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Association between nonalbumin proteinuria and renal tubular damage of *N*-acetyl-β-D-glucosaminidase and its clinical relevance in patients with type 2 diabetes without albuminuria

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ABSTRACT

Aim: Although albuminuria and urinary *N*-acetyl-β-D-glucosaminidase (uNAG) are known as progression markers of diabetic kidney disease, there is limited information regarding the association between urinary nonalbumin proteinuria (NAP) and uNAG and the clinical relevance thereof in patients without albuminuria.
Methods: This cross-sectional study included samples from 244 consecutive patients with type 2 diabetes mellitus (T2D) without albuminuria. Proteinuria and albuminuria were defined according to protein-to-creatinine ratio (uPCR) and albumin-to-creatinine ratio (uACR), respectively. NAP was indirectly calculated by the difference between uPCR and uACR.
Results: NAP and uNAG excretion were significantly correlated ($r = 0.525$, $P < 0.001$). Individuals whose NAP levels were in the highest tertile had a longer duration of diabetes, uncontrolled hyperglycemia, and impaired insulin stimulation (all $P < 0.05$), although more patients in the highest NAP tertile were prescribed insulin and sulfonylurea. Multiple linear regression analyses revealed associations among uNAG, diabetes duration, and waist circumference.
Conclusions: T2D patients without albuminuria excrete proteinuria and that presence of the protein in urine is associated with uNAG. NAP was positively correlated with T2D duration and waist circumference, but negatively correlated with body mass index. Lean, but centrally obese, T2D patients in late diabetes experience more tubular damage, regardless of the presence of albuminuria.

1. Introduction

Diabetes has increasingly become an epidemic, and the incidence of type 2 diabetes mellitus (T2D)-related complications has also become a public health issue. Diabetic kidney disease (DKD), affecting approximately 40% of diabetic patients, is a leading cause of chronic kidney disease (CKD) and end-stage renal disease that must be treated with renal replacement therapy or kidney transplantation.[1,2] The high mortality and morbidity of DKD, as well as CKD-related complications, are expected to have severe socioeconomic consequences.

Although the risk factors for DKD have been reported,[2,3] there is still a need for regression of the DKD. Therefore early diagnosis and monitoring of kidney function are necessary for DKD management.[4] Currently, clinical evaluation and monitoring of DKD are based on estimated glomerular filtration rate (eGFR) and albuminuria content. The equation-based eGFR can be altered by age, sex, race, and serum creatinine measurements.[5] There is also no consensus on a normal eGFR range. Nevertheless, abnormal increases in eGFR renal hyperfiltration have been shown to be indicative of early DKD and to be related with other metabolic abnormalities.[6,7] Albuminuria, defined as a urinary albumin-to-creatinine ratio (uACR) ≥ 30 mg/g is widely used as an early detector of DKD.[4,8] However, a recent study of autopsies reported discrepancies in DKD prevalence between individuals diagnosed with DKD based on biopsies and individuals clinically diagnosed with DKD; moreover, the authors noted histologically the presence of DKD without albuminuria,[9] suggesting the need for other strategies.

In urine, total protein amounts are primarily composed of albumin, although other pathophysiological proteins and nonalbumin proteins are also present. High molecular weight proteins in urine have been shown to be associated with progression of CKD.[10] Since the presence of albuminuria suggests glomerular damage, nonalbumin proteinuria could indicate kidney injury of a tubular origin. Urinary *N*-acetyl-β-D-glucosaminidase (uNAG) is a renal proximal tubule injury marker,[11] which increases with hyperglycemia and reflects glucose fluctuations, even in individuals with normoalbuminuria.[12,13] In addition, renal hyperfiltration can be explained by tubular glomerular feedback,

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suggesting the initial role of tubules in DKD.[6] However, previous reports on DKD have emphasized studies of albuminuria, and reports on nonalbumin proteinuria (NAP) and its potential association with uNAG are limited.

We hypothesized that NAP might be increased in early DKD and that tubular damage from DKD would be reflected as increases in uNAG and NAP levels. The aims of this study were to determine whether NAP is associated with uNAG excretion and to determine the clinical relevance of NAP in relation to glycemic and other metabolic markers in T2D patients.

