

Acylcarnitines: Can They Be Biomarkers of Diabetic Nephropathy?

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Abstract: Diabetic nephropathy (DN), one of the most serious microvascular complications of diabetes mellitus (DM), may progress to end-stage renal disease (ESRD). Current biochemical biomarkers, such as urinary albumin excretion rate (UAER), have limitations for early screening and monitoring of DN. Recent studies have identified some metabolites as candidate biomarkers for early detection of DN. In this review, we summarize the role of dysregulated acylcarnitines (AcylCNs) in DN pathophysiology. Lower abundance of short- and medium-chain AcylCNs and higher long-chain AcylCNs often occurred in DM with normal albuminuria and microalbuminuria, compared with advanced stages of DN. The increase of long-chain AcylCNs was supposed to be an adaptive compensation in fat acids (FAs) oxidation in the early stage of DN. Conversely, the decrease of long-chain AcylCNs was due to incomplete oxidation of FAs in advanced stage of DN. Thus, AcylCNs may serve as sensitive biomarkers in predicting the risk of DN.

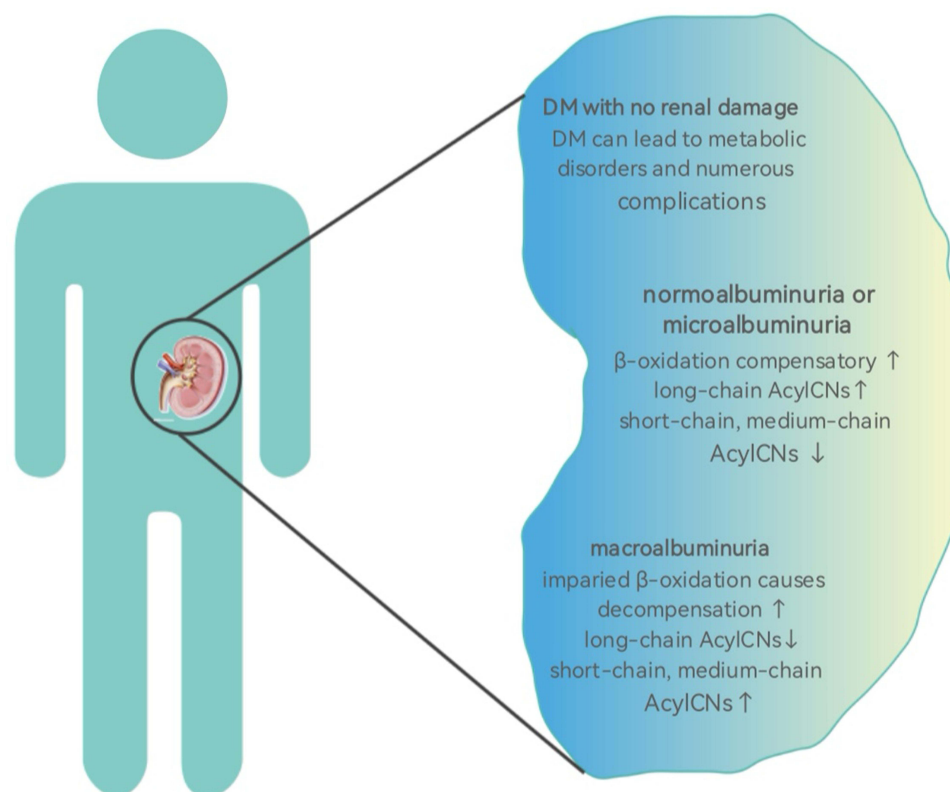
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Introduction

The incidence of diabetes mellitus (DM) continues to rise worldwide. DM is one of the most important public health concerns with high rates of morbidity and mortality.¹⁻³ Diabetic nephropathy (DN) is a severe complication of DM, which is a leading cause of end-stage renal disease (ESRD) in the developed world.⁴⁻⁷ Approximately 25–40% of DM patients develop DN.⁸ Accordingly, an urgent need exists for early biomarkers to predict DN among individuals with DM.

