

Metabolomics in diabetic nephropathy: Unveiling novel biomarkers for diagnosis (Review)

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Abstract. Diabetic nephropathy (DN) also known as diabetic kidney disease, is a major microvascular complication of diabetes and a leading cause of end-stage renal disease (ESRD), which affects the morbidity and mortality of patients with diabetes. Despite advancements in diabetes care, current diagnostic methods, such as the determination of albuminuria and the estimated glomerular filtration rate, are limited in sensitivity and specificity, often only identifying kidney damage after considerable morphological changes. The present review discusses the potential of metabolomics as an approach for the early detection and management of DN. Metabolomics is the study of metabolites, the small molecules produced by cellular processes, and may provide a more sensitive and specific diagnostic tool compared with traditional methods. For the purposes of this review, a systematic search was conducted on PubMed and Google Scholar for recent human studies published between 2011 and 2023 that used metabolomics in the diagnosis of DN. Metabolomics has demonstrated potential in identifying metabolic biomarkers specific to DN. The ability to detect a broad spectrum of metabolites with high sensitivity and specificity may allow for earlier diagnosis and better management of patients with DN, potentially reducing the progression to ESRD. Furthermore, metabolomics

pathway analysis assesses the pathophysiological mechanisms underlying DN. On the whole, metabolomics is a potential tool in the diagnosis and management of DN. By providing a more in-depth understanding of metabolic alterations associated with DN, metabolomics could significantly improve early detection, enable timely interventions and reduce the health-care burdens associated with this condition.

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1. Introduction

Diabetic nephropathy (DN), also known as diabetic kidney disease (DKD) is a leading cause of end-stage renal disease (ESRD) and is associated with increased morbidity and mortality in patients with diabetes. As a microvascular complication of diabetes, DN affects 20–40% of individuals with diabetes worldwide (1). This condition manifests as a gradual loss of kidney function, often leading to the necessity for life-sustaining treatments, such as dialysis or transplantation (2). The economic and social burden associated with DN is considerable, affecting not only the quality of life of patients, but also imposing substantial costs on healthcare systems worldwide.

Despite advances in diabetes care, the incidence and prevalence of DN continue to increase, largely due to the increasing global incidence of type 2 diabetes mellitus (T2DM) associated with obesity and lifestyle changes. Current diagnostic methods for DN, primarily based on measuring albuminuria and the estimated glomerular filtration rate (eGFR), are limited in their sensitivity and specificity. These methods often detect kidney damage only after significant morphological changes have occurred, which may then be too late for effective

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Abbreviations: CKD, chronic kidney disease; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; DKD, diabetic kidney disease; ESKD, end-stage kidney disease; NMR, nuclear magnetic resonance; GC-MS, gas chromatography-mass spectrometry; LC-MS, liquid chromatography-mass spectrometry; eGFR, estimated glomerular filtration rate; DN, diabetic nephropathy

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intervention (3). Hence, there is a clear need for the identification of effective biomarkers that can predict early-stage kidney injury and detect the progression of DN.

Currently, metabolomics is emerging as a dynamic and rapidly evolving field that offers novel insight and potential breakthroughs in the understanding and management of DN. This approach provides a comprehensive analysis of metabolites, which are small molecules produced as end-products of cellular processes. Using high-throughput analytical platforms, metabolomics can assess a wide array of biological samples including blood, urine and tissue fluids. These platforms are capable of detecting a broad spectrum of metabolites with high sensitivity and specificity (4). Metabolomics has the potential to identify metabolic markers specific to DN, which could result in the development of non-invasive, highly sensitive and specific diagnostic tests. Metabolite biomarkers could enable the diagnosis of DN at earlier stages than current methods allow (5). The present review aimed to assess and summarize the existing research on the impact of metabolomics on DN studies, highlighting key findings, discussing challenges and limitations, and suggesting future directions for this field.

2. Data collection methods

The present review focuses on human studies that used a metabolomics approach to assess DN, published between 2011 and 2023. To gather the relevant literature, a systematic search was conducted using PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) and Google Scholar (<https://scholar.google.com/>) databases, using a number of key words, including 'metabolomics', 'metabolites', 'diabetic nephropathy', 'diabetic kidney disease' and 'biomarker'. The search was limited to articles written in the English language. Furthermore, the bibliographies of the articles identified were assessed to further expand the pool of references, ensuring a comprehensive review of contemporary research in this field.

3. Limitations of current biomarkers of DN

The diagnosis and monitoring of DN have traditionally been dependent on clinical variables, including eGFR and albuminuria. While these are essential for accurately predicting future renal conditions in conjunction with other standard clinical variables (6), their ability to detect the DN in its early stages is limited. eGFR, a key indicator of kidney function, is calculated from variables, including serum creatinine levels, age and other factors (7). Despite its usefulness, the reliability of eGFR is often questioned due to its indirect nature and dependence on factors such as muscle mass and diet, which can vary among individuals (8). eGFR typically reflects changes only when significant kidney damage has occurred, often failing to identify the early stages of DN when intervention could be most beneficial. Albuminuria, the presence of albumin in the urine, is another standard marker for assessing kidney damage. However, this marker alone lacks both sensitivity and specificity. Moreover, a number of patients do not exhibit increased albumin levels until the disease has progressed to an advanced stage, and transient increases in albuminuria can result from a number of factors unrelated to DN, such as physical exercise, infection or hypertension (9).