2. Materials and methods

2.1. Study population

In this retrospective cross-sectional study, T2D patients were defined according to the International Classification of Diseases 10th revision,[14] (E11.xxx) and patient use of insulin and/or oral hypoglycemic agents was identified by reviewing patient case notes using the electronic medical records at Severance Hospital between March 2015 and March 2017. Patients were excluded if they fulfilled any one of the following criteria: (1) <20 years of age; (2) type 1 diabetes; (3) uACR ≥30 mg/g; (4) pregnant women; (5) renal diseases other than DKD (e.g., intrinsic renal disease [nephritis or nephrotic syndrome], acute renal failure due to septic shock, contrast agents, use of drugs, or postrenal disease); and (6) those not fully satisfying the inclusion criteria. We finally enrolled 244 patients ≥20 years old with T2D who had been tested for blood glucose components, including insulin and C-peptide, using the standardized mixed meal test, and urinary markers, including NAG, albumin, protein, creatinine for uNAG, protein-creatinine ratio (uPCR), and uACR. The study protocol received ethical approval from the institutional review board at the Yonsei University College of Medicine (4-2017-0820) and adhered to the tenets of the Declaration of Helsinki.

2.2. Measurement of urine and blood parameters

Overnight (≥8 h) fasting blood and spot urine samples were obtained during the early morning. HbA1c, glycated albumin, basal glucose, and stimulated glucose values were analyzed to assess glycemic status. HbA1c was determined by an immunoassay using an Integra 800 CTS analyzer (Roche, Hercules, CA, USA). HbA1c levels were measured by an enzymatic method (Lucia GA-L; Asahi Kasei Pharma, Tokyo, Japan) using a Hitachi 7600 automatic analyzer (Hitachi, Tokyo, Japan). Basal glucose and creatinine were measured using a Hitachi 7600 analyzer (Hitachi). Serum insulin and C-peptide were measured by an electrochemiluminescence immunoassay using a Cobas e601 analyzer (Roche Diagnostics, Basel, Switzerland). Postprandial glucose (designated as stimulated glucose) was evaluated by collecting blood samples 90 min after ingestion of two containers (400 mL total, 400 kcal, 18 g fat, 44 g carbohydrate, and 20 g protein) of a standardized mixed meal (Mediwell Diabetic Meal™; Meail Dairies, Chungbuk, Republic of Korea). Pancreatic β-cell function and insulin sensitivity were assessed using homeostasis model assessment (HOMA-IR) and the homeostasis model assessment of insulin resistance.[15] Postprandial C-peptide-to-glucose ratio (PCGR) was calculated as stimulated C-peptide (ng/mL)/stimulated glucose (mg/dL) × 100.[16] eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.[17]

NAG, albumin, and protein levels were adjusted according to urine creatinine levels and were expressed as the NAG-to-creatinine ratio, uACR, and uPCR. Urine levels of albumin were measured by an immunoturbidimetric method using the AU680 automated analyzer (Beckman Coulter, Brea, CA, USA). Urine creatinine levels were also measured using the AU680 analyzer (Beckman Coulter) by the kinetic Jaffe method. uNAG levels were measured by a colorimetric method using a reagent from Nittobo Medical (Tokyo, Japan) and a JCA-BM 6010/c automated analyzer (JEOL, Tokyo, Japan). Protein concentrations in urine were measured by an immunoturbidimetric method using a Hitachi 7180 auto analyzer (Hitachi). NAP was indirectly calculated from the difference between uPCR and uACR using the formula: NAP (mg/g) = uPCR (mg/g) - uACR (mg/g).[18] Cardiovascular disease was defined as a positive history of angina, carotid artery atherosclerosis, or myocardial infarction documented by electronic medical records.

