

# Lipidomics: New Insight Into Kidney Disease

Ying-Yong Zhao<sup>\*,†,1</sup>, Nosratola D. Vaziri<sup>†</sup>, Rui-Chao Lin<sup>‡</sup>

<sup>\*</sup>Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education, The College of Life Sciences, Northwest University, Xi'an, Shaanxi, PR China

<sup>†</sup>Division of Nephrology and Hypertension, School of Medicine, University of California, Irvine, California, USA

<sup>‡</sup>School of Chinese Materia Medica, Beijing University of Chinese Medicine, Beijing, PR China

<sup>1</sup>Corresponding author: e-mail address: zhaoyybr@163.com; zyy@nwu.edu.cn

## Contents

1. Introduction	150
2. Kidney Disease	151
3. Lipids and Lipidomics	152
3.1 Definition, classification, and biologic function of lipids	152
3.2 Lipidomics	154
3.3 Analytical methods for lipidomics	155
3.4 Analysis of lipidomics data	157
4. Applications of Lipidomics in Kidney Diseases	157
4.1 Lipidomics in clinical kidney disease	158
4.2 Lipidomics in animal models or cell models	160
5. Concluding Remarks and Perspectives	162
Acknowledgments	163
References	163

## Abstract

Due to the incidence of type-2 diabetes and hypertension, chronic kidney disease (CKD) has emerged as a major public health problem worldwide. CKD results in premature death from accelerated cardiovascular disease and various other complications. Early detection, careful monitoring of renal function, and response to therapeutic intervention are critical for prevention of CKD progression and its complications. Unfortunately, traditional biomarkers of renal function are insufficiently sensitive or specific to detect early stages of disease when therapeutic intervention is most effective. Therefore, more sensitive biomarkers of kidney disease are needed for early diagnosis, monitoring, and effective treatment. CKD results in profound changes in lipid and lipoprotein metabolism that, in turn, contribute to progression of CKD and its cardiovascular complications. Lipids and lipid-derived metabolites play diverse and critically important roles in the structure and function of cells, tissues, and biofluids. Lipidomics is a branch of metabolomics, which encompasses the global study of lipids and their biologic function

in health and disease including identification of biomarkers for diagnosis, prognosis, prevention, and therapeutic response for various diseases. This review summarizes recent developments in lipidomics and its application to various kidney diseases including chronic glomerulonephritis, IgA nephropathy, chronic renal failure, renal cell carcinoma, diabetic nephropathy, and acute renal failure in clinical and experimental research. Analytical technologies, data analysis, as well as currently known metabolic biomarkers of kidney diseases are addressed. Future perspectives and potential limitations of lipidomics are discussed.



## 1. INTRODUCTION

Due to the incidence of type-2 diabetes and hypertension, chronic kidney disease (CKD) has emerged as a major public health issue worldwide. CKD results in disability and premature death from accelerated cardiovascular disease and its attendant complications [1]. Numerous pathologic conditions include genetic, metabolic, toxic, immunologic, infectious, hemodynamic, mechanical, and other disorders that lead to development and progression of kidney disease. Early detection, careful monitoring of renal function, and response to therapeutic intervention are critical for timely diagnosis and prevention of progression of CKD and its complications. Unfortunately, traditional markers of renal function are insufficiently sensitive or specific to detect CKD and its cardiovascular or other complications at an early stage when therapeutic intervention is most effective. For example, the most commonly used biomarkers, i.e., serum creatinine and urea and creatinine clearance, are heavily influenced by factors independent of intrinsic kidney function and structure. In this context, muscle mass significantly affects creatinine, protein intake and fluid balance modulates urea, and use of angiotensin converting enzyme inhibitors or angiotensin receptor blockers as well as dietary protein intake affects creatinine clearance. It is, therefore, necessary to develop sensitive and specific biomarkers for early detection of kidney disease and monitoring of its progression and response to therapeutic intervention. Insight into the dynamic differences in genetic, protein and metabolite regulation, interaction, and function in kidney diseases might identify new diagnostic and prognostic biomarkers and therapeutic targets [2–4].

CKD results in profound changes in lipid and lipoprotein metabolism [5–7]. The associated lipid disorders, in turn, contribute to progression of CKD and its cardiovascular and other complications [8–10]. Lipidomics,

the global study of lipids within cells, tissues, and biofluids, involves the analysis of lipid species and their abundance to elucidate biologic function, subcellular localization, and tissue distribution. Small molecular weight lipids such as fatty acids, glycerolipids, glycerophospholipids (GPs), and sphingolipids serve diverse and complex functions in health and disease. They play important roles in the regulation of normal kidney function and pathogenesis of kidney disease. Previous studies have shown significantly increased glomerular cyclooxygenase-1 or -2 expression in patient and animal models of glomerulonephritis [11–13] and upregulation of glomerular cyclooxygenase-2 expression in patient and animal models of lupus nephritis [13,14]. Cyclooxygenase inhibition has been shown to ameliorate passive Heymann nephritis and lupus nephritis in experimental animals [14–16]. Leukotrienes, associated with inflammatory glomerular injury and lipoxygenase product (12-hydroxyeicosatetraenoic acid), mediated angiotensin II and transforming growth factor- $\beta$ -induced mesangial expansion in diabetic nephropathy (DN) [17]. 20-Hydroxyeicosatetraenoic and epoxyeicosatrienoic acids were involved in several forms of kidney injury, including renal injury in metabolic syndrome [18–20] and ceramides have been shown to play a role in the pathogenesis of acute kidney injury. Taken together there is mounting evidence supporting the role of lipids and lipid-derived metabolites in the pathogenesis of kidney disease. Thus, analysis of key lipid mediators has emerged as an important tool in the diagnosis, prognosis, and treatment of kidney disease.

This article reviews recent advances in the use of lipidomics in elucidating the pathogenesis and potential treatment of kidney disease.



---

## 2. KIDNEY DISEASE

Systems biology allows on-time analysis of regulatory and biologic networks in cell metabolism [21–23]. Comprehensive characterization of renal diseases could provide important and integrative information to better characterize molecular relationships underlying this pathophysiology in order to develop more reliable and specific markers for diagnosis, prognosis, prevention, and therapeutic response [2,24]. The growth of systems biology and development of new experimental and computational tools have enabled the connection of gene–cell–organ regulatory mechanisms at multiple levels to integrate the molecular and cell biology of kidney structure and function [25–29]. Lipids play diverse and important roles in biologic systems including membrane bilayer structure, energy storage, signal

transduction, and also provide functional support for membrane proteins and their interactions [30]. For example, arachidonic acid is the precursor of eicosanoids, which act as signaling molecules via specific receptors leading to inflammatory processes [31]. Triacylglycerides serve as cellular energy storage and play an important role in metabolism and disease [32]. Some lipid species, i.e., lysophosphatidylcholines (LPC), glycerophosphoethanolamines (PE), phosphatidylcholines (PC), and glycerophosphoinositols (PI), appear to be potential kidney disease markers [33]. Here, we provide an overview of the lipidomic approach in kidney disease.