DN initially causes glomerular hyperfiltration, followed by varying degrees of proteinuria, decline in glomerular filtration rate (GFR), and finally ESRD.^{9,10} Although the etiology of DN is not clear, it is mainly due to abnormal glucose metabolism, oxidative stress, activation of innate immune system, and inflammation.¹¹⁻¹⁴ It is generally believed that, once developed, DN is difficult to be reversed. In advanced DN, dialysis or kidney transplant is needed for survival.¹⁵ However, the clinical manifestations of DN are often silent in the early stage.¹⁶ Urinary albumin excretion rate (UAER) is considered to be an early marker of DN.¹⁷ However, the levels of UAER are not increased in stages I and II of DN.^{13,18,19} In addition, renal biopsy is considered the gold standard for diagnosis and determines the degree of DN.²⁰ However, it is invasive and expensive, and its results do not correlate with the clinical outcome.²¹ Therefore, a promising clinical marker for early diagnosis of DN is urgently needed. DM, a metabolic disease defined by chronic elevation of glucose, leads to downstream metabolic dysfunction. Metabolomics is the study of small endogenous molecules from cells and biological systems. The changes of urine or plasma metabolites may predict the development of DN in DM individuals. Among multiple metabolites, acylcarnitines (AcylCNs) highlight unique features in DN. This review emphasizes on the role of AcylCNs in DN, and acting as early predictive biomarkers of DN.

Graphical Abstract



Metabolomics in DN

Metabolomics is a qualitative and quantitative analysis of metabolites in organisms after environmental stimuli or gene alteration, which can be used to identify metabolites closely related to physiological and pathological changes.²² Metabolomics can reveal the metabolites that may be specific to disease and play an important role in the development of disease.^{22,23} Metabolomics techniques, such as nuclear magnetic resonance (NMR) and mass spectrometry (MS), have been applied to the urine and circulating metabolites in DN in a targeted or untargeted manner.²⁴ Targeted metabolomics, the gold standard for metabolite quantification, is to detect only a few groups of metabolites and obtain high sensitivity and selectivity by excluding a large number of mixed signals. It has been used to identify biomarkers for DN.^{25–27} By using targeted metabolomics, Zhang et al found a variety of early metabolic signs in DN and identified 11 closely related metabolites, which helped to further predict and prevent DN in the Chinese population.²⁸ Jiang et al used high-performance liquid chromatography-electrospray ionization tandem MS (HPLC-ESI-MS/MS) to simultaneously quantify eight amino thiols in the metabolic cycle of homocysteine in plasma and identified two sulfur-containing metabolites, namely S-adenosylmethionine and S-adenosylhomocysteine, as potential biomarkers for DN.²⁹ Researchers found that AcylCNS could help identify individuals with proteinuria or renal dysfunction based on novel targeted metabolomics approaches, suggesting the increased importance of AcylCNS as biomarkers of DN. They could improve the predictive capacity of clinical models to identify kidney dysfunction and DN-related outcomes.³⁰

The establishment of sensitive biomarkers for DN may help discover novel therapeutic targets.³¹ Metabolic change in DM patients may be triggers for DN. Previous studies have found that amino acids (AAs), fatty acids (FAs), purines, pyrimidines, and other metabolites were altered in the urine and peripheral blood circulation in DN (Table 1).^{32–37} Metabolomics, especially targeted metabolomics, has been recognized as a powerful tool in the field of biomarker discovery of DN.

Table I Related Studies of Metabolomics in DN

Material	Sample	Method	Major Finding	Reference
Serum	Human	LC-MS	Tryptophan as a surrogate prognostic marker for DN	[38]
Serum	Human	LC-MS/MS	Taurine metabolism pathway potentially affect the pathogenesis of DN from T2DM	[20]
Urine	Human	NMR	Identify novel metabolic difference between DN and non-DN	[39]
Serum	Human	LC-MS/MS	Elevated tyrosine is associated with increased DN risk	[40]
Plasma	Rat	GC-MS	Oleic acid might be the potential biomarker of kidney injury	[41]
Kidney tissue	Rat	LC-MS	Mitochondrial ceramide accumulation may result in podocyte damage	[42]
Plasma	Human	UHPLC-QTOF/MS	Sphingomyelin and phosphatidylcholine species are associated with renal impairment and all-cause mortality in T1DM	[43]
Serum	Human	UPLC-ESI-MS/MS	Linoleic acid, L-Dihydroorotic Acid, and Azoxystrobin Acid especially represented a potential indicator of DM progress	[28]
Kidney tissue	Rat	LC-MS	Linoleic acid metabolism and fatty acids β -oxidation are inhibited in DN	[44]
Plasma	Human	MS	Increased acylcarnitines levels are associated with risk of progression to ESRD	[45]
Blood	Rat	SPE-UPLC-MS/MS	A correlation between metabolic disorders of purine and pyrimidine and DN	[25]
Urine	Human	LC-MS	Xanthosine and NI-methylguanosine can be used to predict nephropathy in patients with T2DM	[46]
Plasma	Human	LC-MS/MS	Increased lipoxigenase metabolites and decreased CYP450 metabolites are significantly associated with the incidence of DN	[47]
Serum	Human	GC/LC-MS	Some modified metabolites were associated with renal function decline and time to ESRD	[48]