2.3. Statistical analysis

Data are presented as a mean ± standard deviation (SD) or median (interquartile range) for continuous variables and as a number or percentage for categorical variables. We analyzed participant characteristics according to NAP tertiles using the one-way analysis of variance to compare continuous variables and the χ^2 test to compare categorical variables, followed by post hoc analyses using the Bonferroni method. Because uNAG, uPCR, NAP, total cholesterol, triglyceride, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), insulin, HOMA-β, and HOMA-IR values were not normally distributed, analyses were performed using natural log-transformed data to obtain approximately symmetrical distributions. The correlations between urinary uNAG, NAP, and glucometabolic parameters were assessed using Pearson's correlation coefficient. Multivariate linear regression models were used to identify determinants of NAP levels and the independent association of uNAG with glucometabolic parameters after adjustment for potent confounders. Statistical analyses were performed using IBM SPSS statistical software for Windows, version 23.0 (IBM, Armonk, NY, USA). All values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Baseline characteristics using nonalbumin proteinuria

The clinical characteristics of the study population are listed in Table 1. The mean age of the study population was 60.5 ± 13.5 years. The mean duration of T2D in the study population was 5.9 years, and 62 (25.4%) of the patients had a T2D duration of ≥10 years. The mean body mass index (BMI) was 25.5 kg/m^2, with 129 (53.3%) patients identified as obese according to Asian-Pacific criteria (BMI ≥ 25 kg/m^2). The mean uPCR, uACR, NAP, and uNAG levels were 108.4 mg/g, 9.4 mg/g, 99.1 mg/g, and 9.0 U/g, respectively.

When patients were compared based on their NAP tertile, patients with the highest NAP were significantly older than patients with a lower NAP (57.2 ± 11.0, 62.1 ± 13.3, and 62.3 ± 15.4 years for the lowest, middle, and highest NAP, respectively) ($P = 0.023$). In addition, the highest NAP group had a significantly longer T2D duration (4.7 ± 6.6, 5.0 ± 7.1, and 8.1 ± 9.1 years, respectively) and lower body mass index (BMI; 26.0 ± 4.2, 26.1 ± 3.5, and 24.5 ± 3.5 kg/m^2, respectively) than patients with a lower NAP (all, $P < 0.05$). Waist circumference, however, was comparable among the three groups. The percentages of males (56.1%, 44.4%, and 45.7%, respectively), hypertension (48.8%, 56.8%, and 60.5%, respectively), cardiovascular disease (19.5%, 32.1%, and 25.9%, respectively), and cigarette smokers (46.5%, 36.4%, and 33.3%, respectively) were statistically insignificant among the different NAP tertiles. Anti-diabetes medication (oral hypoglycemic agents and insulin), anti-hypertensive medication, and lipid-lowering drug use was also comparable among the tertiles.

Regarding glucose parameters, basal glucose (means of 131.8, 129.2, and 150.5 mg/dL, respectively), stimulated glucose (means of 179.9, 181.7, and 207.3 mg/dL, respectively), glycated albumin (means of 17.0%, 17.3%, and 19.7%, respectively), and HbA1c levels (means of 6.9%, 7.0%, and 7.6%, respectively) significantly increased with increases in NAP tertile (all $P < 0.05$) (Table 2). Moreover, the percentage of uncontrolled hyperglycemia (HbA1c ≥ 7.0%) increased from the lower

Table 1
Baseline characteristics of the study population.