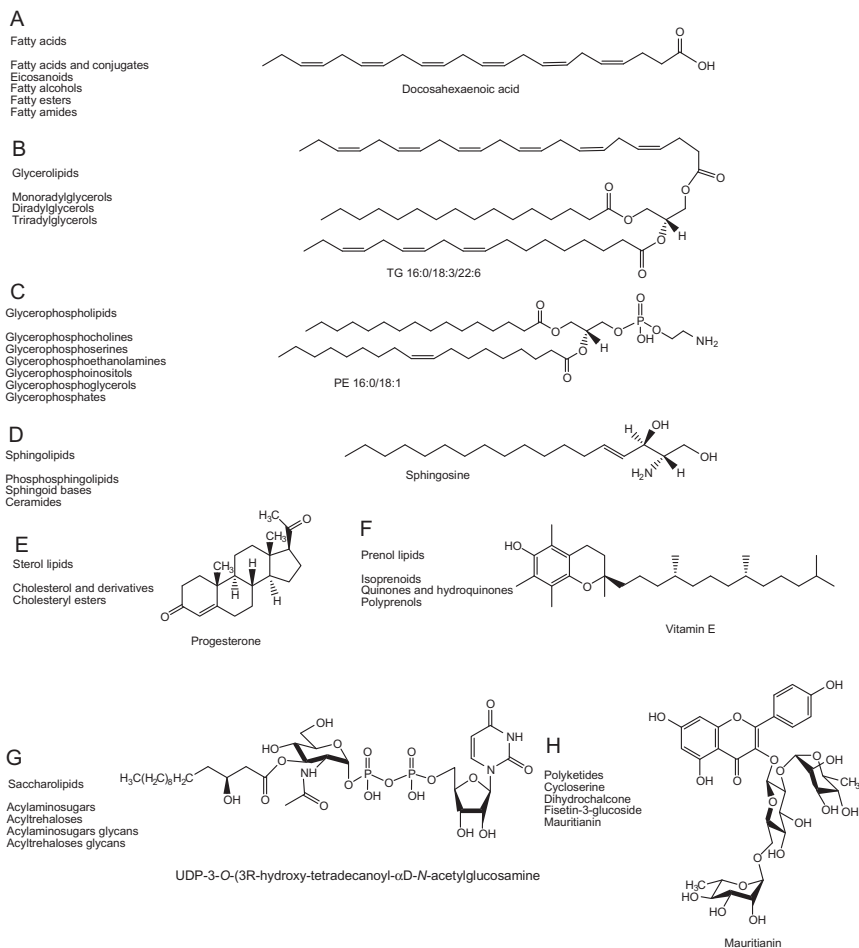


### 3. LIPIDS AND LIPIDOMICS

#### 3.1. Definition, classification, and biologic function of lipids

Lipids, the fundamental components of biologic membranes, are a structurally and functionally diverse class of molecules. Depending on biosynthesis and chemical structure, lipids are defined as hydrophobic or amphiphilic. Amphiphilic lipids exist in vesicles, membranes, or liposomes in an aqueous environment. Biologic lipids originate two distinct types of biochemical subunits: isoprene and ketoacyl groups [34]. Based on this definition, lipids can be divided into eight categories: fatty acids, glycerolipids, sphingolipids, GPs, saccharolipids, sterol lipids, prenol lipids, and polyketides (Fig. 1) [34]. Fatty acids and glycerolipids have relatively simple structure. Fatty acids are one of the most important lipid classes and basic component of all lipids. Fatty acids have saturated or unsaturated straight-carbon chains with lengths of 4–24 carbon atoms and 0–6 double bonds. Fatty acids are precursors of various bioactive lipids. Eicosanoids include leukotrienes, prostaglandins, and thromboxanes which play important roles in the development of inflammatory processes [35]. Glycerolipids are composed of mono-, di-, and tri-substituted glycerols that differ in fatty acid content esterified to the hydroxyl groups of the glycerol backbone [36]. A variety of studies have shown that altered triglyceride synthesis and catabolism play important roles in the occurrence and development of many diseases [37,38]. Sterol lipids, including cholesterol and their derivatives composed of a fused four-ring core structure, are important components of membrane lipids. Sterol lipids have different biologic roles such as regulatory function of cell signaling and modulation of cellular fluid [39].

GP, also known as phospholipids, are ubiquitous in nature and are important components of lipid bilayers and are involved with cell signaling



**Figure 1** Common lipid classes and representative lipid molecular species. (A) Fatty acid: docosahexaenoic acid; (B) glycerolipid: TG(16:0/18:3/22:6); (C) glycerophospholipid: PE(16:0/18:1); (D) sphingolipid: sphingosine; (E) sterol lipid: progesterone; (F) prenel lipid: vitamin E; (G) saccharolipid: UDP-3-O-(3R-hydroxy-tetradecanoyl)- $\alpha$ -D-N-acetylglucosamine; and (H) polyketide: mauritianin.

and metabolism. Based on the nature of the polar head group at the *sn*-3 position of the glycerol backbone in eukaryotes and eubacteria, or the *sn*-1 position in the case of archaeobacteria [40], GP may be subdivided into distinct classes including glycerophosphocholines, glycerophosphatidic acids, glycerophosphoglycerols (PG), glycerophosphoserines (PS), PE, and PI. Brain tissue contains relatively high G, and alterations of their composition have been implicated in neurologic disorders [41]. Some GP such as

LPC, PC, PE, and PI have been identified as potential biomarkers of cancer, kidney, and cardiovascular disease [33,42,43]. Sphingolipids consist of a complex family of compounds composed of a basic backbone of 1,3-dihydroxyl, 2-amino alkane or alkene (sphingoid base). Sphingomyelin (SM) and sphingosine are two important sphingolipids composed of a phosphorylcholine head group and fatty acid linked to a 1-hydroxyl group and 2-amino group of the sphingoid chain, respectively. Earlier studies have demonstrated that ceramides, which belong to *N*-acyl-derivatives of sphingosine, are associated with CKD [44].

### 3.2. Lipidomics

Although a subfraction of the metabolome, its complexity of lipid species, their distinct chemical properties and important biologic activity have made lipidome the focus of considerable research. Metabolomics is defined as “the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiologic stimuli or genetic modification” [45,46]. Metabolomics is a nontargeted quantitative analysis of biofluids and tissues for low molecular mass endogenous metabolites. Lipidomics, as a branch of metabolomics, was first introduced by Han and Gross in 2003 [47]. Lipidomics has been defined as “the full characterization of lipid molecular species and of their biological roles in expression of proteins involved in lipid metabolism and function, including gene regulation” [48]. Lipidomics represents a shift from individual lipid study to examination of global lipid metabolites in a systems-integrated context to more fully understand their role in pathophysiologic processes. In the past 10 years, lipidomics has emerged as a new field in systems biology and has increased interest in disease diagnosis and biomarker discovery (obesity, diabetes, cardiovascular disease, Alzheimer’s disease, pancreatic cancer, etc.), pharmaceutical discovery and development, human food and nutrition research [49–55]. This powerful approach can reveal unique metabolic characteristics of normal, pathologic, or treatment-specific events. Recently, an increased number of lipidomic studies and reviews have been published using mass spectrometry (MS), nuclear magnetic resonance (NMR), and other spectroscopic modalities [56–61]. Separation technologies, gas chromatography (GC), liquid chromatography (LC), super-critical fluid chromatography, and capillary electrophoresis, are critical for lipidomic examination of complex samples [62]. To obtain structural molecular ion information, low collision energy MS is first used followed by higher collision energy MS<sup>2</sup>

conditions to obtain fragment ions. Typically the precursor ion is selected and fragmentation monitored by tandem mass spectrometry (MS/MS). This approach provides greater structural information and detection of individual lipid species in complex biological samples. In addition, MS/MS has been increasingly used to develop quantitative methods for targeted lipidomics [63]. This approach, however, requires information based on a previous full-scan MS. In 2005, Wrona *et al.* [64] introduced the MS<sup>E</sup> technique in which two scanning functions are simultaneous for data collection. MS<sup>E</sup> provided parallel alternating scans for acquisition at low collision energy for precursor ion information (MS) or high collision energy for full-scan accurate mass fragments, precursor ions, and neutral loss information (MS<sup>E</sup>). This approach provided similar information to conventional MS<sup>2</sup> (MS/MS) in a single analytical run and structural information required for identification of unknown biomarkers in untargeted analyses [65–70].

### 3.3. Analytical methods for lipidomics

The traditional methods of lipid analysis usually involve solvent extraction of the biologic samples (blood, tissue, cell, and organism) followed by separation of lipids using thin-layer chromatography, solid-phase extraction, or normal-phase LC and separation of particular classes of lipids into individual molecular species by high-performance liquid chromatography (HPLC)–ultraviolet detector or evaporative light-scattering detector. Using these traditional methods, single molecular species of many lipid classes can be analyzed [71]. Although GC has been used to determine fatty acid content of the various lipids by methyl esters method, this approach tends to be time consuming and involves specimen hydrolysis and derivatization. In general, conventional lipid analysis typically requires large sample quantity because many biologically active species are present in very small amounts. Due to their inherent complexity, sample preparation may involve multiple extractions thus further lowering sensitivity and resolution. In addition, these methods are labor-intensive and often require derivatization thus limiting throughput.

In contrast, direct sample analysis may be used for MS lipidomics [72,73]. Direct-infusion MS technologies have good reproducibility, accuracy, and high sensitivity and are less time consuming than traditional methods. Typically, electrospray ionization quadrupole-time-of-flight (ESI-QTOF) and matrix-assisted laser desorption ionization (MALDI) are the most widely used ion sources in direct-infusion MS analysis [74,75]. Direct-infusion

MS is simple and fast. Its main limitation is ion suppression, which hampers the sensitivity and quantitative accuracy. Unfortunately, this method is unable to identify isobaric and isomeric lipids, whose masses are identical and often produce similar fragmentation patterns. Although direct-infusion MS is relatively limited in searching for novel and unknown compounds from lipid database, it may be useful to screen biochemical pathways in various diseases in the future. Comprehensive reviews of direct-infusion ESI/MS, ESI-QTOF/MS, and MALDI/MS and their applications in lipidomics have been published [74,75].