Kidney injury molecule-1 (KIM-1) is a biomarker specific for tubular damage and is sensitive in the detection of acute kidney injury. However, its use in DN is limited by a delayed response, it is usually elevated 12-24 h following the onset of kidney injury. The specificity of KIM-1 in chronic kidney conditions such as DN may not comprehensively reflect the condition of the kidneys due to its primary association with acute tubular damage (10). Cystatin C is an emerging biomarker which is considered to be less dependent on muscle mass than creatinine, suggesting a more direct measure of glomerular filtration. However, studies have reported variability in its sensitivity and specificity across different populations, thus rendering its diagnostic performance for DN inconsistent (11,12). The heterogeneity in results suggests that cystatin C may be influenced by other patient characteristics or comorbidities, complicating its use in DN (13). Moreover, the biomarker neutrophil gelatinase-associated lipocalin (NGAL) is noted for its rapid response to kidney injury and is useful in acute settings. However, its levels can also be affected by infections, inflammation and cancer, which may coexist with DN. Moreover, NGAL has exhibited variable diagnostic and prognostic accuracy across different populations, which may limit its effectiveness in universally predicting the progression of kidney disease or response to therapy in DN (14).

These limitations underscore the need for more refined and predictive tools that can detect DN at its early stages, providing a window for intervention that could delay or prevent the progression to ESRD. The late diagnosis of DN not only diminishes the quality of life of patients, but also results in substantial economic burden due to the high costs associated with ESRD treatments, such as dialysis and transplantation (15).

In response to these challenges, there is a necessity for the identification of effective biomarkers that are sufficiently sensitive to detect subtle changes in kidney function before irreversible damage occurs. These biomarkers also require sufficient specificity to distinguish DN from other kidney diseases. From this, metabolomics, with its ability to profile a wide array of metabolites, has emerged as a promising avenue. By capturing the metabolic alterations associated with the early stages of DN, metabolomics has the potential to identify novel biomarkers that are more predictive of disease progression. This could also provide deeper insight into the molecular mechanisms of DN, allowing for more targeted and effective treatment strategies to be developed (3,16). Integrating these novel biomarkers with traditional clinical assessments could improve the management of DN, shifting the focus from managing late-stage complications to preventing the onset and progression of the disease. This proactive approach is required to improve the outcomes of patients and to reduce the healthcare burden associated with DN. This approach could represent an effective healthcare strategy with the potential to affect the lives of millions of individuals affected by this condition.

4. Metabolomics: A useful tool for DN

Introduction to metabolomics. Metabolomics is an emerging field in biosciences with profound implications in the understanding and treatment of a number of diseases, including DN.

Metabolomics is the study of metabolites, which are small molecules that are the end products of cellular processes in living organisms (17). These metabolites include a wide range of substances, such as lipids, amino acids and sugars, each offering information regarding the metabolic state of the body at a given moment. Metabolomics is an analytical research method that, through high-throughput analysis, demonstrates interactions within a biological system. Each metabolite has a molecular weight of <1,500 Da, and the metabolites found within a biological sample are referred to as the metabolome, which depicts the state of the biological system via numerous mechanisms (18).

Endogenous metabolites are produced internally within an organism and serve integral roles in reflecting the physiological and metabolic processes of the body. Conversely, exogenous metabolites originate from external sources, such as drugs, environmental pollutants and dietary chemicals, which enter the body through ingestion, inhalation or dermal absorption (19). The examination of both endogenous and exogenous metabolites is required, as it aids researchers in understanding the mechanisms through which organisms interact with their environments and adapt their metabolic pathways in response to external influences. This methodology is used to assess the health of organisms, the mechanisms underlying disease development and the impact of environmental factors (20). Through metabolomic analysis, specific metabolic signatures indicative of DN can be identified, facilitating the development of novel diagnostic markers and therapeutic targets (21).

Metabolomics uses a holistic approach, in contrast to genomics or proteomics, which focus on the outputs from genetic or protein expression. Metabolomics offers a direct view of the physiological state at any given moment. This aspect is useful in DN, where metabolic alterations are both a precursor and a result of the disease. The capacity to track these metabolic changes provides insights into the early stages of DN, potentially before conventional biomarkers, such as the eGFR and albuminuria indicate significant kidney damage (22).

Aspects of metabolite applications in DN. Metabolomic analysis can improve the management of DN via diagnosis at an early stage and prediction of disease severity.

Early biomarker identification. Metabolomics has been used to assess subtle biochemical changes that precede the clinical manifestations of diseases such as DN. In the FinnDiane study (23), the use of mass spectrometry-based metabolomics allowed researchers to identify distinct profiles of metabolites in patients with type 1 diabetes mellitus (T1DM) who later progressed to different stages of albuminuria, a key marker of diabetic kidney disease. Notably, the detection of variations in acyl-carnitines and acyl-glycines indicated that the mitochondrial dysfunction and oxidative stress pathways may have an important role in the development of DN. Moreover, a study by Balint *et al* (24) reported the use of targeted metabolomic analysis in identifying early-stage biomarkers for DKD. This study reported significant variations in these metabolites, demonstrating the biochemical changes associated with early DN. Notably, this previous study was a quantitative and targeted analysis of metabolites derived from gut microbes, and the inclusion of gut microbiota-derived

metabolites provides a novel perspective in understanding the pathophysiology of DKD.

Prediction of disease severity. Niewczas *et al* (25) reported that the levels of specific uremic solutes and essential amino acids were altered in patients progressing to ESRD. Increased uremic solutes, which are typically products of protein catabolism and are excreted by the kidneys in healthy patients, may accumulate due to decreased kidney function. Therefore, the levels of uremic solutes and essential amino acids can indicate the severity of kidney damage. Moreover, Trifonova *et al* (5) identified certain metabolites that are related to amino acid metabolism as potential biomarkers for the late stages of DN. This suggests that changes in these metabolites might not only be applicable in diagnosing DN but also in stratifying the risk levels among diabetic patients, potentially guiding treatment decisions.