	Lowest tertile (N = 82)	Middle tertile (N = 81)	Highest tertile (N = 81)	P value
Demographic data				
Age, year	57.2 ± 11.0	62.1 ± 13.3	62.3 ± 15.4†	0.023
Diabetes duration, year	4.7 ± 6.6	5.0 ± 7.1†	8.1 ± 9.1†,‡	0.008
Diabetes duration ≥10 yrs., N (%)	15 (18.3)	18 (22.2)	29 (35.8)	0.010
Male, N (%)	46 (56.1)	36 (44.4)	37 (45.7)	0.183
BMI, kg/m^2	26.0 ± 4.2	26.1 ± 3.5	24.5 ± 3.5†, ‡	0.009
WC, cm	88.8 ± 10.6	89.6 ± 10.1	88.7 ± 9.9	0.834
SBP, mmHg	124.1 ± 14.4	127.1 ± 15.1	122.5 ± 16.6	0.165
DBP, mmHg	75.9 ± 13.1	79.1 ± 9.8	73.7 ± 11.2‡	0.013
Hypertension, N (%)	40 (48.8)	46 (56.8)	49 (60.5)	0.133
Cardiovascular disease, N (%)	16 (19.5)	26 (32.1)	21 (25.9)	0.348
Smoking, N (%)	33 (46.5)	28 (36.4)	25 (33.3)	0.105
Medication				
Metformin	44 (53.7)	42 (51.9)	39(48.1)	0.483
DPP4 inhibitor	17 (20.7)	17(21.0)	21(25.9)	0.429
SU	26 (31.7)	24 (29.6)	28 (34.6)	0.697
TZD	5 (6.1)	8 (9.9)	5 (6.2)	0.982
Insulin	12 (14.6)	9 (11.1)	19 (23.5)	0.130
ACE inhibitor or ARB	14 (17.1)	16 (19.8)	18 (22.2)	0.409
CCB	11 (13.4)	8 (9.9)	11 (13.6)	0.977
Lipid lowering drug	27 (32.9)	26 (32.1)	19 (23.5)	0.187

Data are presented as a mean ± standard deviation, median [interquartile range], or number (%). Bold characters represent statistically significant values.
Abbreviations: BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; DPP4 inhibitor, dipeptidyl peptidase-4 inhibitor; SU, sulphonylurea; TZD, thiazolidinedione; ACE inhibitor, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CCB, calcium channel blocker.
† $P < 0.05$ by post hoc analyses when compared with lowest tertile.
‡ $P < 0.05$ by post hoc analyses when compared with second tertile.

NAP tertile to the highest NAP tertile (32.9%, 54.3%, and 59.3%, respectively) ($P = 0.001$). Although there were comparable HOMA-IR and HOMA-β levels according to NAP tertile, insulin and C-peptide levels were lowest in the highest NAP tertile (medians of 29.4, 34.9, 22.3 ng/mL, respectively, for insulin increments; and 3.1, 3.4, 2.6 ng/mL, respectively, for the C-peptide). PCGR was lowest in the highest NAP tertile (22.1%, 23.5%, and 16.9%, respectively) ($P = 0.042$). Other laboratory test results, including liver function (AST and ALT), kidney function (blood urea nitrogen, creatinine, and eGFR), and lipid panel (total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol) were similar among the tertile groups.

Regarding urinary markers, uPCR (tertile means of 64.3, 91.9, and 169.8 mg/g, respectively), NAP (57.5, 82.9, and 157.3 mg/g, respectively), and uNAG (5.8, 8.2, and 13.0 U/g, respectively) were significantly higher in the highest NAP tertile than in the other tertiles (all, $P < 0.05$), whereas there was no significant difference in uACR levels among the tertiles ($P = 0.562$), which was attributed to the inclusion criteria of patients without albuminuria (Table 3).

3.2. Correlations between urine markers and glucometabolic parameters

Because an increasingly average uNAG was linked with higher NAP tertiles, uNAG showed a modest positive correlation with urinary NAP ($r = 0.525$, $P < 0.001$) (Table 4). In addition, both uNAG and NAP were positively correlated with the duration of T2D ($r = 0.234$, $P < 0.001$ for uNAG; $r = 0.251$, $P < 0.001$ for NAP), fasting glucose ($r = 0.229$, $P < 0.001$ for uNAG; $r = 0.194$, $P = 0.002$ for NAP), stimulated glucose ($r = 0.357$, $P < 0.001$ for uNAG; $r = 0.184$, $P = 0.004$ for NAP), glycated albumin ($r = 0.370$, $P < 0.001$ for uNAG; $r = 0.252$, $P < 0.001$ for NAP), and HbA1c ($r = 265$, $P < 0.001$ for uNAG; $r = 0.169$, $P = 0.008$ for NAP), whereas the PCGR ($r = -0.249$, $P < 0.001$ for uNAG; $r = 0.150$, $P = 0.021$ for NAP) showed a significant negative correlation with both urinary markers. A distinctive pattern was observed between uNAG and NAP with age and BMI: an elevated uNAG was more closely associated with older age and was not significantly linked with BMI, while the NAP was negatively correlated with a higher BMI and not significantly associated with age. eGFR, increments in insulin, and C-peptide did not show any significant linear correlation with uNAG or NAP.

