News Release

Title

The new mechanism of the development of diabetic kidney disease
～Distinct roles for two enzymes of fructose metabolism～

Key Points

- **We identified the distinct roles of two isoforms of fructokinase (KHK-A and KHK-C), enzymes for fructose, in the development of diabetic kidney disease (DKD).**
- **In diabetes, endogenous fructose production is enhanced through polyol pathway activation. In kidney, enhanced fructose metabolism most likely due to KHK-C induces inflammation, oxidative stress and the increase of nucleotide degradation products, and leads to exacerbate DKD.**
- **Oppositely, lacking KHK-A induces further fructose metabolism, exacerbation of inflammation, and hypoxia in diabetic kidney resulting in more severe tubular injury and renal dysfunction, and thus KHK-A plays a unique protective role against the development of DKD.**
- **Selective inhibition of KHK-C, not KHK-A, may leads to the development of the novel therapy against DKD.**

Summary

The group of researchers, Tomohito Doke (the first author, Nagoya University Graduate School of Medicine (Dean: Prof. Kenji Kadomatsu), Takuji Ishimoto (the corresponding author, Assist. Prof., Nagoya University Hospital), and Shoichi Maruyama (Professor, Department of Nephrology, Nagoya University Graduate School of Medicine) and colleagues identified the new mechanism for the progression of diabetic kidney disease (DKD), which is number one cause of end stage renal disease. This work was published online in the international scientific journal “Metabolism”.

Intake of fructose such as from sugar and high fructose corn syrup has increased dramatically in the last hundred years, and epidemiologically linked with the increase of obesity, diabetes, dyslipidemia, hypertension, NAFLD. Not only from meal (exogenous), but fructose is also endogenously produced via the activation of polyol pathway in diabetes. Our group had reported that metabolic syndrome and DKD were alleviated by suppression of excessive fructose metabolism.

Fructose is primarily metabolized by ketohexokinase (KHK) that has two isoforms, namely, KHK-A and KHK-C. KHK-C is expressed in the liver, intestine, and kidney and rapidly metabolites fructose, whereas KHK-A is expressed in various tissues, including the kidney, and slowly metabolizes fructose. The detailed function of KHK-A remains to be unclear. This study investigated the respective role of KHK-A and KHK-C in DKD. We found the fructose

metabolism induced renal tubular injury through increases of oxidative stress and nucleotide degradation products, and thus endogenous fructose metabolism most likely due to KHK-C exerted deleterious effects in DKD. On the other hand, deletion of KHK-A induced further fructose metabolism, exacerbation of inflammation, and hypoxia, which led to more severe renal tubular injury and renal dysfunction. Hence, we newly elucidated KHK-A plays a unique protective role against the development of DKD, which was attributed from the distinct function of KHK-A as a protein kinase.

This study demonstrates the opposite roles of KHK-A and KHK-C in the progression of DKD. The development of the selective inhibitor for KHK-C would be expected to be a new treatment DKD and metabolic syndrome.

Research Background

Diabetic nephropathy is the number one cause of end stage renal disease in Japan, and its prevalence has been increasing. Diabetic nephropathy is typically characterized by prominent proteinuria, edema, and rapid decrease of renal function. In recent years, patients with renal failure without prominent proteinuria has been increased, and newly, the concept of diabetic kidney disease (DKD) has been suggested. In DKD, the main legions out of glomerular such as inflammation in tubulointerstitial and fibrosis, stiffness of arterioles are observed, however, the mechanism of tubular injury remains to be elucidated. Although many drugs have been used for diabetes, it is still difficult to stop the progression of DKD. Therefore, it has been warranted to clarify the pathophysiology and develop the new treatment for DKD.