Notes: The above table lists some recent studies on metabolomics of DN. In the process of diabetic kidney damage, the metabolic disorders of various substances appear in the body. It is not difficult to find that researchers have begun to focus on metabolomics of DN in recent years, so as to further clarify the mechanism of early diabetic renal damage, which may help to identify preventive and treatment measures for DN.

Abbreviations: DN, diabetic nephropathy; DM, diabetes mellitus; ESRD, end-stage renal disease; UAER, urinary albumin excretion rate; AcylCNs, acylcarnitines; FA, fat acid; GFR, glomerular filtration rate; NMR, nuclear magnetic resonance; MS, mass spectrometry; HPLC-ESI-MS/MS, high performance liquid chromatography-electrospray ionization tandem mass spectrometry; AAs, amino acids; OXPHOS, oxidative phosphorylation; TCA, tricarboxylic acid; CN, carnitine; MS/MS, tandem mass spectrometry; C2, acetyl-CoA; T2DM, type 2 diabetes mellitus; eGFR, estimated glomerular filtration rate; T1DM, type 1 diabetes mellitus; CKD, chronic kidney disease; ACC, acetyl-CoA carboxylase; ACR, albumin/creatinine ratio.

AcylCNs—Potential Biomarkers of DN

It is suggested that mitochondrial dysfunction plays an indispensable role in DN.^{49,50} Mitochondria, known as power-house of the cell, are the basic subcellular organelles in eukaryotic cells to maintain metabolic homeostasis. Neurodegenerative, neoplastic, endocrine, and cardiovascular diseases are associated with mitochondrial dysfunction.^{37,51} Mitochondria are essential for ATP synthesis via oxidative phosphorylation (OXPHOS).⁵² The number of mitochondria in the kidneys is second only to the heart. Tricarboxylic acid (TCA) cycle, the hub of three nutrients, is carried out in mitochondria.^{53,54} Therefore, dysfunction of the TCA cycle causes metabolic disorders of AAs, FAs, and purines (Figure 1). Previous studies have showed that mitochondrial dysfunction in DN causes β -oxidation of FA disorder, ultimately leading to abnormal deposition of lipids in DN.^{44,53} AcylCNs serve as markers of mitochondrial function, which play a critical role in the pathogenesis of DN, specifically for β -oxidation of FA.⁵⁵

Metabolic Process Related to AcylCNs in Organisms

AcylCNs contain an acyl esterified to carnitine (CN), which allows long-chain FAs to pass through the mitochondrial membrane for FA β -oxidation.^{56–58} AcylCNs, intermediate metabolites in FA and AA oxidation, are located in tissues and body fluids. Tandem mass spectrometry (MS/MS) detection of AAs and AcylCNs has been widely used for screening neonatal metabolic diseases.^{55,59,60} AcylCNs are synthesized by carnitine palmitoyl transferase I, which is responsible for transporting FAs to the mitochondrial matrix. AcylCNs enter the mitochondria where they are subjected to carnitine palmitoyl transferase II in the inner mitochondrial membrane, which transforms them to acyl-CoA and releases carnitine.⁶¹ Figure 2 shows the metabolic pathway for production of AcylCNs. Medium-chain and long-chain acyl-