Prediction risk of end-stage kidney disease (ESKD) or associated mortality. Sharma *et al* (26) reported an association between elevated urinary adenine levels and an increased risk of both ESKD and mortality among patients with diabetes. This association was higher in patients who had not yet developed macroalbuminuria. Urinary adenine was significantly elevated in patients with diabetes at high risk of kidney failure and all-cause mortality at all levels of albuminuria, suggesting that adenine could potentially serve as an early detection marker for kidney damage. This study used advanced spatial metabolomics (a mass spectrometry imaging technique for identifying spatial information of metabolites on the basis of qualitative and quantitative metabolites) and single-cell transcriptomics to link adenine to specific kidney pathologies, which involved the mTOR pathway in adenine-induced kidney injury. This multi-omics approach demonstrated the potential of metabolomics to facilitate early diagnosis and predict disease severity with the potential to inform therapeutic interventions. This work demonstrates the role of metabolomics in the medical field.

Technological advancements in metabolomics. The field of metabolomics has had notable technological advancements in recent years, increasing its capacity to detect and analyze metabolites with greater accuracy and sensitivity. These advancements are required for the identification of subtle metabolic changes associated with early-stage DN.

High-resolution spectroscopic techniques, such as mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are primary technologies used in metabolomics. NMR is a non-destructive technique that allows for the direct detection of metabolites in biological samples without the need for complex sample preparation (27). Recent advances in NMR technology, including higher magnetic field strengths and improved detection sensitivity, have increased its use in metabolomics. These improvements enable researchers to detect a broader range of metabolites, and obtain more detailed information about their concentrations and structures, rendering NMR an invaluable tool for metabolic profiling in DN research.

There have also been improvements in MS, particularly in terms of resolution and sensitivity, leading to a marked improvement in its analytical capabilities. Gas chromatography-MS (GC-MS) is valued for its efficiency,

reproducibility and widespread use. GC-MS is used in the analysis of complex samples by combining the robust and selective nature of GC with the detection capabilities of MS, supported by metabolite databases (28). Liquid chromatography-MS (LC-MS) is another pivotal technique, merging the separation capabilities of LC with the detection potential of MS. This combination is used to identify and quantify a wide range of substances in a number of matrices, particularly in clinical settings (29). Capillary electrophoresis-MS is a tool in metabolomics used for profiling polar and charged metabolites. Advancements in the interfacing techniques, metabolic coverage and profiling in volume-restricted samples has increased its applicability (30). Lastly, liquid chromatography-tandem MS (LC-MS/MS) further improves upon LC-MS by offering increased selectivity and sensitivity. It is particularly effective in separating analytes from matrix components, thus enhancing precision and reducing interference in complex sample analyses (29).

Metabolomics can be broadly categorized into targeted and untargeted approaches. While the targeted approach focuses on analyzing specific metabolites of interest, untargeted metabolomics provides a comprehensive profile of all detectable metabolites in a sample, which can be particularly useful in discovering novel biomarkers for DN (31,32).

Furthermore, the integration of computational and bioinformatics tools can be used in metabolomics. A large amount of complex data are generated in metabolomic studies, which are analyzed by algorithms and software, facilitating the identification of patterns and correlations between specific metabolites and DN. These computational tools also enable the integration of metabolomic data with other omics data, including genomics, proteomics and transcriptomics, providing a more comprehensive understanding of the molecular mechanisms underlying DN.

The development and maintenance of a comprehensive, high-quality kidney tissue reference database is crucial for expanding the use and clinical integration of multiomics kidney tissue data (33). Databases, such as the Human Metabolome Database, provide an extensive repository of information on human metabolites, including their chemical structures, biological functions and links to diseases. Access to this comprehensive data is used to identify potential biomarkers to assess the metabolic pathways involved in DN. Moreover, The Kidney Precision Medicine Project was established with the goal of creating a comprehensive kidney atlas reference (34). This atlas aims to provide a detailed and consolidated resource that includes clinical information, histopathological data, biomarkers from blood and urine samples and proteomics and metabolomics data from kidney tissue. Furthermore, integrating artificial intelligence and machine learning with metabolomics data may improve data analysis and interpretation, potentially providing novel insights into the pathogenesis and progression of the disease (34).

In summary, the technological advancements in metabolomics have increased its role as a tool in DN research. These technologies enable the detection and quantification of a wide array of metabolites and provide insights into the complex metabolic networks in diseases. Advances in these technologies may allow early diagnosis and personalized treatment of DN.

Potential biomarkers of DN uncovered through the application of metabolomics. The search for reliable biomarkers for the early detection of DN and monitoring of its progression has led to a growing interest in metabolites as potential indicators. With increased understanding of DN, metabolomics has emerged as a promising tool, providing insight into the molecular mechanism of the disease. A number of metabolites identified as potential biomarkers for DN are discussed in this review, drawing on recent advancements in the field.

