Association of Succinate and Adenosine Nucleotide Metabolic Pathways with Diabetic Kidney Disease in Patients with Type 2 Diabetes Mellitus

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**Highlights**
- Identifying DKD biomarkers is crucial for early ESRD prevention in high-risk patients.
- Urinary succinate consistently correlated independently with DKD and albuminuria.

**Conclusion**
Among several potential metabolites, only urinary succinate was independently associated with DKD.
Association of Succinate and Adenosine Nucleotide Metabolic Pathways with Diabetic Kidney Disease in Patients with Type 2 Diabetes Mellitus

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Background: Although the prevalence of diabetic kidney disease (DKD) is increasing, reliable biomarkers for its early detection are scarce. This study aimed to evaluate the association of adenosine and succinate levels and their related pathways, including hyaluronic acid (HA) synthesis, with DKD.

Methods: We examined 235 participants and categorized them into three groups: healthy controls; those with diabetes but without DKD; and those with DKD, which was defined as estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m². We compared the concentrations of urinary adenosine, succinate, and HA and the serum levels of cluster of differentiation 39 (CD39) and CD73, which are involved in adenosine generation, among the groups with DKD or albuminuria. In addition, we performed multiple logistic regression analysis to evaluate the independent association of DKD or albuminuria with the metabolites after adjusting for risk factors. We also showed the association of these metabolites with eGFR measured several years before enrollment. This study was registered with the Clinical Research Information Service (https://cris.nih.go.kr; Registration number: KCT0003573).

Results: Urinary succinate and serum CD39 levels were higher in the DKD group than in the control and non-DKD groups. Correlation analysis consistently linked urinary succinate and serum CD39 concentrations with eGFR, albuminuria, and ΔeGFR, which was calculated retrospectively. However, among the various metabolites studied, only urinary succinate was identified as an independent indicator of DKD and albuminuria.

Conclusion: Among several potential metabolites, only urinary succinate was independently associated with DKD. These findings hold promise for clinical application in the management of DKD.

Keywords: Albuminuria; Biomarkers; Diabetes mellitus, type 2; Diabetic nephropathies

INTRODUCTION

Diabetic kidney disease (DKD) is the leading cause of end-stage renal disease (ESRD) worldwide. It is associated with a high mortality rate and increased medical costs in patients with type 2 diabetes mellitus (T2DM) [1,2]. Moreover, several studies have identified potential biomarkers of DKD progression, including kidney injury molecule 1, neutrophil gelatin-
Disease Kidney Disease (DKD) is characterized by persistent albuminuria followed by a gradual decline in glomerular filtration rate [6]. However, evidence accumulated over the last decade suggests that the course of DKD is clinically heterogeneous and that various phenotypes of DKD exist [7]. One of these phenotypes is a rapid decline in eGFR before the onset of albuminuria [8,9]. Moreover, numerous studies have suggested that a rapid decline in eGFR is associated with high mortality risk and adverse outcomes [10]. Therefore, understanding the pathophysiological changes in rapidly progressing DKD and identifying relevant biomarkers may help stratify high-risk patients and enable early intervention to prevent ESRD.

Interest in the search for novel biomarkers using omics-based approaches to identify patients at risk of rapid DKD progression has recently grown [11]. In our previous study, we showed 21 DKD-related extracellular vesicle microRNAs (miRNAs) and 17 differentially expressed target mRNAs, including three target mRNAs (ribonuclease reductase M2 subunit, ecto-5' -nucleotidase [NT5E], and UDP-glucose dehydrogenase [UGDH]) [12]. Based on these data, we performed a protein interaction network analysis and found that the succinate dehydrogenase complex iron–sulfur subunit B (SDHB) is connected to NT5E and UGDH (Supplementary Fig. 1). The NT5E gene encodes a cluster of differentiation 73 (CD73), which catalyzes adenosine 5'-monophosphate to produce extracellular adenosine, and UGDH is an essential protein-coding gene for hyaluronic acid (HA) synthesis [13]. SDHB is crucial for succinate oxidation and mitochondrial electron transport [12].

Herein, we measured the products and intermediates of the NT5E-UGDH-SDHB pathway because of the unknown pathophysiological mechanisms associated with a rapid decline in kidney function and the metabolites involved. We also assessed the serum activities of adenosine-producing enzymes (CD73 and CD39), as well as urinary levels of adenosine, HA, and succinate, to extend our previous findings. Furthermore, we conducted a comprehensive analysis to explore the association of these metabolites with DKD. In addition, we evaluated whether the levels of these metabolites are associated with a rapid decline in eGFR, which was calculated retrospectively using previous data.