Intake of fructose such as from sucrose and high fructose corn syrup in soft drink and processed foods has increased dramatically in the last hundred years, and epidemiologically linked with the increase of obesity, diabetes, dyslipidemia, hypertension, NAFLD. In diabetes, hyperglycemia activate polyol pathway and produce fructose from glucose endogenously. Ketohexokinase, which metabolize fructose to fructose-1-phosphate, has two isoforms, namely, KHK-A and KHK-C. In kidney, both isoforms exist only in proximal renal tubules. KHK-C localizes in the liver, intestine, and kidney, and metabolizes fructose rapidly, whereas KHK-A is slower metabolizer of fructose, and expressed systemically. Our group had reported that excessive fructose metabolism by KHK induced obesity, fatty liver, hyperinsulinemia, and progression of DKD with ATP depletion, increases of nucleotide degradation products including uric acid, inflammation, and oxidative stress, but those were prevented in mice lacking both KHK-A and KHK-C. In contrast, we had also reported that those were more exacerbated in in mice lacking KHK-A compared to wild type mice, suggesting that KHK-A and KHK-C have distinct roles. Moreover, it has been reported that KHK-A function as a protein kinase which phosphorylates and activates phosphoribosyl pyrophosphate synthetase 1 (PRPS1) to promote pentose phosphate pathway-dependent *de novo* nucleic acid synthesis in hepatocellular carcinoma. Our study investigated the respective role of KHK-A and KHK-C in the development of DKD.

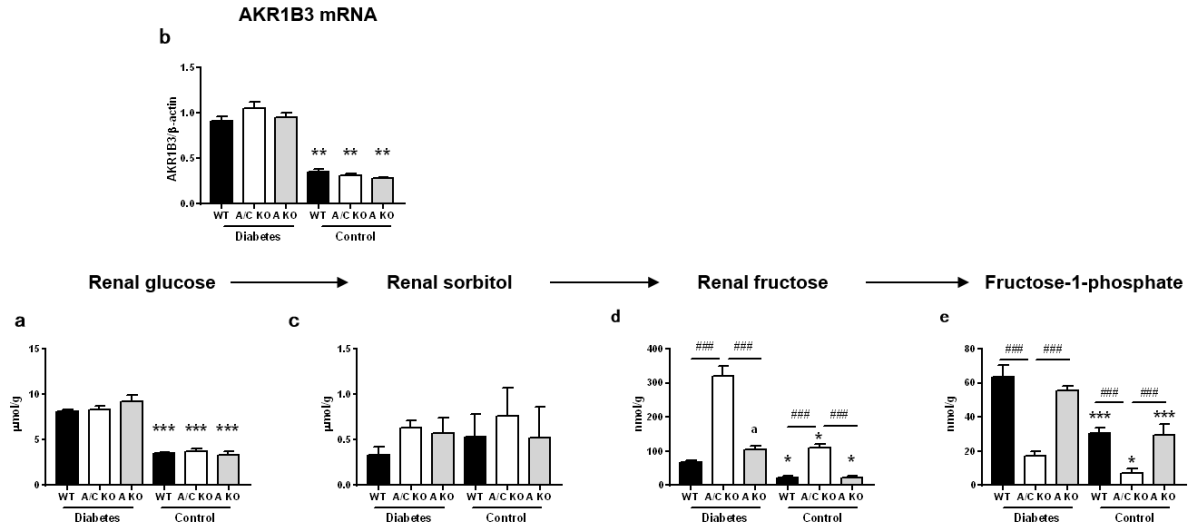
Research Results

We used diabetic wild type mice (D-WT), diabetic KHK-A/ KO mice (D-KHK-A/C KO), and diabetic KHK-A KO mice (D-KHK-A KO), and non-diabetic mice of each genotype, and investigated renal function, tubular injury, inflammation, oxidative stress, and hypoxia. In addition, we conducted metabolomics analysis using renal tissues, serum, and urine to analyze the metabolisms of fructose, TCA cycle, nucleotide, and NAD. In all diabetic groups, renal glucose, renal fructose, and expression of aldose reductase were significantly increased compared to non-diabetic groups, confirming the activation of polyol pathway in kidney (Figure 1). There were D-WT and KHK-A KO showed renal swelling, renal tubular injury accompanied with increases of oxidative stress and nucleotide degradation products compared to D-KHK-A/C KO mice which were unable to metabolize fructose (Figure 2). No significant difference in glomerular injury among all diabetic groups. It has been known fructose metabolism consumes ATP with subsequent increase of AMP, followed by the activation of nucleotide degradation pathway. Consistently, urinary allantoin levels, which is a final product of nucleotide degradation pathway, were significantly correlated with renal AMP contents and urinary NGAL levels, a marker of renal tubular injury (Figure 3). Since both WT and KHK-A KO mice express KHK-C in kidney, these results suggested that fructose metabolism by KHK-C exacerbated renal tubular injury in diabetes.