One of the most notable developments in this area is the identification of urinary and plasma metabolites as potential biomarkers for DN. A previous study using non-targeted metabolomics and machine learning identified potential urinary biomarkers (35). In the logistic analysis conducted for patients with rapid eGFR decline using the urinary metabolome, the odds ratio of C₀₀₃₈, identified as 1-methylpyridin-1-ium (NMP), was 0.1 ($P=0.0003$). Moreover, the odds ratio for trigonelline was determined to be 0.5 ($P=0.0100$). The area under the curve (AUC) was used to assess the discriminative ability of the diagnostic model. Urinary NMP was considered a potential biomarker and its absence could predict the progression of DN since it was revealed to be reduced in patients with rapid decline of eGFR in accordance with the results of the piecewise linear models (AUC urine=0.850). Urinary trigonelline also exhibited good discriminatory ability in handcrafted logistic regression models (mean AUC urine=0.857) (35). Machine learning approaches suggested the roles of these potential biomarkers in advancing prognostic and treatment strategies for DN. These findings highlight the use of advanced analytical and computational techniques in identifying novel biomarkers for DN.

The metabolomics approach has also led to the discovery of numerous small molecule biomarkers of DN, aimed at improving the specificity and sensitivity of diagnosis, and uncovering biochemical mechanisms underlying the cause and progression of the disease. Among these, urinary biomarkers such as acyl-carnitines, acyl-glycines and metabolites related to tryptophan metabolism have been identified with an accuracy of 75% and a precision of 73%, based on a 5.5-year follow-up period in a cohort study (23). This suggests the potential of these metabolites in differentiating between progressive and non-progressive forms of albuminuria in humans.

In addition to urinary metabolites, plasma metabolites have been assessed for their potential as DN biomarkers in patients with T2DM and T1DM. For example, Yoshioka *et al* (36) evaluated plasma and urine samples from 150 patients with DKD using LC-MS and identified saturated fatty acids, such as palmitic (16:0) and stearic (18:0) acids, indicating a shift in lipid metabolism in patients with DN. Similarly, Sharma *et al* (26) assessed 904 patients with T2DM using LC-MS/MS and reported notable differences in metabolites, including adenine, asparagine and isoleucine in urine samples, suggesting that these could be potential markers for DN. Moreover, Vigers *et al* (37) assessed serum samples from 50 patients with T1DM and 20 controls, identifying metabolites such as glycine, histidine and phenylalanine using LC-MS, providing insights into amino acid and energy metabolism alterations in DN. Balint *et al* (24) identified several metabolites in serum and urine as potential biomarkers for

early DKD through untargeted ultra-high-performance liquid chromatography coupled with electrospray ionization-quadrupole-time of flight-MS techniques. Among these, metabolites such as arginine (AUC serum=0.5; AUC urine=0.7); hippuric acid (AUC serum=0.7); and p-cresyl sulfate (AUC urine=0.8), demonstrated promising results. Moreover, Trifonova *et al* (5) evaluated the use of untargeted metabolomics to identify biomarkers for the diagnosis of DN in patients with T1DM. The most robust diagnostic performance for distinguishing between patients with T1DM with and without late-stage DN was achieved by a combination of 12 metabolites with an AUC of 0.99, highlighting their potential use in clinical settings for the effective identification of DN, potentially reducing the progression to kidney failure.

The aforementioned examples represent only a portion of the expanding literature focused on metabolomics research dedicated to DN. A summary of relevant studies published since 2011 is presented in Table I (5,23-26,35-52). These research articles offer a detailed survey, where each study contributes distinct insight into the altered metabolic pathways associated with DN. Using advanced analytical technologies, including GC-MS, LC-MS and NMR, researchers have reported a wide array of potential biomarkers, ranging from specific amino acids and lipids to intricate profiles of urinary and plasma metabolites. These findings increase the current understanding of DN, and broaden the scope of potential diagnostic and therapeutic strategies. The collective research underscores the potential of metabolomics to reveal complex metabolic changes, providing insight that surpasses traditional biomarkers and demonstrating how molecular-level analysis can improve management and treatment of chronic diseases such as DN.

5. Metabolomics pathway analysis in DN metabolomics research

Metabolomics pathway analysis is a useful method to further understand the mechanism underlying the development of DN. This analysis involves evaluating the complex biochemical interactions and pathways in an organism to understand the effects on the cellular biochemistry of a number of conditions, including diseases or drug treatments. This analysis identifies which groups of molecules performing specific biological functions are involved in these conditions, with the aim of mapping identified metabolites to known metabolic pathways, thus enabling the interpretation of complex metabolomics data in a biologically relevant context (53).

The analysis of serum and urine metabolomics in patients with DN demonstrates notable disruptions in amino acids, lipids and energy metabolism, alongside increased oxidative stress. These metabolic alterations provide insights into the pathophysiological mechanisms underlying DN, and highlight potential biomarkers and therapeutic targets for early diagnosis and intervention.

Amino acid metabolism. Amino sugar metabolism, fructose and mannose degradation, gluconeogenesis, glycolysis and the pentose phosphate pathway (PPP) are altered in patients with DN, indicating there is an obstacle to the catabolism of glucose (54).

Lipid metabolism and energy metabolism. Fatty acid metabolism is also altered in DN, leading to the accumulation of toxic lipid intermediates that contribute to nerve damage and inflammation (55). Disruptions in glycerolipid metabolism, which are essential for cell membrane integrity and nerve signal transmission have also been reported (56). This may also exacerbate neuropathy symptoms in patients with diabetes. This shift results in inefficient energy production and increased oxidative stress (56). Moreover, the tricarboxylic acid cycle, which is essential for ATP production is impaired, limiting energy availability for nerve function and increasing the production of reactive oxygen species, contributing to nerve damage (55).

Oxidative stress and antioxidant defense. Changes in glutathione metabolism, a major antioxidant in the body, have been reported in previous studies (55,56). Reduced glutathione levels lead to increased oxidative stress, damaging nerve cells and contributing to the progression of DN (56). Bile acid biosynthesis, taurine and hypotaurine metabolism were also reported to be dysregulated, with decreased taurine levels compromising the antioxidant defense system and exacerbating oxidative nerve damage (56).