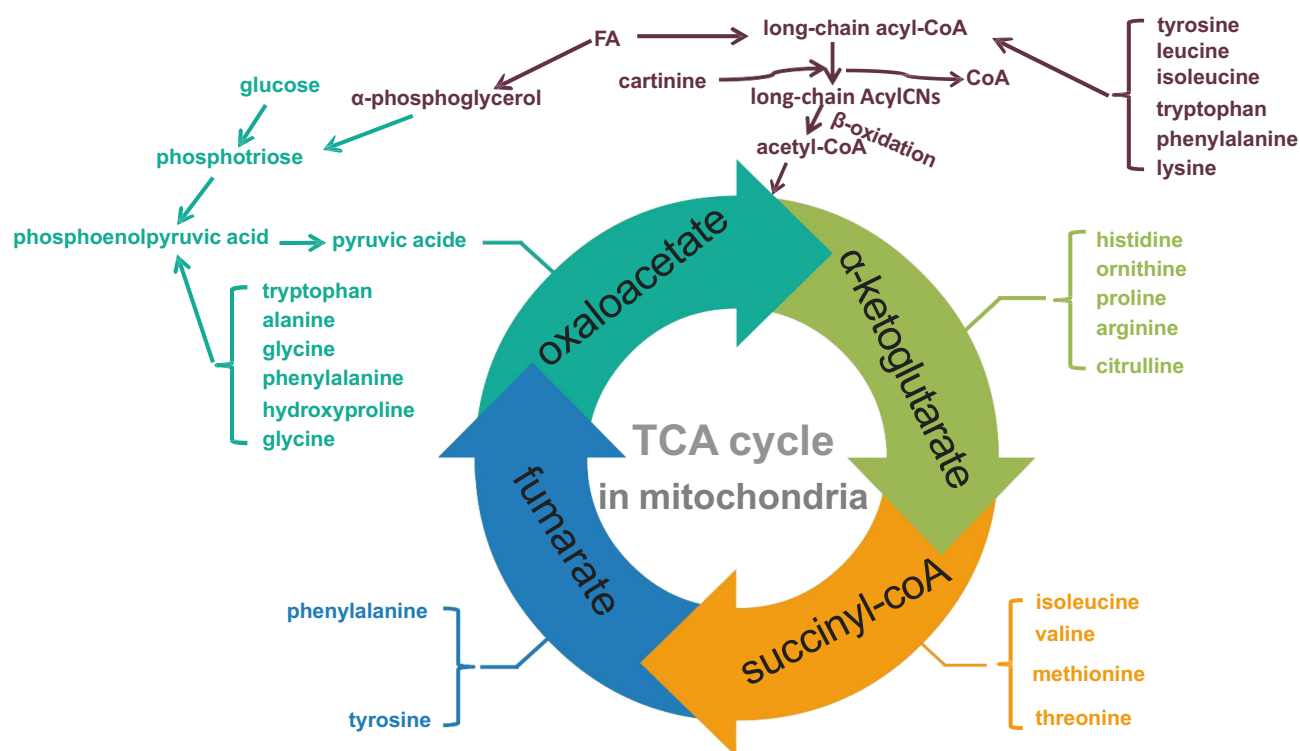


Figure 1 Tricarboxylic acid cycle—three metabolic hubs.

Notes: TCA cycle is the common pathway for the decomposition capacity of the three major nutritional metabolites (glucose, FA and AA), also is the hub of their metabolism. TCA cycle needs to be carried out in mitochondria. When DN causes mitochondrial dysfunction and AGEs increase, it is bound to cause metabolic disorders. **Abbreviations:** TCA cycle, tricarboxylic acid cycle; FA, fatty acid; AA, amino acid; AGEs, advanced glycation end products.

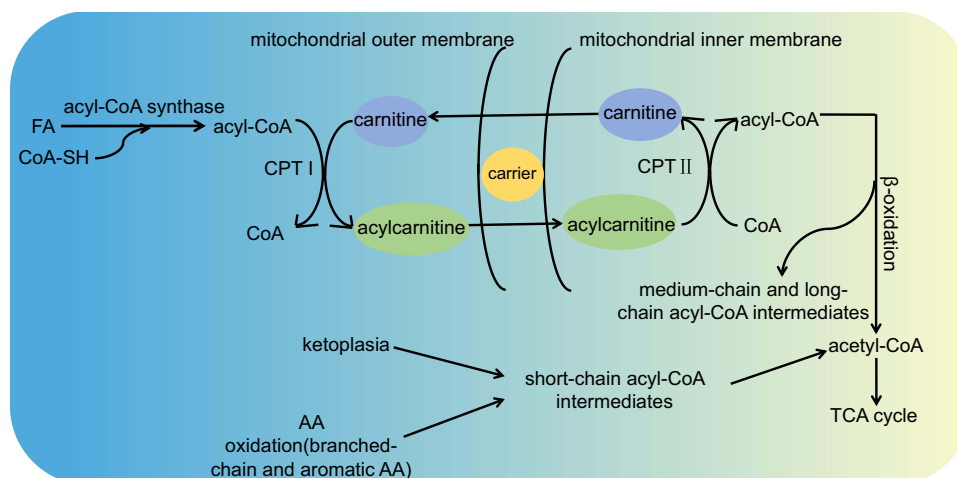


Figure 2 The metabolic process of AcylCNs in organisms.