The primary finding of the present study was that kidney function was more impaired with severe inflammation and tubular damage in the kidney of D-KHK-A KO mice than in D-WT mice. Metabolomics analysis showed increased intrarenal fructose, dihydroxyacetone phosphate (DHAP), and intermediates of TCA cycle levels in D-KHK-A KO mice compared to D-WT mice. These results showed renal fructose metabolism was more enhanced in D-KHK-A KO mice (Figure 4). Moreover, we found significant decrease of renal NAD accompanied with increases of renal HIF1α expression and urinary lactate levels indicating renal hypoxia D-KHK-A KO mice compared to D-WT mice (Figure 5). The decrease of NAD inactivates sirtuin1, and then increases HIF1α expression. Because PRPP is necessary to generate NAD, it might be possible that KHK-A deficiency mediated the paucity of PRPP generation and subsequent reduction of NAD production, resulted in renal hypoxia.

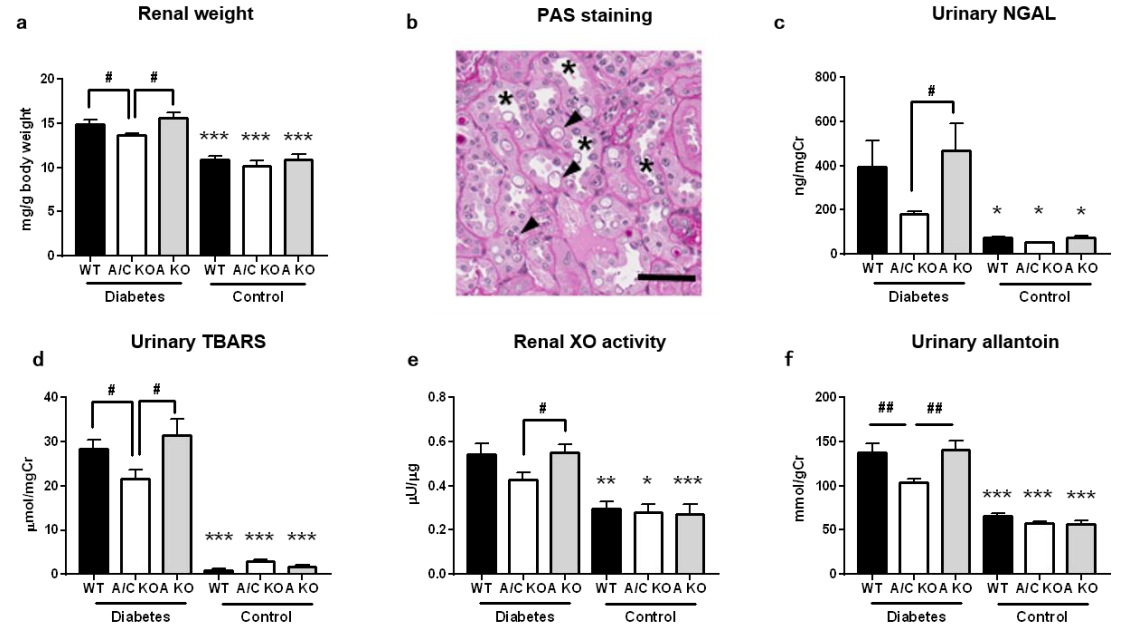
Our study demonstrated that KHK-C might play a deleterious role, and that KHK-A plays a unique protective role in the development of DKD (Figure 6).

Figure 1: Analysis of renal polyol pathway



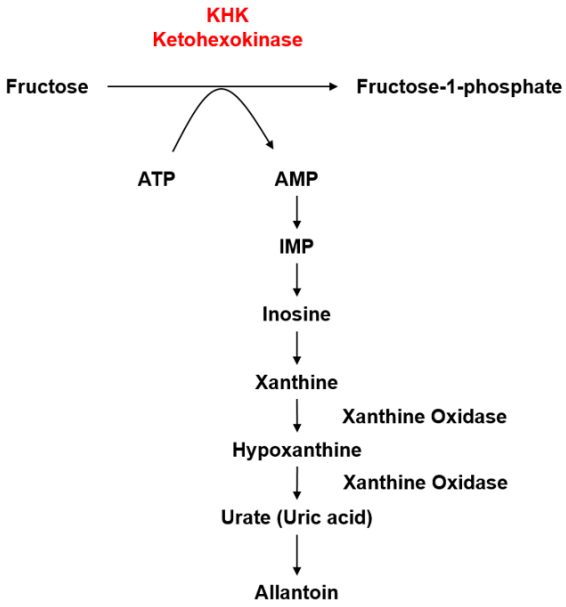
mRNA expression of renal glucose, renal fructose, and the enzyme of aldose reductase (AKR1B3) are significantly increased in each diabetes group compared to each control group, which indicated the enhancement of polyol pathway. The fructose metabolism was suppressed in KHK-A/C KO mice.