Table 2
Blood chemistry and glucose parameters according to NAP tertiles.

	Lowest tertile (N = 82)	Middle tertile (N = 81)	Highest tertile (N = 81)	P value
Basal glucose, mg/dL	131.8 ± 28.4	129.2 ± 37.8†	150.5 ± 63.9†, ‡	0.006
Stimulated glucose, mg/dL	179.9 ± 61.0	181.7 ± 57.9	207.3 ± 84.8†, ‡	0.020
Glycated albumin, %	17.0 ± 4.1	17.3 ± 3.7	19.7 ± 6.0†, ‡	<0.001
HbA1c, %	6.9 ± 1.2	7.0 ± 1.1	7.6 ± 1.9†, ‡	0.004
HbA1c ≥7.0, N (%)	27 (32.9)	44 (54.3)	48 (59.3)	0.001
AST, IU/L[a]	25.8 ± 12.3	23.3 ± 10.0	24.5 ± 13.3	0.313
ALT, IU/L[a]	29.7 ± 19.9	25.1 ± 17.1	25.9 ± 16.4	0.170
Blood urea nitrogen, mg/dL	15.0 ± 4.5	15.5 ± 4.7	16.9 ± 7.8	0.103
Creatinine, mg/dL	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.3	0.504
eGFR, mL/min/1.73 m^2	92.8 ± 16.3	91.7 ± 15.7	91.3 ± 23.7	0.875
Total cholesterol, mg/dL[a]	171.9 ± 40.5	163.7 ± 42.6	169.1 ± 47.4	0.385
Triglyceride (mg/dL)[a]	140.0 ± 75.3	134.2 ± 80.2	142.0 ± 82.8	0.634
High density lipoprotein cholesterol, mg/dL[a]	45.4 ± 12.6	47.1 ± 11.4	48.7 ± 18.2	0.503
Low density lipoprotein cholesterol, mg/dL[a]	98.2 ± 37.8	89.7 ± 34.2	92.0 ± 33.5	0.528
HOMA-IR[a]	3.4 ± 2.5	3.4 ± 3.3	3.5 ± 2.4	0.705
HOMA-β, %[a]	59.9 ± 43.8	76.5 ± 107.6	59.7 ± 48.7	0.236
Basal insulin (ng/mL)	7.8 [5.2–14.4]	8.7 [5.6–10.8]	8.63 [6.1–13.8]	0.876
Stimulated insulin (ng/mL)	36.7 [22.6–60.0]	43.8 [28.6–65.1]	30.5 [21.7–52.7]‡	0.032
Δ insulin (ng/mL)	29.4 [15.8–47.9]	34.9 [20.6–49.3]	22.3 [12.9–44.0]‡	0.014
Basal C peptide (ng/mL)	2.4 [1.6–3.0]	2.2 [1.8–2.7]	2.5 [1.8–3.1]	0.446
Stimulated C peptide (ng/mL)	5.6 [4.2–7.2]	5.6 [4.4–7.3]	5.1 [3.8–6.9]	0.334
Δ C peptide (ng/mL)	3.1 [2.0–4.3]	3.4 [2.3–4.6]	2.6 [1.8–4.0]‡	0.042
PCGR (%)	22.1 [12.9–35.2]	23.5 [14.9–42.1]	16.9 [10.1–30.0]‡	0.018

Data are presented as a mean ± standard deviation, median [interquartile range], or number (%). Bold characters represent statistically significant values.
Abbreviations: AST. aspartate transaminase; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; HOMA-IR, homeostasis model assessment of insulin resistance; PCGR, postprandial C-peptide-to-glucose ratio.
a Log-transformed to achieve normal distribution.
† $P < 0.05$ by post hoc analyses when compared with lowest tertile.
‡ $P < 0.05$ by post hoc analyses when compared with second tertile.