MS is commonly combined with LC for the lipidomics and LC-MS-based studies in lipidomics have been reviewed [76]. Typically, the advantages of the LC-MS approach are good reproducibility, accuracy, and high sensitivity for identification of known or novel lipids. Over the past decade, HPLC-MS has been widely used for both targeted and untargeted analyses in metabolomics and lipidomics using single quadrupole, hybrid, and high-resolution instruments. For global profiling, combinations of ultra-performance liquid chromatography (UPLC) coupled with QTOF/MS or tandem ion mobility TOF/MS are popular choices [77-80]. These provide rapid analysis with high-resolution MS. UPLC uses sub-2  $\mu\text{m}$  sized particles and operates at increased pressures (6000-15,000 psi) thus providing high chromatographic resolution versus conventional HPLC with 5  $\mu\text{m}$  particles [81]. Increased resolution results from improved signal/noise ratio and narrow peak width versus conventional HPLC. This approach is advantageous for metabolic profiling because an enormous number of metabolites may be detected at physiologic concentrations. Although lipids from various biologic sources may be separated by UPLC-MS [82], matrix effects have important influence on global profiles [83]. Unfortunately, sensitivity is typically not as high as targeted lipidomics. In addition, the experimental conditions of each separated compound cannot be optimized. Typically, triple quadrupole MS is used for targeted analyses by UPLC-MS with selective ion monitoring. Targeted lipid methods may include sterols and eicosanoids such as bile acids and steroids [84,85]. GC-based methods are suitable for volatile components and cannot be used for most lipids. Interestingly, GC-MS is the most widely used method for analysis of free fatty acids, esterified fatty acids, and steroids. Free fatty acids and steroids require derivatization or silylation, whereas esterified fatty acids are often analyzed as methyl esters [86]. Supercritical fluid chromatography is another high-resolution technique that can be used for the separation of various lipids. Supercritical fluid chromatography MS can be used for comprehensive lipid profiling of large sample numbers [87].



Ion mobility MS (IM-MS) and multidimensional methodologies are regarded as novel methodologies and have been used in lipidomics [88,89]. Isomers, conformers, and enantiomers can be rapidly separated by IM-MS and has proven useful in the analysis of complex biological samples [78]. The development of imaging MS has also played an important role in the development of imaging ion mobility spectrometry with MS for lipid analysis. Ion mobility spectrometry with MS combined with molecular dynamics computational modeling can be useful for future characterization of the structure and stability of lipid-incorporated complexes. In addition, comprehensive multidimensional LC-MS is an attractive emerging approach for comprehensive lipidomic characterization of complex biologic samples [90].

### 3.4. Analysis of lipidomics data

Lipidomics produces enormous data and its analysis plays a key role, especially in untargeted studies. As such, robust bioinformatics is critical. Prior to statistical analysis, data preprocessing including signal processing, data normalization and transformation are required, such that raw data are transformed into a format compatible with statistical data analysis [91,92]. Given the large degree of lipid variation, the first step of unsupervised and supervised statistical analysis is data reduction. This may be accomplished by a number of methods including orthogonal partial least squares-discriminate analysis, principal components analysis (PCA), and partial least squares-discriminate analysis (PLS-DA). Both unsupervised and supervised methods can be used, depending on the goal of the specific analysis. In unsupervised data analysis, unknown information about different groups is used by PCA and hierarchical cluster analysis. In the supervised approach, each sample or metabolite is associated with known compounds and this prior information is then used for analysis via principal component regression and neural networks [91,92]. Other regression methods including Elastic Net and Least Absolute Shrinkage and Selection Operator are also available for analysis of lipidomic data sets to ascertain the relationship between variables [93].



## 4. APPLICATIONS OF LIPIDOMICS IN KIDNEY DISEASES

Phospholipids represent a class of important cellular constituents that participate in numerous biologic processes and pathways reflective of metabolic status in health and disease. Lipidomics is a suitable tool for disease biomarker discovery in systems biology [94,95]. Comprehensive understanding of its applications is critically important for lipidomics. Many studies have demonstrated that metabolic disorders or abnormalities of various

lipids lead to kidney disease [96–99]. Using chronic renal failure (CRF), renal cell carcinoma (RCC), chronic glomerulonephritis, IgA nephropathy, and DN, we discuss lipidomics in kidney disease in humans and animal- and cell-model studies.

## 4.1. Lipidomics in clinical kidney disease

### 4.1.1 Effect of chronic kidney disease and glomerulonephritis

Lipid abnormalities are common in kidney disease [100,101] and contribute to a high incidence of cardiovascular disorders in this population. Plasma and erythrocyte lipid profiles were examined in CRF patients on hemodialysis for 30 months [102]. Increased triglyceride was observed in plasma and erythrocyte membranes. Increased plasma palmitic acids and monounsaturated fatty acids and decreased plasma polyunsaturated fatty acids were also observed in CRF. Lipid abnormalities were apparent at 18 months and became more profound at 30 months. Plasma and erythrocyte membrane lipid patterns did not change during the dialysis period. CRF patients under regular hemodialysis showed a gradual deterioration in the triglyceride and fatty acid profiles. In another study, HPLC–MS was used to profile plasma phospholipids in patients with chronic glomerulonephritis and CRF without renal replacement therapy [103]. The results showed that primary chronic glomerulonephritis and CRF had abnormal metabolic phospholipid profiles. A number of phospholipids ( $n = 19$ ) were identified as potential biomarkers. A possible mechanism leading to this abnormality included phosphatidylinositol (PI) hydrolysis via activation PI-specific phospholipase C, leading to production of two second messengers, inositol (1,4,5)-trisphosphate (IP3) and diacylglycerol [104], that participate in signal transduction independently. IP3 increases cytoplasmic  $\text{Ca}^{2+}$  by stimulating release of  $\text{Ca}^{2+}$  from sarcoplasmic reticulum [105]. Protein kinase C (PKC) is activated by phosphatidylserine,  $\text{Ca}^{2+}$ , and diacylglycerol. Activation of intracellular PKC signal transduction system, in turn, triggers a series of physiologic and physiochemical reactions.

Based on morphologic and genetic features, RCC is classified into various subtypes. RCC prognosis varies and metastatic or recurrent RCC associated with poor prognosis with rare long-term survival. Desorption ESI/MS was used in an imaging mode to study the lipid profiling of thin tissue sections of human papillary RCC versus adjacent normal tissue (11 sample pairs) and clear cell RCC versus adjacent normal tissue (9 sample pairs) [106]. Increased GP and free fatty acids were observed in the tumor region. PLS-DA distinguished tumor in papillary and clear cell RCC and papillary from

clear cell RCC. Altered GP tissue composition occurs in cancer [107] and appears integrally associated with malignant transformation [108]. Micro-LC-QTOF/MS was used to investigate urine lipids in RCC versus healthy subjects. Thirty-five lipid species were tentatively identified including lipidomic changes in urinary exosomes [109]. Tissue GP and their enzymatic by-products appear related to malignant transformation [110,111] and significantly increased PI has been observed in transformed cells [112].

#### **4.1.2 Effect of DN**

DN is a serious problem worldwide. Phospholipids and their metabolites are closely related to pathogenesis and progression of DN. Untargeted lipidomics of serum phospholipids using normal-phase LC-TOF/MS and ion trap-MS/MS was performed on DN patients [113]. Comparison with healthy subjects revealed eight lipids in seven phospholipid classes as potential DN biomarkers. Two novel biomarkers including PI (18:0/22:6) and SM (d18:0/20:2) effectively discriminated DN patients. Predictably, the same phospholipid class has similar variation trend with DN progression. Upregulated LPC, PE, PG, SM, one PC and one PI and downregulated PE, PS and two PC were noted. A number of studies have shown lipid accumulation in the kidneys of diabetic experimental animals and humans and that lipids influenced the DN pathogenesis [114,115]. It was reported that lipid phosphatase promoted podocyte apoptosis leading to DN and lipid phosphatase was increased before histologic change [116]. Additional evidence has shown that abnormal lipid metabolism and accumulation of lipids in the kidney played an important role in DN pathogenesis [117–119] and oxidized PC species were related to renal dysfunction [120]. Possible mechanisms involve lipid deposition due to increased serum concentration as well as glomerular filtration of protein bound lipids associated with proteinuria. Accumulated lipids increased expression of vascular endothelial growth factor and transforming growth factor- $\beta$  as well as promotion of proteinuria and diabetic glomerulosclerosis [121]. On the other hand, the presence of abnormal phospholipids may promote activation of the sorbitol pathway, oxidative stress, and activation of PKC [122–124]. In DN, decreased PI was related to activation of the sorbitol pathway leading to degradation of intracellular inositol, reduction of myoinositol, and reduction of PI synthesis.