Figure 2: Assessment of renal swelling, oxidative stress, nucleotide metabolism, and tubular injury



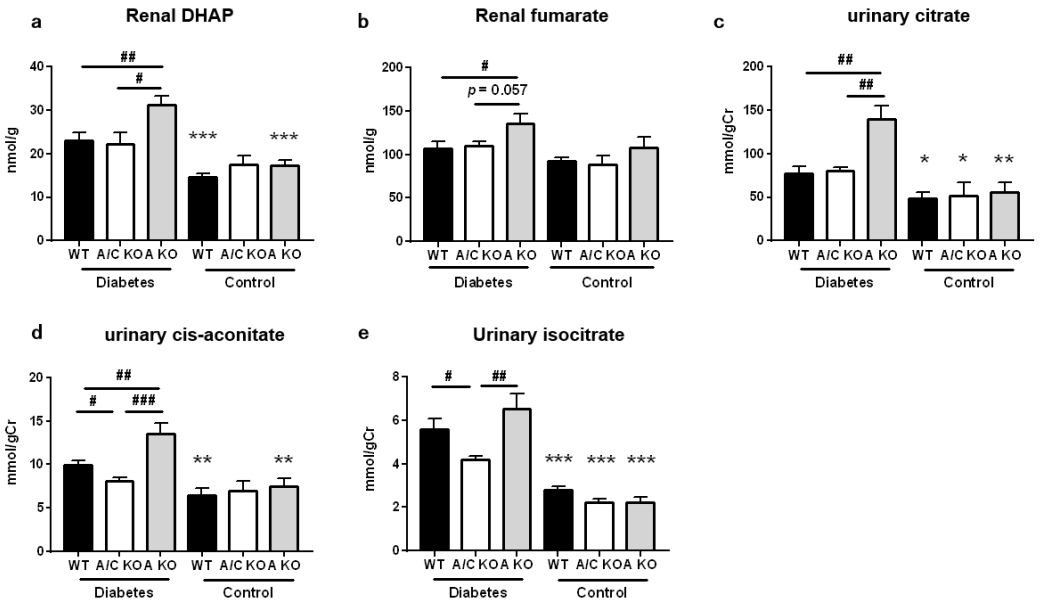
There were significantly increase of urinary NGAL as a marker of tubular injury, urinary TBARS and renal XO activity as a marker of oxidative stress, and urinary allantoin, the final product of nucleotide metabolism, in D-WT and D-KHK-A KO compared to D-KHK-A/C KO. Moe severe degeneration of renal epithelial tubule cell, and tubular dilation ware found in D-KHK-A KO mice.

Figure 3: The relationship between KHK and fructose metabolism and nucleotide degradation



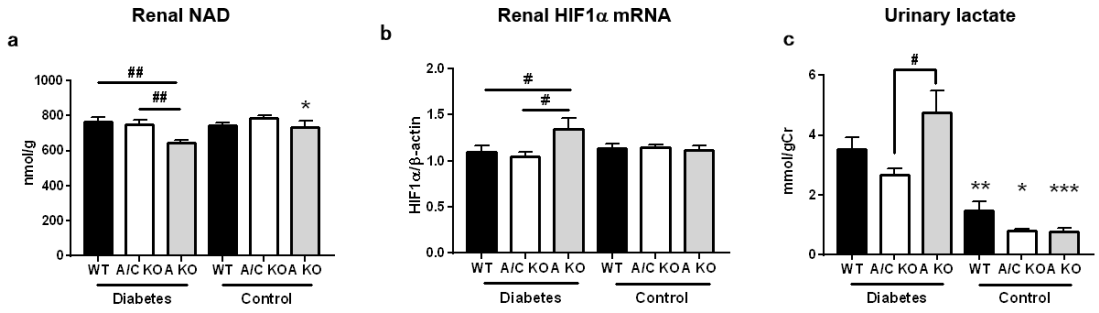
When fructose is metabolized by KHK, ATP is consumed, followed by enhancement of nucleotide degradation. The final product is uric acid in human, however, uric acid is converted to allantoin in rodents, and excreted to urine.