**METHODS**

**Study population**

Among the patients with T2DM who attended the outpatient clinic of either Gachon University Gil Medical Center or Korea University Ansan Hospital between June 2016 and December 2018, the following were selected for enrollment based on the following inclusion criteria: (1) patients with T2DM diagnosed after the age of 30 years; (2) patients <65 years at enrollment; (3) patients who had previously measured eGFR and tested albuminuria more than five times at intervals of at least 3 months between past outpatient visits; and (4) patients receiving angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker for blood pressure (BP) control or albuminuria, provided that stable doses had been administered for at least 2 weeks at enrollment. The exclusion criteria were as follows: (1) patients with ESRD, patients with a history of diabetic ketoacidosis; (2) patients with confirmed chronic kidney disease (CKD) unrelated to diabetes (e.g., polycystic kidney disorder and focal segmental glomerulosclerosis); (3) patients with a history of dialysis at enrollment; (4) patients with a history of cardiovascular disease (CVD) (e.g., acute myocardial infarction, unstable angina, peripheral arterial disease, and stroke) within 12 weeks of examination; (5) patients who used contrast agents within 30 days of examination; (6) patients without creatinine (Cr) results for two consecutive visits; (7) patients receiving steroids or immune-suppressants for >2 weeks; (8) pregnant women; (9) patients with body mass indices (BMIs) <18.5 kg/m²; and (10) patients with a history of malignancy within the last 5 years. Among visitors from the health examination center, 30 age- and sex-matched healthy individuals without diabetes were recruited as the healthy control group.

After excluding those with missing data, 235 participants were identified. We classified participants into three groups: healthy controls without diabetes (n=30), those with diabetes but without DKD (n=131), and those with DKD (n=74), which was defined as an eGFR <60 mL/min/1.73 m² at study enrollment. Participants were also divided into three groups according to their urine albumin-to-creatinine ratio (UACR; mg/g Cr) at study enrollment: <30 mg/g (A1, n=90), 30–299 mg/g (A2, n=36), and ≥300 mg/g (A3, n=67) [14].

Subsequently, we analyzed the association between metabolites and past renal disease progression by categorizing the participants according to the retrospectively calculated eGFR decline. For this analysis, we collected eGFR data more than five
Urinary succinate and adenosine: DKD

Study design and source of data

Eligible participants were selected through a review of medical records, and informed consent was obtained to establish a retrospective cohort. This study was approved by the Institutional Review Board of Korea of University Ansan Hospital (IRB No. 2019AS0226). All participants provided written informed consent prior to sample collection. This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was registered with the Clinical Research Information Service (https://cris.nih.go.kr, registration number: KCT0003573) in accordance with the World Health Organization International Clinical Trials Registry Platform. All personal information was deleted, and only unidentifiable data were included in the analysis.

Anthropometric and laboratory measurements

At enrollment, data on the patients’ medical history and medication use were collected using a self-administered questionnaire and medical record review. Physical examination results and serum biochemical parameters were obtained by trained staff after 12 hours of fasting. Height, body weight, and waist circumference were measured with the participants wearing light clothing. BMI was defined as body weight in kilograms divided by the square of height in meters. Systolic and diastolic BPs were measured using a standardized sphygmomanometer. Glycosylated hemoglobin (HbA1c), fasting plasma glucose, and fasting insulin concentrations were measured 12 hours after fasting. All participants were asked to collect their first urine sample in the morning. Samples of urine (50 mL) and plasma (1 mL) from each participant were aliquoted into tubes and stored at −80°C until further analysis. Albuminuria was quantified by calculating the UACR, and the eGFR was calculated using the CKD Epidemiology Collaboration (CKD-EPI) Cr equation [16]. The urinary concentrations of succinate and adenosine were measured using succinate colorimetric assay kits (MAK184; Sigma-Aldrich, St. Louis, MO, USA) and adenosine fluorometric assay kits (ab211094; Abcam, Cambridge, MA, USA), respectively, following the manufacturer’s instructions. Urinary HA excretion was measured using an enzyme-linked immunosorbent assay (ELISA) kit (MBS2886836; MyBioSource Inc., San Diego, CA, USA). ELISA kits MBS4503745 (MyBioSource) and ab213761 (Abcam) were used to measure the serum concentrations of ectonucleotidases (CD39 and CD73) in accordance with the manufacturers’ instructions.