#### **4.1.3 Effects of renal replacement modalities**

Clinical complications associated with peritoneal dialysis have become increasingly evident. An online two-dimensional LC-QTOF/MS was

developed for plasma lipid profiling in peritoneal dialysis patients [125]. This comprehensive study included 10 lipid classes and 190 lipid species. Thirty biomarkers were identified included PE and PC as indicators of malnutrition, inflammation, and atherosclerotic syndrome. This study also examined differences in lipid profiles in plasma from individuals with poor fluid control and those with good volume status. Significantly increased PC and PE (and plasmalogen subclasses of PC and PE) were observed in those with poor volume status. Interestingly, another similar study showed that the incidence of malnutrition was associated with plasmalogen phospholipids [126]. These findings supported an association between volume and nutritional status in peritoneal dialysis [127]. GC-MS was used to quantify F<sub>2</sub>-isoprostanes in hemodialysis patients with end-stage renal disease [128]. F<sub>2</sub>-isoprostanes were increased ~100-fold after iron/ascorbate-induced oxidative stress and 2- to 4-fold after pentylenetetrazol-induced seizures in hemodialysis patients. Both human and experimental studies support an association between F<sub>2</sub>-isoprostanes and inflammation.

## 4.2. Lipidomics in animal models or cell models

### 4.2.1 Effect of IgA nephropathy

IgA nephropathy is the most common form of glomerulonephritis and can progress to end-stage renal failure. To identify markers of progression, HPLC-MS with PCA and PLS-DA were used to evaluate phospholipid metabolic profiles in plasma in an experimental Balb/c mouse model [129]. PC, LPC, PI, PS, PE, and SM lipid classes including 90 lipid species were identified. PS(18:0/18:0), PS(18:0/22:5), and PI(18:0/20:4) were identified as potential biomarkers. The relationship of phospholipids and intercellular adhesion molecule-1 (ICAM-1) expression was also examined. The latter highly correlated to proteinuria. Another study identified ICAM-1 expression as an indicator of disease progression and suggested PS(18:0/18:0), PS(18:0/22:5), and PI(18:0/20:4) as possible biomarkers of IgA nephropathy [130].

Imaging MS lipidomics is useful for visualizing localization of various lipids in kidney and other tissue [131,132]. Recently the molecular distribution of lipids was analyzed in hyper-IgA murine kidneys using MALDI-quadrupole ion trap-TOF-based imaging MS [133]. Two PC, PC(18:2/22:6) and PC(16:0/22:6), were primarily found in the cortex and two triacylglycerols, TAG(18:1/18:2/18:1) and TAG(16:0/18:2/18:1), were found in the hilum. However, several other lipids were observed in hyper-IgA kidneys, especially in the tubular region. Two hyper-IgA-specific

lipids were O-PC including PC(O-18:1/22:6) and PC(O-16:0/22:6). It was reported that PC(O-18:1/22:6) and PC(O-16:0/22:6) were analogues of plasmalogen and platelet-activating factor, respectively [134,135]. This study also indicated that all the hyper-IgA-specific lipids were derived from urine and that stagnation due to unilateral urethral obstruction caused hyper-IgA-specific distribution of lipids in renal tubules.

A possible mechanism involved activation of PKC pathway leading to expansion of extracellular matrix and thickening of glomerular basement membrane [136]. In fact, PKC activation has been shown to increase permeability of endothelial monolayer to albumin [137]. Epithelial cell and the basement membrane form the glomerular capillary barrier. PKC activation has been shown to damage the glomerular capillary barrier leading to proteinuria [138,139].

#### **4.2.2 Effect of DN**

Rapamycin was shown to prevent DN development in streptozotocin-induced diabetic rats. MALDI-TOF/MS of the renal cortex revealed three classes of sphingolipids including ceramides, SM, and ceramide monohexosides [140]. One ceramide metabolite was significantly increased whereas three disappeared. Sphingolipid composition was greatly altered by rapamycin treatment. Increased ceramide(d18:0/16:0), ceramide monohexoside(d18:1/15:0), SM(d16:1/18:0), and SM(d18:1/18:0) were reversed by rapamycin. The previous study showed that ceramide increase in diabetic kidney and decrease after rapamycin treatment and the long established relationship of ceramide and apoptosis support ceramide as a reasonable biomarker candidate [141]. Streptozotocin significantly increased synthesis of many sphingolipids that was inhibited by rapamycin. Other studies showed that ceramide inhibition, via blocking ceramide synthase or serine palmitoyltransferase, effectively decreased cell death caused by hypoxia-reoxygenation, chemical hypoxia, and radiocontrast media in renal tubular epithelial cells [142–144].

#### **4.2.3 Effect of acute renal failure**

Inflammation plays a key role in the pathogenesis of acute renal failure [145,146]. LC-MS lipidomics was used to investigate impact of short-term dietary  $\omega$ -3 or  $\omega$ -6 polyunsaturated fatty acids on ischemic renal injury and renal lipid autacoid circuits [147]. Renal ischemia (30 min) resulted in significantly reduced renal function and significantly increased serum creatinine in mice fed a  $\omega$ -6 supplemented diet but remained normal in mice

fed a  $\omega$ -3 supplemented diet. Moreover extension of renal ischemia (45 min) caused 100% mortality in  $\omega$ -6 supplemented mice but no death in the  $\omega$ -3 supplemented group. Protective effect of  $\omega$ -3 polyunsaturated fatty acids against ischemic renal injury was associated with decreased polymorphonuclear leukocyte recruitment, chemokine and cytokine production, abrogated formation of lipoxygenase- and cyclooxygenase-derived eicosanoids, and increased protectin D1 expression [148]. Systemic treatment with protectin D1 decreased kidney polymorphonuclear leukocyte influx and upregulated the expression of heme oxygenase-1 protein and mRNA in injured and uninjured kidneys. Protectin D1 appeared effective in prevention of acute kidney injury as well as the effect of dietary  $\omega$ -3 and  $\omega$ -6 polyunsaturated fatty acids on autacid formation in the kidney and outcome of ischemic renal injury [149].

#### 4.2.4 Cell research

ESI/MS lipidomics was used to identify phospholipid changes in human embryonic kidney (HEK293) and human kidney carcinomas (Caki-1) cell death [150]. Significantly decreased PC(14:0/16:0) and PC(16:0/16:0) was observed in cisplatin-treated HEK293 and Caki-1 cells. Bromoenol lactone treatment prior to cisplatin exposure further decreased PC(14:0/16:0), plasmenylcholine(16:0/16:1), and plasmenylcholine(16:0/18:1) in HEK293 and inhibited cisplatin-induced increases in plasmenylcholine(16:1/22:6) in Caki-1. Treatment with bromoenol lactone prior to cisplatin exposure also increased several arachidonic containing phospholipids including PC(16:0/20:4), PC(18:1/20:4), and PC(18:0/20:4) versus cisplatin only treatment. These results demonstrated that phospholipase A<sub>2</sub> inhibition protected against chemotherapy-induced cell death in multiple human renal cell lines and also identified phospholipids that were specifically altered during cell death. The results further demonstrated that alterations in these phospholipids correlated with protection against cell death in the presence of phospholipase A<sub>2</sub> inhibitors. Masood and coworkers used normal and reversed phase LC-MS/MS to quantitate multiple sphingolipid classes in HEK293 cells [151]. These results showed that more than 75% of the ceramides, monohexosylceramides, and SM exist as d18:1<sup>Δ4</sup> c16:0, d18:1<sup>Δ4</sup> c24:1, and d18:1<sup>Δ4</sup> c24:0.



## 5. CONCLUDING REMARKS AND PERSPECTIVES

Novel lipidomics is an emerging methodology which holds promise for systematic and comprehensive study of lipids and their derivatives in

health and disease. Various kidney diseases are associated with significant changes in metabolism and plasma concentration of lipids and lipoproteins, as well as lipid-related metabolites and metabolic pathways. These changes play an important role in the pathogenesis of local and systemic inflammation, impaired energy metabolism, and progression of kidney disease. The combination of lipid profiling and multivariate statistics is useful for discovery of potential biomarkers and novel therapeutic modalities, as well as monitoring response to therapeutic intervention.

Recent advances in MS-based technologies and rapid improvements in chromatography, especially UPLC–MS combined with bioinformatics, have improved our understanding the role of lipid-derived metabolites in the pathogenesis and progression of kidney disease. Although currently available tools allow identification of lipid-derived metabolite structure with high resolution, further advances in analytical techniques and data handling are clearly needed for more effective data preprocessing, data mining, statistical analysis, biomarker identification, and interpretation of biochemical pathways.

## ACKNOWLEDGMENTS

This study was supported by the Program for New Century Excellent Talents in University (NCET-13-0954) and Changjiang Scholars and Innovative Research Team in University (IRT1174) from Ministry of Education of China, National Natural Science Foundation of China (J1210063, 81202909, 81274025, 81001622), the project “As a Major New Drug to Create a Major National Science and Technology Special” (2014ZX09304307-002), China Postdoctoral Science Foundation (2012M521831, 2014T70984), National Innovation Training Plan Program (201310697004), Key Program for the International S&T Cooperation Projects of Shaanxi Province (2013KW31-01), Natural Science Foundation of Shaanxi Provincial Education Department (2013JK0811), and the Administration of Traditional Chinese Medicine of Shaanxi (13-ZY006).