Notes: When β -oxidation or AA oxidation is incomplete, or ketone body formation is insufficient, acyl-CoA intermediates accumulate and produce less C2. Due to the cytotoxicity of excess acyl-CoA, they are converted into AcylCNs and released into the plasma, eventually excreted into the urine. AcylCNs are important markers of peroxisome and mitochondrial oxidative disorder, which can serve as biomarkers for the metabolic syndrome.

Abbreviations: AcylCNs, acylcarnitines; TCA cycle, tricarboxylic acid cycle; FA, fatty acid; AA, amino acid; C2, acetyl-CoA; CPT I, carnitine palmitoyl transferase I; CPT II, carnitine palmitoyl transferase II.

CoA intermediates are generated during FA oxidation, whereas short-chain acyl-CoA intermediates are generated during AA oxidation (branched amino acids and aromatic amino acids) and ketone body formation.^{62,63} During the active oxidation of FAs and AAs, acyl-CoA intermediates are produced, which are immediately converted into short products

and acetyl-CoA (C2), thus entering the TCA cycle.⁶⁴ With incomplete oxidation or insufficient ketone body formation, acyl-CoA intermediates accumulate and produce less C2. Due to the cytotoxicity of excess acyl-CoA, they are converted to AcylCNs, released into plasma, and eventually excreted into urine.⁶⁵

AcylCNs are important markers of peroxisome and mitochondrial oxidative disorders and can serve as biomarkers for metabolic syndrome.^{66–71} Some scholars used targeted metabolomics to study a group of AcylCNs, evaluated their relationship with the incidence of DM, and incorporated them into the established DM risk model.⁷² The results showed the accuracy and applicability of the model improved when added metabolomic data. In addition, some related literature indicated that AcylCNs were related to type 2 DM (T2DM), suggesting that AcylCNs accumulation could cause imbalance of insulin synthesis and secretion, which further led to β cell dysfunction.^{73,74} Moreover, researchers highlighted the importance of AcylCNs as biomarkers in clinical predictive models associated with rapid estimated GFR (eGFR) decline in type 1 DM (T1DM) combined with traditional risk factors.⁷⁵ The study of AcylCNs metabolic spectrum is of great significance for understanding the occurrence of DM and its complications.^{76–78} Plasma AcylCNs were also significantly associated with prognosis and treatment response in IgA nephropathy patients. Baseline levels of AcylCNs were associated with 1-year eGFR decline.⁷⁹ In different stages of chronic kidney disease (CKD), the abundance of AcylCNs was constantly fluctuating, and the ratio of long-to-intermediate AcylCNs was gradually decreasing as the kidney disease progresses. The trend of long-to-intermediate AcylCNs ratio decreased gradually from CKD stage 2 to 5.⁸⁰ It was supposed that the early increase of FA oxidation may be an adaptive phenomenon during the progression of CKD to cope with the elevated saturated FA, but impaired β -oxidation continued to prevail as the disease progressed and formed a vicious circle. In this case, impaired oxidation caused intracellular FA accumulation, further impaired mitochondria.⁸⁰ Based on the above studies, AcylCNs are considered to be important metabolites of DM and DM complications.