Statistical analysis

The number of missing participants varied for each variable in the analyses. Among the 235 participants, 183, 162, 178, and 226 were included in the adenosine, succinate, HA, and CD39 (CD73) groups, respectively. Continuous variables are represented as the mean±standard deviation or median (interquartile range). Categorical variables are expressed as percentages.

Urinary metabolite excretion was calculated by dividing the metabolite concentration in milligrams by the Cr concentration in grams (mg/g). Skewed variables (UACR, urinary adenosine/Cr, urinary succinate/Cr, and urinary HA/Cr ratios) were subjected to logarithmic transformation for the analysis. The clinical characteristics between groups were compared using one-way analysis of variance for continuous variables and the chi-square test for categorical variables. Between-group comparisons were performed as post hoc tests using the Tukey-Kramer test. The Kruskal-Wallis test was performed to compare the control, non-DKD, and DKD groups. Additionally, the Wilcoxon rank-sum test with t-approximation was used to compare the NPs and RPs. Correlations were evaluated using Pearson’s correlation coefficients. Skewed variables underwent a logarithmic transformation for analysis. The odds ratios (ORs) and 95% confidence intervals (CIs) for the association between urinary metabolites and DKD progression or the presence of UACR >30 mg/g were examined using logistic regression analysis, with the following variables adjusted for the logistic regression models: age, sex, systolic BP, diabetes duration, HbA1c, UACR, urinary adenosine/Cr, succinate/Cr, HA/Cr, and serum CD39 and CD73 concentrations. Considering that the serum concentrations of CD73 were low, we calculated the odds of the dependent variables as a change in 100 units of CD73. All statistical analyses were performed using SAS version 9.4 for Windows (SAS Institute Inc., Cary, NC, USA). All reported P values were two-tailed, and P<0.05 was considered to be statistically significant.
RESULTS

The characteristics of the study participants are listed in Table 1. The mean age of the participants with diabetes was >59 years, and the duration of diabetes was >15 years. The participants in the DKD group had lower eGFRs and higher UACRs than those in the non-DKD group. Statistically significant differences in BMI were found among the groups, with the DKD group exhibiting a higher BMI than the control and non-DKD groups. The participants in the DKD group had higher proportions of comorbid diabetic retinopathy, hypertension, and CVD and were treated with insulin more frequently than those in the non-DKD group. The mean ΔeGFR/year values of the participants in the DKD and non-DKD groups during the previous years until the enrollment were −6.9 and 0.4 mL/min/1.73 m²/year, respectively.

Several noteworthy findings were observed in our investigation of urinary metabolites and serum ectonucleotidases (CD39 and CD73) in the DKD group (Fig. 1). The participants in the DKD group had higher urinary succinate/Cr values than those in the control and non-DKD groups. Similarly, the urinary adenosine/Cr and HA/Cr values were higher in the DKD group than in the control group (Fig. 1). Although urinary adenosine/Cr values were highest in the non-DKD group, the difference among the groups was not statistically significant. CD39 and CD73, the enzymes involved in adenosine production, showed an opposite relationship with DKD progression; the concentrations of CD39 were higher, but those of CD73 were lower in the DKD group than in the control group (Fig. 1). Additionally, we reclassified these participants into control, NP, and RP groups according to the change in eGFR during the several years prior to enrollment and compared these metabolites among these groups (Supplementary Fig. 2). The results were similar to those obtained in the comparison among the control, non-DKD, and DKD groups.

We also analyzed the associations between metabolites and albuminuria stage. Urinary succinate concentrations were highest in group A3 (UACR ≥300 mg/g), but adenosine concentra-