Other notable pathways. Changes in the PPP have also been reported. The PPP generates NADPH for reductive biosynthesis and for maintaining glutathione in its reduced form. Impairments in this pathway contribute to oxidative stress and neuronal damage in DN (56).

Database of metabolomics pathways. There are a number of resources available for mapping metabolites to biochemical pathways. Some of the currently available resources include the BioCyc database collection (57), the Kyoto Encyclopedia of Genes and Genomes pathway database (58), MetaboAnalyst (59), the Small Molecule Pathway Database (60) and Recon3D (61). Using these resources, researchers can identify and use metabolites to influence phenotypes, providing new avenues for the understanding and treatment of DN.

6. Challenges and future directions

Challenges and opportunities. The integration of metabolomics into DN research holds promise, despite challenges that need addressing to enhance its practical and effective use. In the field of metabolomics, a major challenge arises from the vast diversity and dynamic range of metabolite concentrations present in biological samples, which are influenced by a number of factors. One factor is genetics, as this determines the heritability and levels of specific metabolites. The gut microbiome is another major factor, which determines a substantial portion of the variance in metabolite levels across individuals. Dietary intake also impacts metabolite concentrations, with a study noting that specific dietary intakes, such as leafy green vegetables or sugar-sweetened beverages, can lead to noticeable changes in metabolite profiles (62). Likewise, lifestyle choices such as smoking have been reported to influence metabolite levels (63). Circadian rhythms are endogenously generated but can be adjusted by external cues, such as light, thereby

Table I. Metabolites identified in metabolomics studies of serum, plasma and urine of diabetic patients with diabetic nephropathy.

First author, year	Study population	Sample source	Analysis platform	Metabolites	(Refs.)
Van der Kloet <i>et al</i> , 2012	52 patients with T1DM	Urine	GC-MS; LC-MS	Predicting progressive and non-progressive DKD: Acyl-carnitines ↑, acyl-glycines ↑ and metabolites related to tryptophan metabolism ↑	(23)
Niewczas <i>et al</i> , 2014	40 progressors to ESRD; 40 controls	Plasma	MS	Diagnosing late stages of DN: Putative uremic solutes ↑ and essential amino acids ↓	(25)
Lee <i>et al</i> , 2016	56 controls; Group 1: 39 patients with non-DM CKD and 66 patients with DM; Group 2: 46 patients with non-DM CKD and 66 patients with DM; Group 3: 30 patients with non-DM CKD and 45 patients with DM	Serum	NMR	Diagnosing CKD: Trimethylamine oxide ↑, Cr ↑, urea ↑ and glucose ↑; arginine ↓, leucine ↓, valine ↓, glutamine ↓, tyrosine ↓, pyruvate ↓, citrate ↓, acetate and formate ↓ Distinguishing between DM-CKD and non-DM CKD: formate ↑, tyrosine ↑, VLDL/LDL CH3 ↑, valine ↑, lactate ↑, glucose ↑, leucine ↑ and choline ↑; acetate ↓, arginine ↓, urea ↓, Cr ↓, pyruvate ↓, N-acetyl-glycoprotein ↓ and citrate ↓	(38)
Niewczas <i>et al</i> , 2017	158 patients with T1DM	Serum	GC-MS; LC-MS	Diagnosing ESRD: C-glycosyltryptophan ↑, pseudouridine ↑, O-sulfotyrosine ↑, N-acetylthreonine ↑, N-acetylserine ↑, N6-carbamoylthreonyladenosine ↑ and N6-acetyllysine ↑	(39)
Pena <i>et al</i> , 2017	150 patients with T2DM	Urine	GC-MS	Predicting eGFR decline in DKD: Aconitic acid ↓, citric acid ↓, glycolic acid ↓, homovanillic acid ↓, 2-ET-3-OH-propionate ↓, 3-OH-isobutyrate ↓, 3-OH-isovalerate ↓, 2-3-OH-propionate ↓ and uracil ↓	(40)
Liu <i>et al</i> , 2017	Patients with T2DM: 126 with early DKD, 154 with overt DKD and 129 with no DKD	Serum	GC-MS; LC-MS	Diagnosing DKD: 5 phosphatidylcholine ↑, short dicarboxylacylcarnitine subfamily ↑, sphingomyelin ↑, ceramide ↑, glucosylceramide ↑ and four sphingomyelin-ceramide metabolites ↓ (sphingomyelin 18:1/16:1, ceramide 18:1/16:0, glucosylceramide 18:1/18:0 and sphingosine)	(41)
Saulnier <i>et al</i> , 2018	62 patients with T2DM	Urine	GC-MS	Diagnosing the early stage of DKD: 2-ethyl-3-hydroxy propionate ↓, aconitic acid ↓, 3-hydroxy propionate ↓, 3-hydroxy isobutyrate ↓, homovanillic acid ↓, uracil ↓, tiglylglycine ↓, 3-methyl-crotonyl glycine ↓, 3-methyl adipic acid ↓, citric acid ↓, 3-hydroxy isovalerate ↓ and glycolic acid ↓	(42)

Table I. Continued.