## REFERENCES

- [1] A. Levin, N.R. Powe, J. Rossert, et al., Chronic kidney disease as a global public health problem: approaches and initiatives—a position statement from Kidney Disease Improving Global Outcomes, *Kidney Int.* 72 (2007) 247–259.
- [2] K. Makris, N. Kafkas, Neutrophil gelatinase-associated lipocalin in acute kidney injury, *Adv. Clin. Chem.* 58 (2012) 141–191.
- [3] X.B. Ling, E.D. Mellins, K.G. Sylvester, H.J. Cohen, Urine peptidomics for clinical biomarker discovery, *Adv. Clin. Chem.* 51 (2010) 181–213.
- [4] T.K. Sigdel, R.B. Klassen, M.M. Sarwal, Interpreting the proteome and peptidome in transplantation, *Adv. Clin. Chem.* 47 (2009) 139–169.
- [5] N.D. Vaziri, Dyslipidemia of chronic renal failure: the nature, mechanisms and potential consequences, *Am. J. Physiol. Renal Physiol.* 290 (2006) 262–272.
- [6] N.D. Vaziri, J. Yuan, Z. Ni, S.B. Nicholas, K.C. Norris, Lipoprotein lipase deficiency in chronic kidney disease is accompanied by down-regulation of endothelial GPIHBP1 expression, *Clin. Exp. Nephrol.* 16 (2012) 238–243.

- [7] N.D. Vaziri, Molecular mechanisms of lipid disorders in nephrotic syndrome, *Kidney Int.* 63 (2003) 1964–1976.
- [8] N.D. Vaziri, Lipotoxicity and impaired HDL-mediated reverse cholesterol/lipid transport in chronic kidney disease, *J. Ren. Nutr.* 20 (2010) S35–S43.
- [9] N.D. Vaziri, K. Norris, Lipid disorders and their relevance to outcomes in chronic kidney disease, *Blood Purif.* 31 (2011) 189–196.
- [10] N.D. Vaziri, Role of dyslipidemia in impairment of energy metabolism, oxidative stress, inflammation and cardiovascular disease in chronic kidney disease, *Clin. Exp. Nephrol.* 18 (2014) 265–268.
- [11] C. Waldner, G. Heise, K. Schror, P. Heering, COX-2 inhibition and prostaglandin receptors in experimental nephritis, *Eur. J. Clin. Invest.* 33 (2003) 969–975.
- [12] A. Hartner, A. Pahl, K. Brune, M. Goppelt-Struebe, Upregulation of cyclooxygenase-1 and the PGE2 receptor EP2 in rat and human mesangioproliferative glomerulonephritis, *Inflamm. Res.* 49 (2000) 345–354.
- [13] S. Tomasoni, M. Noris, S. Zappella, et al., Upregulation of renal and systemic cyclooxygenase-2 in patients with active lupus nephritis, *J. Am. Soc. Nephrol.* 9 (1998) 1202–1212.
- [14] C. Zoja, A. Benigni, M. Noris, et al., Mycophenolate mofetil combined with a cyclooxygenase-2 inhibitor ameliorates murine lupus nephritis, *Kidney Int.* 60 (2001) 653–663.
- [15] T. Takano, A.V. Cybulsky, W.A. Cupples, et al., Inhibition of cyclooxygenases reduces complement-induced glomerular epithelial cell injury and proteinuria in passive Heymann nephritis, *J. Pharmacol. Exp. Ther.* 305 (2003) 240–249.
- [16] G. Heise, B. Grabensee, K. Schrör, P. Heering, Different actions of the cyclooxygenase 2 selective inhibitor flosulide in rats with passive Heymann nephritis, *Nephron* 80 (1998) 220–226.
- [17] Z.G. Xu, S.L. Li, L. Lanting, et al., Relationship between 12/15-lipoxygenase and COX-2 in mesangial cells: potential role in diabetic nephropathy, *Kidney Int.* 69 (2006) 512–519.
- [18] A. Dey, R.S. Williams, D.M. Pollock, et al., Altered kidney CYP2C and cyclooxygenase-2 levels are associated with obesity-related albuminuria, *Obes. Res.* 12 (2004) 1278–1289.
- [19] X. Zhao, J.E. Quigley, J. Yuan, et al., PPAR- $\alpha$  activator fenofibrate increases renal CYP-derived eicosanoid synthesis and improves endothelial dilator function in obese Zucker rats, *Am. J. Physiol.* 290 (2006) H2187–H2195.
- [20] Y. Zhou, S. Lin, H.H. Chang, et al., Gender differences of renal CYP-derived eicosanoid synthesis in rats fed a high-fat diet, *Am. J. Hypertens.* 18 (2005) 530–537.
- [21] Y.Y. Zhao, R.C. Lin, Metabolomics in nephrotoxicity, *Adv. Clin. Chem.* 65 (2014) 69–89.
- [22] M.H. Rosner, Urinary biomarkers for the detection of renal injury, *Adv. Clin. Chem.* 49 (2009) 73–97.
- [23] R.G. Fassett, S.K. Venuthurupalli, G.C. Gobe, et al., Biomarkers in chronic kidney disease: a review, *Kidney Int.* 80 (2011) 806–821.
- [24] D. Leberherz-Eichinger, C.G. Krenn, G.A. Roth, Keratin 18 and heat-shock protein in chronic kidney disease, *Adv. Clin. Chem.* 49 (2009) 73–97.
- [25] Y.Y. Zhao, R.C. Lin, UPLC-MS<sup>E</sup> application in disease biomarker discovery: the discoveries in proteomics to metabolomics, *Chem. Biol. Interact.* 215 (2014) 7–16.
- [26] R.H. Weiss, K. Kim, Metabolomics in the study of kidney diseases, *Nat. Rev. Nephrol.* 8 (2011) 22–33.
- [27] V. Fanos, C. Fanni, G. Ottonello, et al., Metabolomics in adult and pediatric nephrology, *Molecules* 18 (2013) 4844–4857.



- [28] M.H. Hanna, J.L. Segar, L.M. Teesch, et al., Urinary metabolomic markers of aminoglycoside nephrotoxicity in newborn rats, *Pediatr. Res.* 73 (2013) 585–591.
- [29] W.L. Yang, Q. Bai, D.D. Li, et al., Changes of urinary phospholipids in the chronic kidney disease patients, *Biomarkers* 18 (2013) 601–606.
- [30] S. Subramaniam, E. Fahy, S. Gupta, et al., Bioinformatics and systems biology of the lipidome, *Chem. Rev.* 111 (2011) 6452–6490.
- [31] R. Naresh Kumar, R. Sundaram, P. Shanthi, P. Sachdanandam, Protective role of 20-OH ecdysone on lipid profile and tissue fatty acid changes in streptozotocin induced diabetic rats, *Eur. J. Pharmacol.* 698 (2013) 489–498.
- [32] X. Han, D.R. Abendschein, J.G. Kelley, R.W. Gross, Diabetes-induced changes in specific lipid molecular species in rat myocardium, *Biochem. J.* 352 (2000) 79–89.
- [33] Y.Y. Zhao, Metabolomics in chronic kidney disease, *Clin. Chim. Acta* 422 (2013) 59–69.
- [34] E. Fahy, S. Subramaniam, R.C. Murphy, et al., Update of the LIPID MAPS comprehensive classification system for lipids, *J. Lipid Res.* 50 (2009) S9–S14.
- [35] C.D. Funk, Prostaglandins and leukotrienes: advances in eicosanoid biology, *Science* 294 (2001) 1871–1875.
- [36] R.A. Coleman, D.P. Lee, Enzymes of triacylglycerol synthesis and their regulation, *Prog. Lipid Res.* 43 (2004) 134–176.
- [37] T.E. Graham, Exercise, postprandial triacylglyceridemia, and cardiovascular disease risk, *Can. J. Appl. Physiol.* 29 (2004) 781–799.
- [38] D.J. Wu, B.J. Zhu, X.D. Wang, Metabonomics-based omics study and atherosclerosis, *J. Clin. Bioinforma.* 1 (2011) 30.
- [39] M. Traini, W. Jessup, Exploring sterol and lipid biology in the functional genomic era, *Curr. Pharm. Biotechnol.* 13 (2012) 341–351.
- [40] P.T. Ivanova, S.B. Milne, M.O. Byrne, Y. Xiang, H.A. Brown, Glycerophospholipid identification and quantitation by electrospray ionization mass spectrometry, *Methods Enzymol.* 432 (2007) 21–57.
- [41] A.A. Farooqui, L.A. Horrocks, T. Farooqui, Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders, *Chem. Phys. Lipids* 106 (2000) 1–29.
- [42] J.L. Spratlin, N.J. Serkova, S.G. Eckhardt, Clinical applications of metabolomics in oncology: a review, *Clin. Cancer Res.* 15 (2009) 431–440.
- [43] A. Giovane, A. Balestrieri, C. Napoli, New insights into cardiovascular and lipid metabolomics, *J. Cell. Biochem.* 105 (2008) 648–654.
- [44] Y.Y. Zhao, X.L. Cheng, F. Wei, et al., Serum metabonomics study of adenine-induced chronic renal failure in rats by ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry, *Biomarkers* 17 (2012) 48–55.
- [45] J.K. Nicholson, J.C. Lindon, E. Holmes, ‘Metabonomics’: understanding the metabolic responses of living systems to pathophysiological stimuli via multivariate statistical analysis of biological NMR spectroscopic data, *Xenobiotica* 29 (1999) 1181–1189.
- [46] J.K. Nicholson, J. Connelly, J.C. Lindon, E. Holmes, Metabolomics: a platform for studying drug toxicity and gene function, *Nat. Rev. Drug Discov.* 1 (2002) 153–161.
- [47] X. Han, R.W. Gross, Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics, *J. Lipid Res.* 44 (2003) 1071–1079.
- [48] F. Spener, M. Lagarde, A. Geloën, M. Record, What is lipidomics? *Eur. J. Lipid Sci. Technol.* 105 (2003) 481–482.
- [49] C. Hu, R. van der Heijden, M. Wang, et al., Analytical strategies in lipidomics and applications in disease biomarker discovery, *J. Chromatogr. B* 877 (2009) 2836–2846.