### Table 1. Characteristics of study participants at study enrollment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Control</th>
<th>Non-DKD</th>
<th>DKD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>30</td>
<td>131</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>57.0±6.5</td>
<td>59.4±7.5</td>
<td>60.1±8.6</td>
<td>0.1790</td>
</tr>
<tr>
<td>Male sex</td>
<td>18 (60.0)</td>
<td>77 (58.8)</td>
<td>42 (56.8)</td>
<td>0.9415</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.1±3.4</td>
<td>25.1±3.2</td>
<td>26.7±3.2</td>
<td>0.0002</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>131.5±18.0</td>
<td>134.6±17.3</td>
<td>140.2±19.7</td>
<td>0.0389</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>83.3±9.6</td>
<td>80.2±11.7</td>
<td>80.0±11.7</td>
<td>0.3589</td>
</tr>
<tr>
<td>Duration of DM, yr</td>
<td>-</td>
<td>15.7±7.5</td>
<td>18.5±8.7</td>
<td>0.0168*</td>
</tr>
<tr>
<td>Use of insulin</td>
<td>-</td>
<td>27 (20.6)</td>
<td>39 (54.2)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.5±0.3</td>
<td>7.9±1.4</td>
<td>7.8±1.6</td>
<td>0.8746*</td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m²</td>
<td>98.0±8.7</td>
<td>92.9±12.7</td>
<td>32.2±13.2</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>ΔeGFR, mL/min/1.73 m²/yr</td>
<td>-</td>
<td>0.4±2.0</td>
<td>−6.9±6.1</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>UACR, mg/g</td>
<td>4.4 (3.3–10.7)</td>
<td>10.5 (4–66.6)</td>
<td>1,222.9 (287.8–2,948.3)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Hypertension</td>
<td>2 (6.7%)</td>
<td>67 (51.2)</td>
<td>64 (86.5)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>DM retinopathy</td>
<td>-</td>
<td>45 (34.4)</td>
<td>59 (79.7)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Known CVD</td>
<td>2 (6.7%)</td>
<td>27 (20.6)</td>
<td>29 (39.2)</td>
<td>0.0041*</td>
</tr>
<tr>
<td>RAS blocker</td>
<td>1 (6.7%)</td>
<td>72 (54.9)</td>
<td>46 (63.9)</td>
<td>0.2174*</td>
</tr>
<tr>
<td>SGLT2 inhibitor</td>
<td>-</td>
<td>5 (3.8)</td>
<td>1 (1.4)</td>
<td>0.3285*</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation, number (%), or median (interquartile range).

DKD, diabetic kidney disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; HbA1c, glycosylated hemoglobin; eGFR, estimated glomerular filtration rate; UACR, urine albumin-to-creatinine ratio; CVD, cardiovascular disease; RAS, renin-angiotensin system; SGLT2, sodium-glucose co-transporter 2.

*DKD and non-DKD groups were compared within the diabetic patient group.
tions were highest in group A2 (UACR 30–299 mg/g). As albuminuria levels increased, urinary succinate excretion and serum CD39 concentrations increased, whereas serum CD73 concentrations decreased (Supplementary Fig. 3).

Results of Pearson correlation analysis revealed no significant correlation between urinary adenosine excretion and eGFR (Table 2). However, urinary succinate excretion was negatively correlated with eGFR (r = −0.440, P < 0.0001) and ΔeGFR (r = −0.254, P = 0.0017), indicating that the DKD group had higher urinary succinate concentrations than the other groups. Urinary succinate levels were significantly positively correlated with the UACR (r = 0.317, P < 0.0001). Serum CD39 concentrations correlated negatively with impaired renal function represented by eGFR and ΔeGFR, and positively with increased albumin excretion. However, CD73 expression showed the opposite correlation (Table 2).

We performed a multivariate logistic regression analysis to identify the factors independently associated with DKD (Table 3). Additionally, UACR and urinary succinate/Cr ratio were significantly associated with DKD (OR, 1.947; 95% CI, 1.483 to 2.557; P < 0.0001) and ΔeGFR (OR, 2.862; 95% CI, 1.423 to 5.757; P = 0.0032, respectively). Conversely, the urinary adenosine/Cr ratio was linked to a decreased risk (OR, 0.507; 95% CI, 0.278 to 0.925; P = 0.0267). DKD showed no significant associations with DKD and urinary HA/Cr or serum ectonucleotidases CD39 and CD73, cluster of differentiation 73.

DISCUSSION

In this study, we investigated the association between the succinate and adenosine nucleotide metabolic pathways and DKD.
Urinary succinate and serum CD39 concentrations were associated with DKD and albuminuria, whereas urinary HA and serum CD73 levels showed limited significance. Regression analysis revealed that only urinary succinate levels were significantly associated with DKD. We also confirmed that urinary succinate and serum CD39 levels were associated with a rapid decline in eGFR, which was calculated retrospectively.