Table 3
Urinary parameters according to NAP tertiles.

	Lowest tertile (N = 82)	2nd tertile (N = 81)	Highest tertile (N = 81)	*P* value
uPCR (mg/g Cr)[a]	64.3 ± 10.1	91.9 ± 9.9	169.8 ± 90.5[†, ‡]	<0.001
uACR (mg/g Cr)[a]	6.8 ± 5.4	9.0 ± 5.4	12.5 ± 9.2	0.562
NAP (mg/g Cr)[a]	57.5 ± 7.9	82.9 ± 7.0[†]	157.3 ± 90.5[†, ‡]	<0.001
NAG/Cr (U/g)[a]	5.8 ± 2.8	8.2 ± 3.9[†]	13.0 ± 8.8[†, ‡]	<0.001

Data are presented as a mean ± standard deviation, median [interquartile range], or number (%). Bold characters represent statistically significant values.
Abbreviations: uPCR, urine protein to creatinine ratio; uACR, urine albumin-to-creatinine ratio; NAP, nonalbumin proteinuria; NAG, *N*-acetyl-β-D-glucosaminidase.
[a] Log-transformed to achieve normal distribution.
[†] $P < 0.05$ by post hoc analyses when compared with lowest tertile.
[‡] $P < 0.05$ by post hoc analyses when compared with second tertile.

3.3. Determinants of NAP excretions

Independent determinants of NAP levels were assessed using multivariate regression models in T2D patients (Table 5). In model 1, a longer T2D duration, lower BMI, and higher waist circumference were significantly associated with elevated NAP excretion. When glycated albumin and PCGR were included in the regression model (model 2), T2D duration, BMI, and waist circumference were still associated with NAP levels. In a fully adjusted model (model 3), uNAG, duration of T2D, and waist circumference were positively correlated with NAP, while BMI was negatively associated with NAP.

4. Discussion

Assessment of proteinuria is essential in investigating CKD. Its involvement in DKD, however, is uncertain.[19] Currently, the evaluation and monitoring of DKD rely on uACR and eGFR.[4,8] While the presence of albuminuria in the absence of proteinuria might be found in some diabetic patients, the opposite (proteinuria in normoalbuminuria patients) is also occasionally detected,[19] although its cause and clinical relevance are still unclear. To clarify the role of these parameters in DKD, we recruited a T2D population with a normal range of albuminuria (uACR <30 mg/g) and determined the clinical relevance of NAP levels on clinical, glucometabolic, and renal parameters. The major finding of this study of T2D patients without albuminuria was that the source of isolated NAP, supported by increases thereof together with uNAG, might have originated from renal tubules. Additionally, we found that NAP was positively correlated with T2D duration, impaired glycemic status, and decreased insulin secretion potential, but negatively associated with the BMI in T2D patients with normoalbuminuria.

Table 4
Correlation between NAP, urinary NAG, and glucometabolic parameters.

	NAP[a]		uNAG[a]	
	r	*P*	r	*P*
Age, year	0.100	0.118	0.236	<0.001
BMI, kg/m^2	−0.190	0.003	−0.099	0.124
Sex, female	0.047	0.463	−0.004	0.954
WC, cm	0.018	0.817	0.099	0.187
Diabetes duration, year	0.251	<0.001	0.234	<0.001
Basal glucose, mg/dL	0.194	0.002	0.229	<0.001
Stimulated glucose, mg/dL	0.184	0.004	0.357	<0.001
Glycated albumin, %	0.252	<0.001	0.370	<0.001
HbA1c, %	0.169	0.008	0.265	<0.001
Δ insulin (ng/mL)[a]	−0.118	0.070	−0.091	0.166
Δ C peptide (ng/mL)[a]	−0.114	0.080	−0.101	0.125
PCGR (%)[a]	−0.150	0.021	−0.249	<0.001
eGFR, mL/min/1.73 m^2	−0.013	0.840	−0.080	0.215
uNAG[a]	0.525	<0.001	–	–
NAP[a]	–	–	0.525	< 0.001

Abbreviations: NAP, nonalbumin proteinuria; NAG, *N*-acetyl-β-D-glucosaminidase; BMI, body mass index; WC, waist circumference; PCGR, postprandial C-peptide-to-glucose ratio; eGFR, estimated glomerular filtration rate.
[a] Log-transformed to achieve normal distribution for calculating Persons coefficients.