- [50] X. Han, An update on lipidomics: progress and application in biomarker and drug development, *Curr. Opin. Mol. Ther.* 9 (2007) 586–591.
- [51] A. Lamaziere, C. Wolf, P.J. Quinn, Application of lipidomics to assess lipogenesis in drug development and pre-clinical trials, *Curr. Pharm. Biotechnol.* 13 (2012) 736–745.
- [52] S.A. Murphy, A. Nicolaou, Lipidomics applications in health, disease and nutrition research, *Mol. Nutr. Food Res.* 57 (2013) 1336–1346.
- [53] T. Hyötyläinen, I. Bondia-Pons, M. Orešič, Lipidomics in nutrition and food research, *Mol. Nutr. Food Res.* 57 (2013) 1306–1318.
- [54] H. Miao, H. Chen, X. Zhang, et al., Urinary metabolomics on the biochemical profiles in diet-induced hyperlipidemia rat using ultra-performance liquid-chromatography coupled with quadrupole time-of-flight SYNAPT high-definition mass spectrometry, *J. Anal. Methods Chem.* 2014 (2014) 184162.
- [55] H. Chen, H. Miao, Y.L. Feng, Y.Y. Zhao, R.C. Lin, Metabolomics in dyslipidemia, *Adv. Clin. Chem.* 66 (2014) 101–119.
- [56] P.D. Rainville, C.L. Stumpf, J.P. Shockcor, R.S. Plumb, J.K. Nicholson, Novel application of reversed-phase UPLC-oeTOF-MS for lipid analysis in complex biological mixtures: a new tool for lipidomics, *J. Proteome Res.* 6 (2007) 552–558.
- [57] S.J. Blanksby, T.W. Mitchell, Advances in mass spectrometry for lipidomics, *Annu Rev Anal Chem (Palo Alto, Calif)* 3 (2010) 433–465.
- [58] M. Li, L. Yang, Y. Bai, H. Liu, Analytical methods in lipidomics and their applications, *Anal. Chem.* 86 (2014) 161–175.
- [59] W.J. Griffiths, Y. Wang, Mass spectrometry: from proteomics to metabolomics and lipidomics, *Chem. Soc. Rev.* 38 (2009) 1882–1896.
- [60] A. Grélard, C. Loudet, A. Diller, E.J. Dufourc, NMR spectroscopy of lipid bilayers, *Methods Mol. Biol.* 654 (2010) 341–359.
- [61] F. Vilella, L.B. Ramirez, C. Simón, Lipidomics as an emerging tool to predict endometrial receptivity, *Fertil. Steril.* 99 (2013) 1100–1106.
- [62] Y.Y. Zhao, S.P. Wu, S. Liu, Y. Zhang, R.C. Lin, Ultra-performance liquid chromatography-mass spectrometry as a sensitive and powerful technology in lipidomic applications, *Chem. Biol. Interact.* 220 (2014) 181–192.
- [63] N. Zehethofer, D.M. Pinto, Recent developments in tandem mass spectrometry for lipidomic analysis, *Anal. Chim. Acta.* 627 (2008) 62–70.
- [64] M. Wrona, T. Mauriala, K.P. Bateman, R.J. Mortishire-Smith, D. O'Connor, 'All-in-One' analysis for metabolite identification using liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry with collision energy switching, *Rapid Commun. Mass Spectrom.* 19 (2005) 2597–2602.
- [65] Y.Y. Zhao, X.L. Cheng, N.D. Vaziri, et al., UPLC-based metabolomics applications for discovering biomarkers of diseases in clinical chemistry, *Clin. Biochem.* 47 (2014) 16–26.
- [66] Y.Y. Zhao, J. Liu, X.L. Cheng, X. Bai, R.C. Lin, Urinary metabolomics study on biochemical changes in an experimental model of chronic renal failure by adenine based on UPLC Q-TOF/MS, *Clin. Chim. Acta* 413 (2012) 642–649.
- [67] Y.Y. Zhao, X.L. Cheng, F. Wei, X. Bai, R.C. Lin, Application of faecal metabolomics on an experimental model of tubulointerstitial fibrosis by ultra performance liquid chromatography/high-sensitivity mass spectrometry with MS<sup>E</sup> data collection technique, *Biomarkers* 17 (2012) 721–729.
- [68] Y.Y. Zhao, L. Zhang, F.Y. Long, et al., UPLC-Q-TOF/HSMS/MS<sup>E</sup>-based metabolomics for adenine-induced changes in metabolic profiles of rat faeces and intervention effects of ergosta-4,6,8(14),22-tetraen-3-one, *Chem. Biol. Interact.* 301 (2013) 31–38.

- [69] Y.Y. Zhao, P. Lei, D.Q. Chen, Y.L. Feng, X. Bai, Renal metabolic profiling of early renal injury and renoprotective effects of *Poria cocos* epidermis using UPLC Q-TOF/HSMS/MS<sup>E</sup>, *J. Pharm. Biomed. Anal.* 81–82 (2013) 202–209.
- [70] Z.H. Zhang, Y.Y. Zhao, X.L. Cheng, et al., General toxicity of *Pinellia temata* (Thunb.) Berit. in rat: a metabolomic method for profiling of serum metabolic changes, *J. Ethnopharmacol.* 149 (2013) 303–310.
- [71] B.L. Peterson, B.S. Cummings, A review of chromatographic methods for the assessment of phospholipids in biological samples, *Biomed. Chromatogr.* 20 (2006) 227–243.
- [72] X. Han, Multi-dimensional mass spectrometry-based shotgun lipidomics and the altered lipids at the mild cognitive impairment stage of Alzheimer's disease, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1801 (2010) 774–783.
- [73] X. Han, R.W. Gross, Shotgun lipidomics—electrospray ionization mass spectrometric analysis and quantitation of cellular lipidomes directly from crude extracts of biological samples, *Mass Spectrom. Rev.* 24 (2005) 367–412.
- [74] M. Ståhlman, C.S. Ejsing, K. Tarasov, et al., High-throughput shotgun lipidomics by quadrupole time-of-flight mass spectrometry, *J. Chromatogr. B* 877 (2009) 2664–2672.
- [75] J. Schiller, R. Süss, J. Arnhold, et al., Matrix-assisted laser desorption and ionization time-of-flight (MALDI-TOF) mass spectrometry in lipid and phospholipid research, *Prog. Lipid Res.* 43 (2004) 449–488.
- [76] M. Li, Z. Zhou, H. Nie, Y. Bai, H. Liu, Recent advances of chromatography and mass spectrometry in lipidomics, *Anal. Bioanal. Chem.* 399 (2011) 243–249.
- [77] Y.Y. Zhao, H.T. Li, Y.L. Feng, X. Bai, R.C. Lin, Urinary metabolomic study of the surface layer of *poria cocos* as an effective treatment for chronic renal injury in rats, *J. Ethnopharmacol.* 148 (2013) 403–410.
- [78] V. Shah, J.M. Castro-Perez, D.G. McLaren, et al., Enhanced data-independent analysis of lipids using ion mobility-TOFMS<sup>E</sup> to unravel quantitative and qualitative information in human plasma, *Rapid Commun. Mass Spectrom.* 27 (2013) 2195–2200.
- [79] Y.Y. Zhao, X. Shen, X.L. Cheng, et al., Urinary metabolomics study on the protective effects of ergosta-4,6,8(14),22-tetraen-3-one on chronic renal failure in rats using UPLC Q-TOF/MS and a novel MS<sup>E</sup> data collection technique, *Process Biochem.* 47 (2012) 1980–1987.
- [80] J. Sun, A. Baker, P. Chen, Profiling the indole alkaloids in yohimbe bark with ultra-performance liquid chromatography coupled with ion mobility quadrupole time-of-flight mass spectrometry, *Rapid Commun. Mass Spectrom.* 25 (2011) 2591–2602.
- [81] I.D. Wilson, J.K. Nicholson, J. Castro-Perez, et al., High resolution “ultra performance” liquid chromatography coupled to oa-TOF mass spectrometry as a tool for differential metabolic pathway profiling in functional genomic studies, *J. Proteome Res.* 4 (2005) 591–598.
- [82] D. Chen, X. Yan, J. Xu, X. Su, L. Li, Lipidomic profiling and discovery of lipid biomarkers in *Stephanodiscus* sp. under cold stress, *Metabolomics* 9 (2013) 949–959.
- [83] L. Denoroy, L. Zimmer, B. Renaud, S. Parrot, Ultra high performance liquid chromatography as a tool for the discovery and the analysis of biomarkers of diseases: a review, *J. Chromatogr. B* 927 (2013) 37–53.
- [84] J. Song, X. Liu, J. Wu, et al., A highly efficient, high-throughput lipidomics platform for the quantitative detection of eicosanoids in human whole blood, *Anal. Biochem.* 433 (2013) 181–188.
- [85] I. Bobeldijk, M. Hekman, J. de Vries-van der Weij, et al., Quantitative profiling of bile acids in biofluids and tissues based on accurate mass high resolution LC-FT-MS: compound class targeting in a metabolomics workflow, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 871 (2008) 306–313.