Several studies have proposed that succinate and succinate receptors play roles in DKD pathogenesis [17]. Local stress conditions, including hyperglycemia and hypoxia, are recognized to inhibit or partially reverse succinate dehydrogenase and other tricarboxylic acid (TCA) cycle enzymes, leading to the succinate accumulation characteristic of DKD. This accumulation and activation of the succinate receptor G protein-coupled receptor 91 (GPR91) promote renin release, contributing to early glomerular hyperfiltration and renin–angiotensin system (RAS) activation in individuals with diabetes [18-20]. These studies, along with the results of the current study, highlight that succinate plays an important role in the rapid deterioration of renal function in patients with diabetes by altering the TCA cycle metabolism. However, human studies on the association between succinate and DKD have shown inconsistent results. Feng et al. [21] observed that urinary succinate concentrations decrease in an albuminuric DKD group.

By contrast, Liu et al. [22] observed increased levels of urinary fumarate converted from succinate and proposed that this increase may predict DKD progression in patients with T2DM. Discrepancies in the results of human studies can be attributed to several factors. First, the characteristics and definitions of patients vary across studies. DKD is a complex disease with varying stages and clinical manifestations, and this variation in patients can contribute to the inconsistent results regarding the association between succinate and DKD. Additionally, the dynamic changes in metabolites during the early and late stages of DKD can be challenging to detect and quantify. Because DKD is a progressive disease, investigation of these dynamic changes requires repeated measurements and a longitudinal study design.

In the present study, we investigated the association of CD39 and CD73 with DKD. CD39 and CD73 are ectonucleotidases responsible for the sequential conversion of adenosine triphosphate to adenosine (Supplementary Fig. 1). Numerous studies have suggested that adenosine is crucial in water and sodium transport, tubuloglomerular feedback (TGF), inflammation, renin secretion, and renal fibrosis [23-26]. Furthermore, several experimental studies have reported the anti-inflammatory roles of CD39/CD73 and adenosine signaling in DKD [27,28]. Furthermore, the beneficial effect of extracellular adenosine might result from the regulation of renal hemodynamic conditions through renal TGF [29]. However, large cohort studies investigating the association of urinary adenosine and serum CD39/CD73 concentrations with DKD progression are rare. In the present study, although CD39, CD73, and adenosine are involved in the same pathway, they exhibited different patterns of association with DKD. CD39 concentrations increased with DKD, whereas CD73 concentrations decreased. Although CD39 expression was not statistically significant in the regression analysis, it significantly increased in the participants with DKD and macroalbuminuria. Additionally, the participants in the non-DKD group had the highest urinary adenosine concentration, but the difference was not statistically significant (Fig. 1). This finding indicates the potential activation of the adenosine pathway in the early stages of DKD, which is consistent with the results of previous studies demonstrating an early increase in urinary adenosine levels in an animal model of DKD [30]. Given the prior evidence of renal protection associated with adenosine [27-29], the elevated levels of CD39 in the DKD and A3 groups may represent a defensive mechanism.

**Table 3. Association of multiple biomarkers and the presence of DKD in multivariate logistic analysis**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.022</td>
<td>0.947–1.102</td>
<td>0.5801</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.507</td>
<td>0.497–4.572</td>
<td>0.4688</td>
</tr>
<tr>
<td>SBP</td>
<td>1.005</td>
<td>0.972–1.040</td>
<td>0.7568</td>
</tr>
<tr>
<td>Duration of diabetes</td>
<td>1.084</td>
<td>1.011–1.163</td>
<td>0.0230</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.820</td>
<td>0.579–1.161</td>
<td>0.2625</td>
</tr>
<tr>
<td>UACR</td>
<td>1.947</td>
<td>1.483–2.557</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Adenosine/Cr</td>
<td>0.507</td>
<td>0.278–0.925</td>
<td>0.0267</td>
</tr>
<tr>
<td>Succinate/Cr</td>
<td>2.862</td>
<td>1.423–5.757</td>
<td>0.0032</td>
</tr>
<tr>
<td>HA/Cr</td>
<td>0.442</td>
<td>0.158–1.243</td>
<td>0.1219</td>
</tr>
<tr>
<td>CD39</td>
<td>0.763</td>
<td>0.427–1.366</td>
<td>0.3635</td>
</tr>
<tr>
<td>CD73</td>
<td>1.008</td>
<td>0.990–1.027</td>
<td>0.3581</td>
</tr>
</tbody>
</table>

All 11 variables were incorporated into a multivariate logistic regression analysis to estimate their association with the risk of DKD progression. Skewed variables underwent a logarithmic transformation for analysis.