NAP includes several types of proteins, such as alpha-1 microglobulin, beta-2 microglobulin, cystatin C, and matrix metalloproteinase-9,[18,20] The prevalence of patients with isolated NAP was 10.1% in the United States, and increased up to 36.2% after normoalbuminuria subgroup analyses.[21] It is generally accepted that the clinical relevance of NAP may either be its role in renal tubular damage or kidney disease development and progression.[22] The presence of NAP, in contrast to albuminuria, which indicates a glomerular injury, suggests renal tubule damage. In 1011 patients, a lower ratio of albumin to protein in the urine was a reasonable predictor of tubulointerstitial disorders (area under the curve 0.84).[23] Although renal pathology results were available in only 68 patients, a lower uACR to uPCR ratio was associated with a histological diagnosis of tubular damage. Investigating the predictive role of NAP-extracted albuminuria from total proteinuria (total proteinuria - albuminuria) in relation to graft loss and death, a study of renal transplantation recipients showed that the presence of NAP predicted an approximately five-fold risk for death and 14-fold risk for graft loss.[18] Furthermore, the tubular hypothesis in renal hyperfiltration is based on deterioration of proximal tubules, followed by glomerulus alterations, which support the concept of NAP in the development of DKD.[24] Moreover, based on our finding of a positive correlation between worsening glycemic parameters and NAP in normoalbuminuria and the proven value of NAP to uACR in predicting DKD progression,[25] NAP might precede microalbuminuria, and it could provide an opportunity with which to identify early kidney involvement prior to the development of microalbuminuria.

Similar to the role of NAP in predicting tubulointerstitial disorders,[26] the NAG that exists in the proximal tubule epithelial cells also represents tubular injury, as a marker of tubular-lysosomal injury. Our study showed that uNAG is moderately correlated with NAP (Pearson's coefficient = 0.525; $P < 0.001$). Based on this correlation, we suggest that the source of isolated NAP in T2D patients with normoalbuminuria might have originated from renal tubules rather than glomeruli. There is accumulating evidence that the levels of uNAG are associated with glycemic status in T2D[13,27] and with diabetic vascular complications in

Table 5
Determinants of NAP in type 2 diabetes patients.

	Model 1		Model 2		Model 3	
Variables	st β	*P*	st β	*P*	st β	*P*
Age, year	0.003	0.962	0.023	0.305	−0.150	0.131
Sex, female	0.055	0.484	0.076	0.351	0.072	0.358
Diabetes duration, year	0.303	<0.001	0.225	0.006	0.213	0.006
BMI, kg/m^2	−0.423	0.002	−0.367	0.007	−0.334	0.010
WC, cm	0.396	0.005	0.373	0.009	0.315	0.023
Glycated albumin, %	–	–	0.197	0.048	0.102	0.300
PCGR %[a]	–	–	−0.017	0.881	0.003	0.975
eGFR, mL/min/1.73 m^2	–	–	–	–	−0.126	0.184
uNAG[a]	–	–	–	–	0.331	<0.001

Abbreviations: NAP, nonalbumin proteinuria; NAG, *N*-acetyl-β-D-glucosaminidase; BMI, body mass index; WC, waist circumference; PCGR, postprandial C-peptide-to-glucose ratio; eGFR, estimated glomerular filtration rate.
[a] Log-transformed to achieve normal distribution for calculating Persons coefficients.