First author, year	Study population	Sample source	Analysis platform	Metabolites	(Refs.)
Colombo <i>et al</i> , 2019	1,174 patients with T1DM	Serum	LC-MS	Predicting DN progression in T1DM: CD27 antigen ↑, KIM-1 ↑ and α1- microglobulin ↑	(43)
Devi <i>et al</i> , 2019	31 patients with T2DM- DN; 29 patients with T2DM; 30 patients with NGT	Serum	LC-MS	Diagnosing DN: (βS)-β-hydroxy-L-tryptophan ↑, (R)-3-hydroxybutyrylcarnitine ↑, 2- amino-9,10-epoxy-8-oxodecanoic acid ↑, 6-oxo-2-piperidinecarboxylic acid ↑, anagestone acetate ↑, apronalide ↑, DL-dipalmitoylphosphatidylcholine ↑, imidazolelactate ↑, N-acetylmethionine ↑, tetrahydrocorticosterone ↑, trigonelline ↑; cholecalciferol ↓, diosgenin ↓, lauramide ↓, primobolan ↓ and xanthoaphin ↓	(44)
Feng <i>et al</i> , 2020	95 patients with T2DM	Urine	LC-MS	Distinguishing between ADKD and NADKD subjects: Linoleic acid ↑, γ-linolenic acid ↑, L-malic acid ↑; L-proline ↓, L- erythro-4-hydroxyglutamate ↓, N- carbamoylputrescine ↓ and spermidine ↓	(45)
Winther <i>et al</i> , 2020	161 patients with T1DM; 50 control patients	Serum	LC-MS	Diagnosing T1DM: Dimethylarginine ↑, α-hydroxybutyrate ↑, β-hydroxybutyrate ↑, N-methylnicotinamide ↑; alanine ↓, L-citrulline ↓, leucine ↓, phenylalanine ↓ and tryptophan ↓ Distinguishing subjects with macroalbuminuria or normo- or microalbuminuria: Indoxyl sulphate ↑, L-citrulline ↑, homocitrulline ↑, L-kynurenine ↑ and tryptophan ↓	(46)
Tan <i>et al</i> , 2021	30 patients with T2DM- N; 30 patients with T2DM-DKD; 30 control patients	Serum	LC-MS	Diagnosing the early stage of DN: Glutamine ↑, phenylacetylglutamine ↑, 3-indoxyl sulfate ↑, acetylphenylalanine ↑, xanthine ↑, dimethyluric acid ↑ and asymmetric dimethylarginine ↑	(47)
Fernandes Silva <i>et al</i> , 2021	8,661 Finnish men without diabetes from the METSIM cohort	Serum	LC-MS	Assessing urinary albumin excretion rate: Xanthurenate ↑, indolelactate ↑, kynurenate ↑, N-acetyltryptophan ↑, N-acetylkynurenine ↑, N- acetylphenylalanine ↑, γ- glutamylphenylalanine ↑, phenyllactate ↑, phenylpyruvate ↑, 3-(4-hydroxyphenyl) lactate ↑, stearidonate (18:4n3) ↑, maleate ↑, pantothenate ↑, linolenate ↑ and palmitoyl-linoleoyl-glycerol (16:0/18:2) ↑	(48)

Table I. Continued.

First author, year	Study population	Sample source	Analysis platform	Metabolites	(Refs.)
Mutter <i>et al</i> , 2022	2,670 patients with T1DM	Urine	NMR	Predicting overall progression of DN: Leucine ↑, valine ↑, isoleucine ↑, pseudouridine ↑, threonine ↑, citrate ↑, 2-hydroxyisobutyrate ↑ and pyroglutamate ↑	(49)
Lecamwasam <i>et al</i> , 2022	119 patients with T2DM-DKD	Serum and urine	NMR	Diagnosing late stage of DKD: Cr ↑, apolipoprotein B/apolipoprotein A1 ↑, LDL- TG ↑, S-VLDL-C ↑, XS-VLDL- TG ↑, IDL-TG ↑; valine ↓, apolipoprotein A1 ↓, HDL-C ↓, HDL2-C ↓ and M-HDL-C ↓	(50)
Peng <i>et al</i> , 2022	60 patients with DKD; 23 controls	Serum	LC-MS	Diagnosing late stage of DKD: L-homocysteine ↑, 3- sulfinylpyruvate ↑, 2,3-diketo-5- methylthiopentyl-1-phosphate ↑, L- cysteine ↑, s-adenosyl-L- methionine ↑, smethyl-5-thio-D- ribose 1-phosphate ↑, Asn-Met- Cys-Ser ↑, Asn-Cys-Pro-Pro ↑ and mercaptopyruvate ↓	(51)
Vigers <i>et al</i> , 2023	50 patients with T1DM; 20 controls	Serum	LC-MS	Diagnosing early kidney dysfunction of T1DM: Glycine ↓, histidine ↓, methionine ↓, phenylalanine ↓, serine ↓, threonine ↓, citrate ↓, fumarate ↓ and malate ↓	(37)
Lucio-Gutiérrez <i>et al</i> , 2022	6 patients with T2DM; 13 patients with mild DKD; 10 patients with moderate DKD; 14 patients with severe DKD; 17 controls	Serum and urine	NMR	Predicting progression of DKD: Trigonelline ↑, hippurate ↑, phenylalanine ↑, glycolate ↑, dimethylamine ↑, alanine ↑, 2- hydroxybutyrate ↑, lactate ↑ and citrate ↑	(52)
Yoshioka <i>et al</i> , 2022	150 patients with DKD	Plasma and urine	LC-MS	Predicting fast progression of DKD: Saturated fatty acids palmitic (16:0) ↑ and stearic (18:0) acids ↑	(36)
Hirakawa <i>et al</i> , 2022	46 patients with UT-DKD with eGFR change rate >0%/year; 34 patients with UT- DKD with eGFR change rate <0 and >-3.3%/year; 39 patients with UT-DKD with eGFR change rate <-3.3 and >-10%/year; 14 patients with UT- DKD with eGFR change rate <-10%/year	Plasma and urine	CE-MS/MS	Predicting progression of DKD: Plasma kynurenine ↑, plasma gluconolactone (gluconate) ↑, urinary threonic acid ↑, urinary 1-palmitoyl-glycero-3- phosphocholine ↑, urinary sphingomyelin (d18:1/16:0) ↑; urinary trigonelline ↓ and urinary 1-methylpyridin-1-ium ↓	(35)

Table I. Continued.