DKD, diabetic kidney disease; CI, confidence interval; SBP, systolic blood pressure; HbA1c, glycated hemoglobin; UACR, urine albumin-to-creatinine ratio; Cr, creatinine; HA, hyaluronic acid; CD39, cluster of differentiation 39; CD73, cluster of differentiation 73.
against DKD progression. However, the fact that adenosine, the final product of this pathway, did not increase in the DKD group suggests that this renoprotective mechanism may not be fully functional or limited to advanced stages of DKD.

We hypothesized that elevated urinary succinate excretion disrupts the CD39/CD73–adenosinergic axis by influencing adenosine methylation. M6A, the most abundant RNA modification, is dynamically regulated by enzymes known as methyltransferases (writers) and demethylases (erasers) at the N6 position of adenine within RNA molecules. This process involves methylation by writers to produce m6A and subsequent removal of these methyl groups by erasers to produce adenosine [31]. Metabolites of the TCA cycle, including succinate and fumarate, inhibit m6A demethylase activity and consequently increase m6A levels [32]. In the present study, the succinate accumulation in the DKD group may represent a potential mechanism contributing to the reduced adenosine levels through increased m6A expression. Recent studies have reported decreased urinary m6A levels in patients with DKD [33]. This suggests that the regulation of adenosine through m6A expression is a complex process, given that m6A is the most widespread and common internal RNA modification throughout the body [31,32].

Regarding urinary HA excretion, the results of a preliminary study showed higher expression of UGDH in the renal tissue of mice with DKD than in that of controls [12]. UGDH is an essential protein-coding gene for HA synthesis and an important structural component of the extracellular matrix (ECM) in the kidneys. In this study, we observed increased urinary HA excretion in the DKD group, as defined by the rate criterion, and the A3 group. Various studies have shown that diabetes promotes HA production in several kidney cells, including proximal tubular cells, fibroblasts, and mesangial cells [34]. Thus, the increased urinary excretion of HA could be due to increased HA synthesis, suggesting a plausible link between hyperglycemia and structural changes in the ECM of patients with DKD. We provide insights into the possible association of the CD39–adenosinergic axis with HA and succinate synthesis pathways in DKD progression. Further mechanistic studies of these regulatory pathways are necessary to gain a deeper understanding of our findings.

Our study has some limitations. First, this was a cross-sectional study. The causal relationship between the metabolites and DKD could not be comprehensively determined. Second, this study did not thoroughly investigate the potential influence of medications, such as sodium-glucose co-transporter 2 (SGLT2) and RAS inhibitors, on DKD progression. The renoprotective effects of SGLT2 inhibitors are partially mediated by increased adenosine production and the resulting restoration of TGF [35-37], suggesting that these are important covariates. Although SGLT2 inhibitors were introduced in Korea at the end of 2013, their use in patients with T2DM was limited during the study period because of government reimbursement restrictions, as evidenced by the literature on prescription patterns [38]. The annual prescription rate of SGLT2 inhibitors in patients with T2DM in 2016 was <5% [39]. Additional analyses of the prescription data should be conducted in future prospective studies using this cohort. Finally, racial and ethnic differences in DKD progression should be considered. Hispanic, black, and Asian patients with diabetes have a higher risk of rapid DKD progression than Caucasian populations [1,40]. The generalizability of our results may be limited by the Asian ethnicity of the participants included in this study.

Despite these limitations, this study has several strengths that distinguish it from previous studies. First, one of the key advantages of our research is the measurement of novel metabolites of DKD, such as adenosine, succinate, and HA, in a considerable number of patients and healthy controls. Second, we further retrospectively divided the participants into those with a rapid decline in kidney function and NPs. This classification enhanced the robustness of our results and consistently highlighted the association between urinary succinate excretion and serum CD39 levels in patients with DKD. Finally, we provide valuable insights into the potential connection between the CD39–adenosinergic axis and HA and succinate synthesis pathways in DKD, a hypothesis previously suggested by our miRNA profiling study [12].

Building on our previous research, we found a robust association of elevated urinary succinate and serum CD39 concentrations with DKD. However, in the regression model, only urinary succinate excretion was independently and consistently associated with DKD and albuminuria. These findings must be confirmed through mechanistic and long-term follow-up prospective cohort studies in larger populations.