patients with T2D.[28,29] The uNAG in the present study was more closely related to old age; a long T2D duration; elevated glycemic markers, including basal glucose; stimulated glucose, glycated albumin, and HbA1c; and dysfunction in insulin secretion. Although we did not find a significant negative correlation with uNAG and BMI, our results were consistent with previous studies.[12,28,29] Considering the physiologically causal sequences of insulin secretory dysfunctions, hyperglycemia increased glucosuria as osmotic diuresis during secretion of uNAG,[30] and the concentration of uNAG could be physiologically elevated in hyperglycemia. In addition, uncontrolled hyperglycemia itself could not only be harmful to renal tubules, but it could also indirectly accelerate the formation of glycated end products, which could also be toxic to kidney tubules. Although previous studies only showed the existence of NAP and its association with renal tubule disorders and limited general renal diseases,[18,26] the present study, to the best of our knowledge, is the first report on the impact of NAP on DKD and its clinical implications in T2D patients. In contrast to previous studies of a positive correlation between uNAG and eGFR,[12,29] we did not find any statistical significance between uNAG and eGFR or between NAP and eGFR. This might be attributed to the inclusion criteria of T2D patients with normoalbuminuria, resulting in the exclusion of CKD patients. The mean eGFR of the entire study population was 91.9 mL/min/1.73 m^2, and 136 (55.7%) patients were in CKD stage 1 (eGFR ≥90 mL/min/1.73 m^2).

We unexpectedly found a negative correlation between BMI and both NAP and uNAG, and found a positive correlation between the waist circumference and NAP. Moreover, both NAP and uNAG had closer relationships to insulin secretory indices (PCGR, Δ insulin, and Δ C-peptide) than HOMA-IR. A previous study of the Korean population showed a negative correlation between uNAG and BMI;[12] however, another Dutch study reported a positive association between uNAG and BMI.[10] This might be explained by the unique characteristics of Asian diabetic populations, involving uncompensated or defective insulin secretion capacity over normal body weight as abdominal adiposity increases.[31] This hypothesis was clearly explained by a 10-year follow-up study of Korean participants. The authors concluded that failure of pancreatic beta cells was observed early and that dysfunction of insulin secretion was more affected than insulin resistance in the course of diabetes.[32] In addition, progression to diabetes or prediabetes was associated with a high waist circumference and relatively low BMI. Thus, we postulate that lean, but relatively centrally obese, T2D patients with late diabetes might have a decreased insulin secretion potential and consequently tend to have uncontrolled hyperglycemia in spite of the use of insulin secretagogues, resulting in more NAP being excreted.

This study has some limitations. First, because of its cross-sectional design, we could not establish direct causality between insulin secretory dysfunction and NAP and between NAP and diabetic tubulopathy progression or initiation. Whether susceptible patients with beta cell dysfunction identified by NAP excretion actually experience progression of DKD independent of the presence of hyperglycemia needs to be confirmed in long-term prospective studies. Second, spot urine samples at single times were measured. Third, with the exception of uNAG, we did not compare the sensitivity of detecting diabetic tubulopathy using other renal markers. In addition, other tubular proteins (e.g., retinol binding protein, alpha-1 microglobulin, cystatin-C, lysozyme, immunoglobulin light chains) that directly reflect tubulointerstitial kidney disease were not analyzed in the current study. Finally, due to the limitations of the current study, retrospective and observational data, we could not confirm the tubular origin proteins by an independent technique, such as SDS-gel electrophoresis or actually quantitating selected tubular proteins.

Despite these limitations, our study had several strengths. First, it utilized data from a relatively large number of patients, guaranteeing statistical reliability in the results. We identified the clinical relevance of NAP in respective demographic, glucometabolic, and nephropathic indices, whereas previous studies only reported the existence of NAP. Finally, standardization of glucose homeostasis assessments resulting from a mixed meal tolerance test provided the necessary data to classify the impairment of insulin secretion and insulin resistance.

In conclusion, our results showed that T2D patients without albuminuria excreted proteinuria and that NAP in the urine is associated with the renal tubular origin of uNAG. Levels of NAP and uNAG excretions were associated with higher glycemic variabilities in T2D patients. We postulate that metabolically obese patients with T2D with a late course of diabetes accompanied with dysfunctions in insulin secretion might have renal tubulopathy. Our findings suggest the potential role of NAP as a renal tubule damage marker in risk stratification of patients with T2D during an early stage of DKD.

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