- [86] O. Quehenberger, A.M. Armando, E.A. Dennis, High sensitivity quantitative lipidomics analysis of fatty acids in biological samples by gas chromatography–mass spectrometry, *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1811 (2011) 648–656.
- [87] T. Bamba, J.W. Lee, A. Matsubara, E. Fukusaki, Metabolic profiling of lipids by supercritical fluid chromatography/mass spectrometry, *J. Chromatogr. A* 1250 (2012) 212–219.
- [88] M. Kliman, J.C. May, J.A. McLean, Lipid analysis and lipidomics by structurally selective ion mobility–mass spectrometry, *Biochim. Biophys. Acta Mol. Cell Biol. Lip.* 1811 (2011) 935–945.
- [89] R.W. Gross, C.M. Jenkins, J. Yang, D.J. Mancuso, X. Han, Functional lipidomics: the roles of specialized lipids and lipid-protein interactions in modulating neuronal function, *Prostaglandins Other Lipid Mediat.* 77 (2005) 52–64.
- [90] X. Guo, E. Lankmayr, Multidimensional approaches in LC and MS for phospholipid bioanalysis, *Bioanalysis* 2 (2010) 1109–1123.
- [91] P.S. Niemelä, S. Castillo, M. Sysi-Aho, M. Orešič, Bioinformatics and computational methods for lipidomics, *J. Chromatogr. B* 877 (2009) 2855–2862.
- [92] M. Orešič, Informatics and computational strategies for the study of lipids, *Biochim. Biophys. Acta Mol. Cell Biol. Lip.* 1811 (2011) 991–999.
- [93] M. Orešič, Bioinformatics and computational approaches applicable to lipidomics, *Eur. J. Lipid Sci. Technol.* 111 (2009) 99–106.
- [94] P.L. Wood, Mass spectrometry strategies for clinical metabolomics and lipidomics in psychiatry, neurology, and neuro-oncology, *Neuropsychopharmacology* 39 (2014) 24–33.
- [95] A.D. Postle, Lipidomics, *Curr. Opin. Clin. Nutr. Metab. Care* 15 (2012) 127–133.
- [96] Y.Y. Zhao, X.L. Cheng, F. Wei, et al., Intrarenal metabolomic investigation of chronic kidney disease and its TGF- $\beta$ 1 mechanism in induced-adenine rats using UPLC Q-TOF/HSMS/MS<sup>E</sup>, *J. Proteome Res.* 12 (2013) 692–703.
- [97] L. Jia, J. Chen, P. Yin, X. Lu, G. Xu, Serum metabolomics study of chronic renal failure by ultra performance liquid chromatography coupled with Q-TOF mass spectrometry, *Metabolomics* 4 (2008) 183–189.
- [98] Y.Y. Zhao, Y.L. Feng, X. Bai, et al., Ultra performance liquid chromatography-based metabolomic study of therapeutic effect of the surface layer of *Poria cocos* on adenine-induced chronic kidney disease, *PLoS One* 8 (2013) e59617.
- [99] Y.Y. Zhao, X.L. Cheng, J.H. Cui, et al., Effect of ergosta-4,6,8(14),22-tetraen-3-one (ergone) on adenine-induced chronic renal failure rat: a serum metabolomic study based on ultra performance liquid chromatography/high-sensitivity mass spectrometry coupled with MassLynx i-FIT algorithm, *Clin. Chim. Acta* 413 (2012) 1438–1445.
- [100] J. Liu, M.H. Rosner, Lipid abnormalities associated with end-stage renal disease, *Semin. Dial.* 19 (2006) 32–40.
- [101] W.F. Keane, J.E. Tomassini, D.R. Neff, Lipid abnormalities in patients with chronic kidney disease: implications for the pathophysiology of atherosclerosis, *J. Atheroscler. Thromb.* 20 (2013) 123–133.
- [102] N.T. de Gómez Dumm, A.M. Giammona, L.A. Touceda, C. Raimondi, Lipid abnormalities in chronic renal failure patients undergoing hemodialysis, *Medicina (B Aires)* 61 (2001) 142–146.
- [103] L. Jia, C. Wang, S. Zhao, X. Lu, G. Xu, Metabolomic identification of potential phospholipid biomarkers for chronic glomerulonephritis by using high performance liquid chromatography–mass spectrometry, *J. Chromatogr. B* 860 (2007) 134–140.
- [104] S.G. Rhee, Regulation of phosphoinositide-specific phospholipase C, *Annu. Rev. Biochem.* 70 (2001) 281–312.
- [105] L.C. Cantley, The phosphoinositide 3-kinase pathway, *Science* 296 (2002) 1655–1657.