**SUPPLEMENTARY MATERIALS**

Supplementary materials related to this article can be found online at https://doi.org/10.4093/dmj.2023.0377.
CONFLICTS OF INTEREST

Ji A Seo has been associate editor of the Diabetes & Metabolism Journal since 2022. Dae Ho Lee has been international editorial board members of the Diabetes & Metabolism Journal since 2023. They were not involved in the review process of this article. Otherwise, there was no conflict of interest.

AUTHOR CONTRIBUTIONS

Conception or design: S.N., D.H.L., N.H.K.
Drafting the work or revising: I.J., D.H.L., N.H.K.
Final approval of the manuscript: all authors.

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Urinary succinate and adenosine: DKD


### Supplementary Table 1. Multivariable logistic regression analysis for the presence of albuminuria

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.951</td>
<td>0.883–1.023</td>
<td>0.1788</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.816</td>
<td>0.630–5.232</td>
<td>0.2693</td>
</tr>
<tr>
<td>SBP</td>
<td>1.060</td>
<td>1.026–1.095</td>
<td>0.0005</td>
</tr>
<tr>
<td>Diabetic duration</td>
<td>1.042</td>
<td>0.971–1.117</td>
<td>0.2510</td>
</tr>
<tr>
<td>HbA1c</td>
<td>1.254</td>
<td>0.862–1.825</td>
<td>0.2363</td>
</tr>
<tr>
<td>Adenosine/Cr</td>
<td>0.684</td>
<td>0.392–1.193</td>
<td>0.1804</td>
</tr>
<tr>
<td>Succinate/Cr</td>
<td>2.034</td>
<td>1.127–3.669</td>
<td>0.0183</td>
</tr>
<tr>
<td>HA/Cr</td>
<td>0.822</td>
<td>0.318–2.122</td>
<td>0.6853</td>
</tr>
<tr>
<td>CD39</td>
<td>1.392</td>
<td>0.875–2.216</td>
<td>0.1628</td>
</tr>
<tr>
<td>CD73</td>
<td>0.978</td>
<td>0.959–0.996</td>
<td>0.0199</td>
</tr>
</tbody>
</table>

All 10 variables were incorporated into multivariate logistic regression analysis to estimate their association with the risk of diabetic kidney disease. Skewed variables (urine adenosine/Cr, urine succinate/Cr, and urine hyaluronic acid/Cr ratios) were subjected to logarithmic transformation.

CI, confidence interval; SBP, systolic blood pressure; HbA1c, glycosylated hemoglobin; Cr, creatinine; HA, hyaluronic acid; CD39, cluster of differentiation 39; CD73, cluster of differentiation 73.
Supplementary Fig. 1. Proposed mechanisms of diabetic kidney disease (DKD) through target mRNAs identified in the analysis of DKD-related urinary miRNA profiles. Metabolites that increased during DKD exacerbation are shown in light pink boxes, and those that decreased are shown in blue boxes. ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; CD39, cluster of differentiation 39; CD73, cluster of differentiation 73; HA, hyaluronic acid; NT5E, ecto-5′-nucleotidase; TEX101, testis-expressed 101; UGDH, UDP-glucose dehydrogenase; SIRT3, sirtuin 3; SDHB, succinate dehydrogenase complex iron–sulfur subunit B.
**Supplementary Fig. 2.** (A) Urinary metabolite and (B) serum ectonucleotidase (cluster of differentiation [CD39] and CD73) concentrations in relation to rapid kidney function decline. Diabetic kidney disease rapid progressors are patients with an estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m² at study enrollment and meeting either of the following criteria: those with ΔeGFR/year ≤ −3 mL/min/1.73 m²/year or those exhibiting an annual rate of eGFR decline of at least 3.3%. Cr, creatinine. *Post hoc* \( P \leq 0.05 \), \( ^{\text{a}}P \leq 0.01 \), \( ^{\text{c}}P \leq 0.0001 \).
Supplementary Fig. 3. Concentrations of (A) urinary metabolites and (B) serum ectonucleotidases according to albuminuria categories. Skewed variables underwent logarithmic transformation for analysis. Cr, creatinine; HA, hyaluronic acid; CD39, cluster of differentiation 39; CD73, cluster of differentiation 73. *Post hoc $P \leq 0.05$, *Post hoc $P \leq 0.01$, *Post hoc $P \leq 0.001$, *Post hoc $P \leq 0.0001$. 