Metabolic Changes in AcylCNs in DN

The study of lipidomics revealed the potential mechanisms underlying the development of DN: impaired mitochondrial β -oxidation and complex lipid remodeling.^{55,81} The concentrations of carnitine, a carrier of FA transport into the mitochondria, and AcylCNs indicate the rate of β -oxidation of FA in the progression of DN.⁸² DN progression is associated with disorders of FA synthesis, desaturation, and oxidation. AcylCNs can accumulate in the plasma due to mitochondrial damage, especially in individuals with reduced eGFR.^{30,83} Previous study suggested that the increased levels of AcylCNs in urine were associated with early kidney injury in DM patients, reflecting the changes in β -oxidation pathway.⁸⁴ Theoretically, all short-, medium-, and long-chain AcylCNs would be elevated in patients with DN. Actually, the concentrations of AcylCNs in different lengths varied between different stages of DN. The plasma short- and medium-chain AcylCNs were increased, whereas long-chain AcylCNs were decreased in DM patients with normoalbuminuria and microalbuminuria, while the plasma short- and medium-chain AcylCNs were decreased, and the long-chain AcylCNs were increased.⁸⁵ A possible explanation is that FA oxidation may cause adaptive compensation (FA prolongation and desaturation) in DM with normoalbuminuria and microalbuminuria.⁵⁵ It converts more toxic lipids (saturated nonesterified FA) into less toxic lipids (polyunsaturated triglycerides) to prevent kidney injury, rather than reducing the utilization of these FA intermediates for mitochondrial oxidation. When DM patients progress to macroalbuminuria, mitochondrial oxidation in the kidneys is impaired in a state of decompensation. It is difficult to compensate for the renal metabolism and pathological changes by strengthening FA oxidation. Thus, the incomplete β -oxidation of long-chain FAs is increased, resulting in an increase in AcylCNs intermediates of plasma short- and medium-chain AcylCNs. What is more, lipid metabolic changes associated with DM are reported to occur at the early stage of DN. Studies of lipid metabolism can help discriminate between progression and non-progression patients and allow risk stratification in DM patients.⁵⁵ In addition, previous study demonstrated that the activation of acetyl-CoA carboxylase (ACC) was a key determinant of DN progression.⁸⁵ Upregulation of ACC suppresses CTPI, which in turn suppresses the cytoplasmic transformation of long-chain acyl-CoA to long-chain AcylCNs, thereby reducing the substrate of carnitine shuttle, impairing β -oxidation of long-chain FAs, and increasing the abundance of cytoplasmic palmitic acid. Long-chain AcylCNs are an important substrate for β -oxidation, so the β -oxidation is downregulated accordingly (Figure 3). Interestingly, the short-chain AcylCNs (C4, C4-OH, C5:1, C5-OH, and C5DC) were observed to be associated with

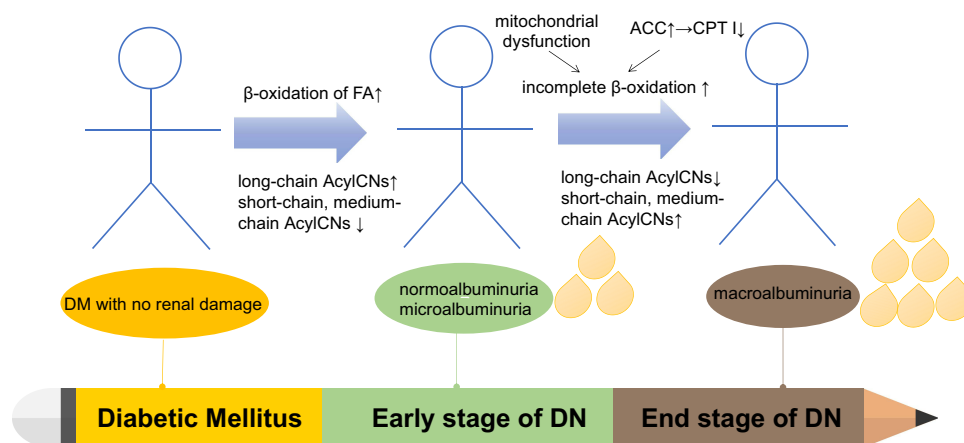


Figure 3 AcylCNs in DN.

Notes: The abundance of AcylCNs have different changes during the progression from DM to DN, even including AcylCNs with different length chains. These changes are mainly due to the increase of long-chain AcylCNs caused by adaptive compensation enhancement in early FA oxidation, on the contrary, the decrease caused by incomplete oxidation enhancement of FAs caused by mitochondrial dysfunction in the late stage. Drips represent the amount of protein in urine.

