10 -15.1 (-18.1, -12.0) (n=30)	p for trend
Age (years)	64.0 (45.8, 73.0)	66.0 (58.3, 71.8)	60.0 (43.8, 69.5)	
Sex (male, female)	28, 18 (60.9%, 39.1%)	33, 39 (45.8%, 54.2%)	16, 14 (53.3%, 46.7%)	Not analyzed
Duration of diabetes (years)	11.5 (6.8, 18.0)	13.5 (5.3, 20.0)	9.5 (3.0, 20.8)	
HbA1c (%)	6.9 (6.5, 7.7)	6.9 (6.5, 7.3)	7.0 (6.5, 7.5)	
BMI (kg/m^2)	27.2 (23.7, 31.8)	24.4 (22.4, 28.6)	27.5 (24.1, 29.2)	
Systolic blood pressure (mmHg)	135 (122, 154)	132 (118, 144)	138 (126, 153)	
Diastolic blood pressure (mmHg)	81 (70, 94)	79 (71, 88)	85 (76, 97)	
eGFR ($mL/min/1.73m^2$)	62 (53, 83) *	63 (52, 77) *	85 (63, 100)	
Smoking status (current, past, never, data missing)	11, 12, 22, 1 (23.9%, 26.1%, 47.8%, 2.2%)	4, 26, 41, 1 (5.6%, 36.1%, 56.9%, 1.4%)	3, 9, 18, 0 (10.0%, 30.0%, 60.0%, 0%)	Not analyzed
Diabetic retinopathy (Non-DR, background DR, proliferative DR, data missing)	26, 5, 8, 7 (56.5%, 10.9%, 17.4%, 15.2%)	44, 9, 13, 6 (61.1%, 12.5%, 18.1%, 8.3%)	18, 6, 5, 1 (60.0%, 20.0%,16.6%, 3.3%)	Not analyzed
Diabetic neuropathy	27 (58.7%)	39 (54.2%)	14 (46.7%)	Not analyzed
Hypertension	37 (80.4%)	55 (76.4%)	19 (63.3%)	Not analyzed
Dyslipidemia	41 (89.1%) *	56 (77.8%)	19 (63.3%)	Not analyzed
Insulin	20 (43.5%)	35 (48.6%)	13 (43.3%)	Not analyzed

Glucagon-like peptide-1 receptor agonist	12 (26.1%)	15 (20.8%)	8 (26.7%)	Not analyzed
Sodium glucose cotransporter 2 inhibitors	15 (32.6%)	11 (15.3%)	6 (20.0%)	Not analyzed
other oral hypoglycemic agents	38 (82.6%)	49 (68.1%)	24 (80.0%)	Not analyzed
Statins	21 (45.7%)	35 (48.6%)	11(36.7%)	Not analyzed
RAS inhibitors	14 (30.4%)	33 (45.8%)	10 (33.3%)	Not analyzed
Calcium channel blockers	9 (18.6%)	10 (13.9%)	5 (16.7%)	Not analyzed

Data are described as the mean ± standard deviation (SD), median (Q1, Q3) or n (%).

*: $p<0.05$; vs. -10 mL/min/1.73m^2

BMI, body mass index; eGFR, estimated glomerular filtration rate; L-FABP, L-type fatty acid binding protein; HMW, high molecular weight; NDR, nondiabetic retinopathy; SDR, simple diabetic retinopathy; PPDR, pre-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; RAS, renin-angiotensin system.

Table G.1. Comparison of basal urinary parameters among three groups categorized according to Δ eGFR of type 2 diabetes

Δ eGFR (n=148)	> 0 (n=46)	≦ 0, > -10 (n=72)	≦ -10 (n=30)	p for trend
u-total-ADPN (μg/ g Cr)	0.87 (0.43, 1.78)	1.22 (0.65, 2.45)	0.92 (0.35, 2.66)	0.396
u-HMW-ADPN (μg/ g Cr)	0.08 (0.04, 0.33)	0.24 (0.05, 0.79)	0.15 (0.07, 0.67)	0.037
u-ACR (mg/g Cr)	13 (7, 44)	10 (5, 99)	24 (7, 109)	0.458
u-L-FABP (μg/ g Cr)	1.5 (0.9, 2.5)	1.4 (0.4, 2.7)	1.9 (0.7, 3.3)	0.432

Data are described as the median (Q1, Q3).

eGFR, estimated glomerular filtration rate; u-total-ADPN, urinary total adiponectin-to-creatinine ratio; u-HMW-ADPN, urinary HMW adiponectin-to-creatinine ratio; u-ACR, urinary albumin-to-creatinine ratio; u-L-FABP, urinary L-FABP-to-creatinine ratio