First author, year	Study population	Sample source	Analysis platform	Metabolites	(Refs.)
Trifonova <i>et al</i> , 2022	80 patients with T1DM	Plasma	GC-LC	Diagnosing both early- and late stages of DN: Cr ↑, L-proline ↑, L-cysteine ↑, 1-methylhistidine ↑, L-arginine ↑, citrulline ↑, oxalosuccinic acid ↑, N-acetyl-b-glucosaminylamine ↑, N2-succinyl-L-ornithine ↑, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid ↑ and creatine ↓ Diagnosing the early stage of DN: Cr ↑; L-proline ↑, L-cysteine ↑, N-formyl-L-aspartate ↑, 1-methylhistidine ↑, oxalosuccinic acid ↑, N-acetyl-b-glucosaminylamine ↑, N2-succinyl-L-ornithine ↑ and 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid ↑ Diagnosing the late stage of DN: Cr ↑, L-cysteine ↑, thiocysteine ↑, 4-guanidinobutanamide ↑, L-proline ↑, 1-methylhistidine ↑, 2-oxo-3-hydroxy-4-phosphobutanoic ↑, citrulline ↑, oxalosuccinic acid ↑, N2-succinyl-L-ornithine ↑, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid ↑ and creatine ↓	(5)
Sharma <i>et al</i> , 2023	CRIC cohort: 904 patients with CKD; SMART2D cohort: 309 patients with T2DM	Urine	LC-MS/MS	Diagnosing ESKD: Adenine ↑, asparagine ↑, aspartic acid ↑, betaine ↑, isoleucine ↑, L.α.aminobutyric acid ↑, lysine ↑, ornithine ↑, phenylalanine ↑, pipecolate ↑, threonine ↑, tryptophan ↑ and valine ↑	(26)
Balint <i>et al</i> , 2023	90 with T2DM (normoalbuminuria: uACR <30 mg/g; microalbuminuria: uACR ≥30 and <300 mg/g; macroalbuminuria: uACR ≥300 mg/g); 20 controls	Serum and urine	Untargeted UHPLC- QTOF-ESI+-MS	Diagnosing the early stage of DKD: Serum arginine ↑, dimethylarginine ↑, hippuric acid ↑, indoxyl sulfate ↑, butenoylcarnitine ↑, sorbitol ↑ and urine p-cresyl sulfate ↑	(24)

LC, liquid chromatography; MS, mass spectrometry; UHPLC, ultra-high performance LC; QTOF, quadrupole time-of-flight; ESI, electrospray ionization process; uACR, urine albumin-to-Cr ratio; Cr, creatinine; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; NGT, normal glucose tolerance; T2DM-N, type 2 diabetes mellitus with normal renal function; CKD, chronic kidney disease; DKD, diabetic kidney disease; ADKD, albuminuric DKD; NADKD, normoalbuminuric DKD; NMR, nuclear magnetic resonance; SMART2D, Singapore Study of Macro-angiopathy and Micro-vascular Reactivity in Type 2 Diabetes; GC, gas chromatography; DN, diabetic nephropathy; eGFR, estimated glomerular filtration rate; CE-MS/MS, capillary electrophoresis tandem mass spectrometry; LC-MS/MS, liquid chromatography-tandem MS; UT-DKD, uncertain type diabetic kidney disease; LDL, low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoproteins; TG, triglyceride; S-VLDL-C, small very-low-density lipoprotein cholesterol; XS-VLDL-TG, very small very-low-density lipoprotein triglyceride; HDL-C, high-density lipoprotein cholesterol; M-HDL-C, medium high-density lipoprotein cholesterol; METSIM, Metabolic Syndrome in Men.

influencing metabolic processes (64). Furthermore, environmental exposure to persistent organic pollutants including polychlorinated biphenyls, dioxins and related compounds impacts human health. These pollutants accumulate in the body and can alter metabolic pathways involved in energy production, fatty acids and amino acids (65).

The analytical techniques themselves, particularly LC-MS/MS are robust, yet demand rigorous calibration and validation to maintain accuracy and reproducibility across studies. The sensitivity of these methods can vary depending on the specific metabolite and the matrix being analyzed, adding complexity to the interpretation of results in DN research (66,67). A previous study demonstrated that the metabolite profiles from plasma and serum are distinctly different, with 104 metabolites exhibiting higher concentrations in serum (66). Another primary limitation is the validation and refinement required for metabolomics biomarkers for early diagnosis to potentially outperform traditional markers, such as eGFR and albuminuria, which are often recognized only following significant kidney damage has occurred (5). Despite these challenges, the potential benefits of using metabolomics in DN research are substantial. Metabolomics can identify novel biomarkers that may enable earlier diagnosis and more accurate patient stratification. This, in turn, facilitates the development of targeted and personalized treatment strategies, potentially reducing long-term healthcare costs by preventing severe complications of DN.