Figure 4: Analysis of downstream products of fructose



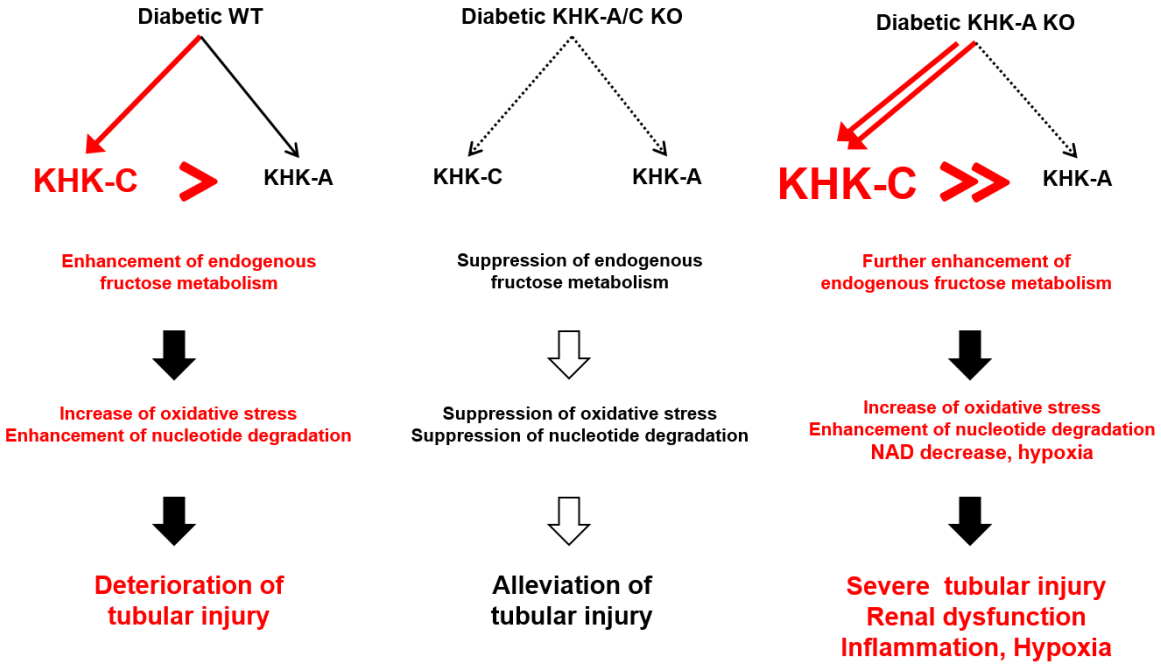
There were significant increase of renal DHAP, renal fumarate, urinary citrate, urinary cis-aconitate, and urinary isocitrate in D-KHK-A KO compared to D-WT and D-KHK-A/C KO. In addition, there were significant increase of urinary cis-aconitate, and urinary isocitrate in D-WT compared to D-KHK-A/C KO. These results indicated fructose metabolism was enhanced in D-KHK-A KO mice.

Figure 5: Analysis of renal hypoxia



Renal NAD was significantly decreased and the expression of renal HIF1α was significantly increased in D-KHK-A KO compared to D-WT and D-KHK-A/C KO. Urinary lactate was significantly increased in D-KHK-A KO compared to D-KHK-A/C KO. These results indicated the renal hypoxia in D-KHK-A KO mice.

Figure 6: The role of KHK-A and KHK-C in DKD



Research Summary and Future Perspective

Our study elucidated the opposing effects of KHK-A and KHK-C on DKD besides metabolic syndrome. Fructose metabolism by KHK-C would be an exacerbating factor, and that KHK-A has a protective role in the development of DKD. It is expected that the development of selective KHK-C inhibitor might be a novel therapy against metabolic syndrome and DKD.

Publication

Tomohito Doke, Takuji Ishimoto, Takahiro Hayasaki, Satsuki Ikeda, Masako Hasebe, Akiyoshi Hirayama, Tomoyoshi Soga, Noritoshi Kato, Tomoki Kosugi, Naotake Tsuboi, Miguel A. Lanaspa, Richard J. Johnson, Kenji Kadomatsu, and Shoichi Maruyama

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