- [106] A.L. Dill, L.S. Eberlin, C. Zheng, et al., Multivariate statistical differentiation of renal cell carcinomas based on lipidomic analysis by ambient ionization imaging mass spectrometry, *Anal. Bioanal. Chem.* 398 (2010) 2969–2978.
- [107] S. Shimma, Y. Sugiura, T. Hayasaka, et al., MALDI-based imaging mass spectrometry revealed abnormal distribution of phospholipids in colon cancer liver metastasis, *J. Chromatogr. B* 855 (2007) 98–103.
- [108] K. Glunde, C. Jie, Z.M. Bhujwalla, Molecular causes of the aberrant choline phospholipid metabolism in breast cancer, *Cancer Res.* 64 (2004) 4270–4276.
- [109] P. Del Boccio, F. Raimondo, D. Pieragostino, et al., A hyphenated microLC-Q-TOF-MS platform for exosomal lipidomics investigations: application to RCC urinary exosomes, *Electrophoresis* 33 (2012) 689–696.
- [110] M. Athar, Oxidative stress and experimental carcinogenesis, *Indian J. Exp. Biol.* 40 (2002) 656–667.
- [111] E.O. Aboagye, Z.M. Bhujwalla, Malignant transformation alters membrane choline phospholipid metabolism of human mammary epithelial cells, *Cancer Res.* 59 (1999) 80–84.
- [112] T. Alonso, E. Santos, Increased intracellular glycerophosphoinositol is a biochemical marker for transformation by membrane-associated and cytoplasmic oncogenes, *Biochem. Biophys. Res. Commun.* 171 (1990) 14–19.
- [113] C. Zhu, Q.L. Liang, P. Hu, Y.M. Wang, G.A. Luo, Phospholipidomic identification of potential plasma biomarkers associated with type 2 diabetes mellitus and diabetic nephropathy, *Talanta* 85 (2011) 1711–1720.
- [114] C. Guijarro, B.L. Kasiske, Y. Kim, et al., Early glomerular changes in rats with dietary-induced hypercholesterolemia, *Am. J. Kidney Dis.* 26 (1995) 152–161.
- [115] H.S. Lee, J.S. Lee, H.I. Koh, et al., Intraglomerular lipid deposition in routine biopsies, *Clin. Nephrol.* 36 (1991) 67–75.
- [116] M.E. Hyvönen, P. Saurus, A. Wasik, et al., Lipid phosphatase SHIP2 downregulates insulin signalling in podocytes, *Mol. Cell. Endocrinol.* 328 (2010) 70–79.
- [117] Z. Wang, T. Jiang, J. Li, et al., Regulation of renal lipid metabolism, lipid accumulation, and glomerulosclerosis in FVBdb/db mice with type 2 diabetes, *Diabetes* 54 (2005) 2328–2335.
- [118] W.F. Keane, The role of lipids in renal disease: future challenges, *Kidney Int. Suppl.* 75 (2000) S27–S31.
- [119] H. Oda, W.F. Keane, Lipids in progression of renal disease, *Kidney Int. Suppl.* 62 (1997) S36–S38.
- [120] S. Jimi, N. Uesugi, K. Saku, et al., Possible induction of renal dysfunction in patients with lecithin: cholesterol acyltransferase deficiency by oxidized phosphatidylcholine in glomeruli, *Arterioscler. Thromb. Vasc. Biol.* 19 (1999) 794–801.
- [121] L. Sun, N. Halahel, W. Zhang, et al., Role of sterol regulatory element-binding protein 1 in regulation of renal lipid metabolism and glomerulosclerosis in diabetes mellitus, *J. Biol. Chem.* 277 (2002) 18919–18927.
- [122] E. Schleicher, A. Nerlich, The role of hyperglycemia in the development of diabetic complications, *Horm. Metab. Res.* 28 (1996) 367–373.
- [123] R.G. Larkins, M.E. Dunlop, The link between hyperglycaemia and diabetic nephropathy, *Diabetologia* 35 (1992) 499–504.
- [124] S.H. Ayo, R. Radnik, J.A. Garoni, D.A. Troyer, J.I. Kreisberg, High glucose increases diacylglycerol mass and activates protein kinase C in mesangial cell cultures, *Am. J. Physiol.* 261 (1991) F571–F577.
- [125] M. Li, B. Feng, Y. Liang, et al., Lipid profiling of human plasma from peritoneal dialysis patients using an improved 2D (NP/RP) LC-QTOF MS method, *Anal. Bioanal. Chem.* 405 (2013) 6629–6638.

- [126] P. Stenvinkel, I. Holmeberg, O. Heimbürger, U. Diczfalusy, A study of plasmalogen as an index of oxidative stress in patients with chronic renal failure. Evidence of increased oxidative stress in malnourished patients, *Nephrol. Dial. Transplant.* 13 (1998) 2594–2600.
- [127] L.T. Cheng, W. Tang, T. Wang, Strong association between volume status and nutritional status in peritoneal dialysis patients, *Am. J. Kidney Dis.* 45 (2005) 891–902.
- [128] I. Wiswedel, F<sub>2</sub>-isoprostanes: sensitive biomarkers of oxidative stress in vitro and in vivo: a gas chromatography–mass spectrometric approach, *Methods Mol. Biol.* 580 (2009) 3–16.
- [129] L. Jia, C. Wang, H. Kon, Z. Cai, G. Xu, Plasma phospholipid metabolic profiling and biomarkers of mouse IgA nephropathy, *Metabolomics* 2 (2006) 95–103.
- [130] P. Arrizabalaga, M. Sole, R. Abellana, Tubular and interstitial expression of ICAM-1 as a marker of renal injury in IgA nephropathy, *Am. J. Nephrol.* 23 (2003) 121–128.
- [131] N.H. Patterson, A. Thomas, P. Chaurand, Monitoring time-dependent degradation of phospholipids in sectioned tissues by MALDI imaging mass spectrometry, *J. Mass Spectrom.* 49 (2014) 622–627.
- [132] S. Fröhlich, B. Putz, H. Schachner, et al., Renopathological microstructure visualization from formalin fixed kidney tissue by matrix-assisted laser/desorption ionization-time-of-flight mass spectrometry imaging, *Balkan J. Med. Genet.* 15 (2012) 13–16.
- [133] Y. Kaneko, Y. Obata, T. Nishino, et al., Imaging mass spectrometry analysis reveals an altered lipid distribution pattern in the tubular areas of hyper-IgA murine kidneys, *Exp. Mol. Pathol.* 91 (2011) 614–621.
- [134] R. Coppo, V. Fonsato, S. Balegno, et al., Aberrantly glycosylated IgA1 induces mesangial cells to produce platelet-activating factor that mediates nephrin loss in cultured podocytes, *Kidney Int.* 77 (2010) 417–427.
- [135] D.R. Robinson, S. Tateno, B. Patel, et al., Dietary marine lipids after the course of autoimmune disease, *Prog. Clin. Biol. Res.* 282 (1988) 295–303.
- [136] M. Fukui, T. Nakamura, I. Ebihara, ECM gene expression and its modulation by insulin in diabetic rats, *Diabetes* 41 (1992) 1520–1527.
- [137] J.J. Lynch, T.J. Ferro, F.A. Blumenslock, Increased endothelial albumin permeability mediated by protein kinase C activation, *J. Clin. Invest.* 85 (1990) 1991–1998.
- [138] P.G. Schmitz, K. Zhang, 1994 Expression of protein kinase C- $\alpha$  in the remnant kidney proposed role in potentiating glomerular capillary hypertension, *J. Am. Soc. Nephrol.* 5 (1994) 611.
- [139] S. Chen, M.P. Cohen, G.T. Lautenslager, et al., Glycated albumin stimulates TGF- $\beta$ 1 production and protein kinase C activity in glomerular endothelial cells, *Kidney Int.* 59 (2001) 673–681.
- [140] G. Liu, F. Han, Y. Yang, et al., Evaluation of sphingolipid metabolism in renal cortex of rats with streptozotocin-induced diabetes and the effects of rapamycin, *Nephrol. Dial. Transplant.* 26 (2011) 1493–1502.
- [141] Y. Yang, J. Wang, L. Qin, et al., Rapamycin prevents early steps of the development of diabetic nephropathy in rats, *Am. J. Nephrol.* 27 (2007) 495–502.
- [142] N. Ueda, G.P. Kaushal, X. Hong, et al., Role of enhanced ceramide generation in DNA damage and cell death in chemical hypoxic injury to LLC-PK1 cells, *Kidney Int.* 54 (1998) 399–406.
- [143] A.G. Basnakanian, N. Ueda, X. Hong, et al., Ceramide synthase is essential for endonuclease-mediated death of renal tubular epithelial cells induced by hypoxia-reoxygenation, *Am. J. Physiol. Renal Physiol.* 288 (2005) F308–F314.
- [144] Y. Itoh, T. Yano, T. Sendo, et al., Involvement of de novo ceramide synthesis in radiocontrast-induced renal tubular cell injury, *Kidney Int.* 69 (2006) 288–297.

- [145] P.K. Datta, S.B. Koukouritaki, K.A. Hopp, et al., Heme oxygenase-1 induction attenuates inducible nitric oxide synthase expression and proteinuria in glomerulonephritis, *J. Am. Soc. Nephrol.* 10 (1999) 2540–2550.
- [146] N. Toda, T. Takahashi, S. Mizobuchi, et al., Tin chloride pretreatment prevents renal injury in rats with ischemic acute renal failure, *Crit. Care Med.* 30 (2002) 1512–1522.
- [147] I.R. Hassan, K. Gronert, Acute changes in dietary omega-3 and omega-6 polyunsaturated fatty acids have a pronounced impact on survival following ischemic renal injury, *J. Immunol.* 182 (2009) 3223–3232.
- [148] B.D. Levy, P. Kohli, K. Gotlinger, et al., Protectin D1 is generated in asthma and dampens airway inflammation and hyperresponsiveness, *J. Immunol.* 178 (2007) 496–502.
- [149] V.A. Mustad, S. Demichele, Y.S. Huang, et al., Differential effects of n-3 polyunsaturated fatty acids on metabolic control and vascular reactivity in the type 2 diabetic ob/ob mouse, *Metabolism* 55 (2006) 1365–1374.
- [150] L. Zhang, B.L. Peterson, B.S. Cummings, The effect of inhibition of  $\text{Ca}^{2+}$ -independent phospholipase  $\text{A}_2$  on chemotherapeutic-induced death and phospholipid profiles in renal cells, *Biochem. Pharmacol.* 70 (2005) 1697–1706.
- [151] M.A. Masood, R.P. Rao, J.K. Acharya, J. Blonder, T.D. Veenstra, Quantitation of multiple sphingolipid classes using normal and reversed-phase LC-ESI-MS/MS: comparative profiling of two cell lines, *Lipids* 47 (2012) 209–226.