Moreover, the impact of the gut microbiome on health and disease is attracting increasing attention, offering a distinct opportunity for metabolomics to assess the role of microbial metabolites in DN. This could identify novel therapeutic pathways. For example, a previous study reported elevated plasma levels of gut-derived solute derivatives of amino acids, such as phenol sulfate, indoleacetate and 3-indoxyl sulfate, in individuals progressing to ESRD. While these variations were not statistically significant, they imply that the gut microbiome may modulate the plasma levels of amino acid-derived uremic solutes, potentially escalating the risk of developing ESRD in patients with T2DM (25).

It is essential to note that further validation of the screened metabolomic biomarkers in larger, independent cohorts is necessary to establish the clinical use of these metabolomic biomarkers. As the field continues to advance, these biomarkers hold the promise of transforming the approach to managing DN, potentially improving the outcomes for patients affected by this condition. Thus, the initial investment in metabolomics could be offset by the potential long-term benefits in the management of DN.

Novelties and limitations of this review. The present review distinguishes itself from previously published reviews on metabolomics in DN by offering several unique contributions and perspectives that enhance the understanding and application of metabolomics in DN. Firstly, the present review specifically focuses on human studies published between 2011 and 2023, ensuring an up-to-date and concentrated analysis. This approach contrasts with previous reviews, such as Cordero-Pérez *et al* (68), which covered a broader range of topics, including numerous complications of diabetes beyond DN and did not necessarily include the most recent studies

published after 2020. Moreover, the present review extends the analysis by integrating a wide range of human studies, which allows for a comprehensive discussion of the application of metabolomics in DN.

Moreover, the present review assesses the technological advancements in metabolomics, such as high-resolution MS and NMR spectroscopy and discusses the improvements in computational tools for data analysis and integration. For example, the enhanced capabilities of NMR technology, including higher magnetic field strengths and improved detection sensitivity, were assessed in the present review, but not addressed in a previous review (69), which significantly increase its utility in metabolomics research. Furthermore, the present review provides insights into the metabolic pathways disrupted in DN, including amino acid, lipid and energy metabolism, and oxidative stress. By emphasizing the connection between identified metabolites and specific biochemical pathways, the present review offers a more granular understanding of the pathophysiology of DN, which is required for developing targeted interventions. This pathway analysis is a distinctive feature of the present review, compared with previous reviews that may not offer such a comprehensive examination of the molecular mechanisms underlying DN (70,71).

One limitation of the present review identifying candidate metabolites as potential biomarkers for DN. The identification of metabolites is crucial for advancing diagnostic and therapeutic strategies. However, comparing these metabolites based on their feasibility and clinical relevance requires consistent experimental and analytical conditions across numerous studies, which is beyond the scope of this review. Experimental studies included in the present review use different methodologies, sample types and patient populations, making direct comparisons challenging. This heterogeneity can lead to variability in the reported significance and use of specific metabolites. Therefore, while the present review provides a comprehensive overview of potential biomarkers, the lack of standardized conditions limits the ability to rank them definitively. Addressing this issue based on standardized methodologies in future research is crucial for enabling the accurate ranking and validation of potential biomarkers.

Future directions. Incorporating metabolomics into the diagnosis of DN may initially increase medical costs due to the steps involved, such as sample preparation, data acquisition and data analysis. These steps are more costly than traditional diagnostic methods, such as serum creatinine measurements or urine tests for albumin. The metabolomics workflow also requires consumables, equipment maintenance and software for data processing, all of which contribute to higher diagnostic costs. For instance, the current GC-MS method for very long chain fatty acid analysis is known to be laborious, time-consuming and expensive to perform (72). However, efforts are being made to reduce these costs. By developing characteristic biomarkers into kits, the overall cost of metabolomics can be lowered. These kits make the process more affordable and convenient for clinical application (73). This advancement could make metabolomics a more viable option for widespread use in diagnosing DN, balancing the initial high costs with long-term benefits and improved patient outcomes.

In the future, advancements are anticipated in the field of metabolomics for DN research, with an increase in sensitivity, specificity and throughput. These improvements will enable the detection of subtle metabolic changes that occur in the early stages of DN. Moreover, integrating metabolomics data with other omics data, including genomics, proteomics, and transcriptomics, and using computational tools will provide a more comprehensive understanding of the molecular mechanisms underlying DN.

Future research should also focus on validating metabolomic biomarkers in larger, independent cohorts to establish their clinical use. Assessing the impact of the gut microbiome on DN through metabolomics could identify novel therapeutic pathways and increase the understanding of disease progression. Moreover, the development of less expensive and more efficient analytical techniques will be essential for the widespread clinical adoption of metabolomics.

While challenges remain, the future of metabolomics in DN research is promising, with the potential to transform the understanding and management of this disease. By overcoming current limitations and advancing technological capabilities, metabolomics could improve early detection, enable timely interventions and reduce the healthcare burdens associated with DN.

7. Conclusions

The integration of metabolomics into DN research offers a promising avenue for increasing the understanding and management of DN. Metabolomics provides a comprehensive view of metabolic alterations at the molecular level, potentially identifying novel biomarkers that can predict disease onset and progression well before traditional clinical signs manifest. This could lead to earlier interventions and more personalized treatment strategies, ultimately improving patient outcomes. The ability of metabolomics to identify subtle changes in metabolic profiles holds significant potential to improve DN diagnosis. However, the practical application of metabolomics in DN faces several challenges, including the need for extensive validation, the high costs and the variability in metabolite profiles due to genetic and environmental factors. Future research should focus on addressing these limitations, improving the affordability and accessibility of metabolomic analyses and integrating these insights with other clinical data to increase diagnostic accuracy and treatment efficacy. Addressing these challenges is required for integrating metabolomics into routine clinical practice and for realizing its full potential in improving the outcomes of patients with DN.

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Competing interests

The authors declare that they have no competing interests.

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