Abbreviations: AcylCNs, acylcarnitines; DM, diabetic mellitus; DN, diabetic nephropathy; FAs, fatty acids; ACC, acetyl-CoA carboxylase; CPT I, carnitine palmitoyl transferase I.

urinary microalbumin levels. In particular, C4 was a positive, independent, and significant predictor of the albumin/creatinine ratio (ACR) levels.³³ During the progression from normoalbuminuria to microalbuminuria, an abundance of C4 entered to the TCA cycle, thereby inhibiting glucose oxidation, exacerbating intracellular demand for carnitine storage. C4 concentration in the plasma was significantly elevated when DM progressed from normoalbuminuria to microalbuminuria. In summary, when stratified according to the levels of albuminuria, patients with normoalbuminuria and microalbuminuria have higher levels of long-chain AcylCNs than those with macroalbuminuria accompanied by β -oxidation activated. As DN progresses, the levels of long-chain AcylCNs reduced as a result of downregulated β -oxidation.

Limitations and Future Perspective of AcylCNs in DN

Metabolomics is the science of measuring small molecules in body fluids, which can probe the biomarkers to understand the pathogenic mechanisms and discover the novel therapeutic targets.^{23,86,87} However, the application of metabolomics has certain limitations, and there are some challenges when AcylCNs serve as biomarkers of DN. First, the metabolic products change in organisms in different nutritional status.^{88,89} It is difficult to confirm that all DM patients have the same dietary conditions. Different lipid diets interfere with changes in AcylCNs concentrations in blood and urine. So, are AcylCNs still reliable as indicators of kidney injury in DM patients with different diet habits? However, in fact, most DM patients have a similar diet when included in the diet management of DM. AcylCNs are not significantly disturbed by the confounding factors of the diet habits. Of course, further studies are needed to confirm whether diet influences the AcylCNs concentrations in DN patients. Meanwhile, we suggest that AcylCNs combined with traditional indicators such as UAER, ACR, eGFR, and even renal biopsy may achieve more precise prediction of DN. Second, the baseline AcylCNs levels in different age groups are actually different.⁹⁰ If we simply judge the AcylCNs levels with a constant standard, it is actually unreasonable. We suggest that the metabolic spectrum of AcylCNs should be standard by age. Finally, the clinical significance of AcylCNs is still not confirmed, and the mechanisms of AcylCNs involved in DN pathogenesis and progression need more studies in the future.²² The abundance of AcylCNs is not only influenced by the production but also by consumption in a nonlinear manner.⁸⁸ It was proposed that isotope tracers combined with metabolomics can reveal the pathway activities to evaluate AcylCNs concentrations. Recent advances in neural network (computer-based prediction method) to metabolomics is promising.⁹¹ It may help to predict the biological activity of AcylCNs in DN progression.

Metabolomics is a promising diagnostic tool in early and pre-symptomatic onset of disease and may supply personalized treatment. For example, previous studies have found that the addition of leucine to the diet reduced insulin resistance, interfered with TCA circulating metabolism, and decreased urinary carnitine levels in db/db mice by urine metabolomics. Furthermore, in these mice, autophagy was increased in the renal cortex, and DN was improved.⁹² Metabolomics can identify biomarkers or genetic phenotypes associated with pathophysiology by regulating the levels of the genome, epigenome, transcriptome, and proteome, which help to intervene by dietary supplementation of an abnormal metabolite or knockout gene phenotype.^{93,94} Although there is no relevant literature to support the supplement of long-chain AcylCNs to relieve DN, it may be a promising therapeutic target in the future. Targeted metabolomics shed light on the early detection of DN and unveiling potential biomarkers.

Conclusion

DN, as well as its onset and progression, is often insidious. UAER and renal biopsy have limitations for the early and accurate diagnosis. The emergence of metabolomics provides insight into DM kidney injury in a non-invasive manner. Based on novel targeted metabolomics strategies, we can identify plasma and urine biomarkers (including AcylCNs) together to elucidate the mechanisms and establish a clinical prediction model.^{30,72} This review mainly describes the changes in AcylCNs in the lipid in DN. Lower abundance of short- and medium-chain AcylCNs, and higher long-chain AcylCNs occur in DM with normal albuminuria and microalbuminuria, while higher abundance of short-chain and medium-chain AcylCNs and lower long-chain AcylCNs occur in DM with macroalbuminuria. Further research is needed to understand the changes, pathogenesis, and the clinical significance of AcylCNs involved in DN. It is recommended to use isotope tracer or neural network combined with targeted metabolomics to quantify AcylCNs of different lengths and the metabolic pathway in DN. Future studies are needed to determine the threshold values of AcylCNs in plasma or urine to predict DN. In summary, AcylCNs are promising biomarkers for early diagnosis of DN.

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Disclosure

The authors report no conflicts of interest in this work.

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