The Updates of Podocyte Lipid Metabolism in Proteinuric Kidney Disease

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Keywords

Podocyte · Lipid accumulation · Proteinuric kidney disease · Diabetes · Inflammation

Abstract

Background: Podocytes, functionally specialized and terminally differentiated glomerular visceral epithelial cells, are critical for maintaining the structure and function of the glomerular filtration barrier. Podocyte injury is considered as the most important early event contributing to proteinuric kidney diseases such as obesity-related renal disease, diabetic kidney disease, focal segmental glomerulosclerosis, membranous nephropathy, and minimal change disease. Although considerable advances have been made in the understanding of mechanisms that trigger podocyte injury, cell-specific and effective treatments are not clinically available. **Summary:** Emerging evidence has indicated that the disorder of podocyte lipid metabolism is closely associated with various proteinuric kidney diseases. Excessive lipid accumulation in podocytes leads to cellular dysfunction which is defined as lipotoxicity, a phenomenon characterized by mitochondrial oxidative stress, actin cytoskeleton remodeling, insulin resistance, and inflammatory response that can

eventually result in podocyte hypertrophy, detachment, and death. In this review, we summarize recent advances in the understanding of lipids in podocyte biological function and the regulatory mechanisms leading to podocyte lipid accumulation in proteinuric kidney disease. **Key Messages:** Targeting podocyte lipid metabolism may represent a novel therapeutic strategy for patients with proteinuric kidney disease.

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Introduction

Proteinuria is not only a typical sign of kidney damage but also importantly contributes to the progression of chronic kidney disease (CKD) such as obesity-related glomerulopathy (ORG), diabetic kidney disease (DKD), focal segmental glomerulosclerosis (FSGS), membranous nephropathy (MN), and minimal change disease (MCD) [1]. Podocyte injury is considered as the most important early event in the pathogenesis of proteinuria and progressive glomerulosclerosis in patients with proteinuric kidney diseases [2]. Although considerable advances have been made in the understanding of mechanisms that trig-

ger podocyte injury, cell-specific and effective treatments are not clinically available.

Emerging evidence has revealed that the disorder of lipid metabolism is linked to renal dysfunction, as well as to several other pathological hallmarks of ORG and DKD [3–7]. In fact, in addition to obesity and diabetes, ectopic lipid accumulation is common in other kinds of proteinuric kidney diseases such as FSGS, MN, and MCD which has been confirmed in numerous experimental animal and clinical studies [8–11]. And different types of renal parenchymal cells have different sensitivities to pathologic lipid accumulation, which make different contributions to disease progression. Podocytes are vulnerable to lipid accumulation, resulting in their dysfunction which is defined as lipotoxicity, a phenomenon characterized by mitochondrial oxidative stress, actin cytoskeleton remodeling, insulin resistance, and inflammatory response. Importantly, pathologic lipid accumulation in podocytes is the consequence of the dysregulation of genes or proteins involved in cellular lipid metabolism, which comprises synthesis, uptake, storage, utilization, and cellular export of lipids rather than the amount of circulating lipids [12], indicating the importance of podocyte lipid metabolism in proteinuric kidney diseases. An increasing number of evidence suggests that not only the quantity of lipids but also the type of accumulated lipids may be responsible for cellular damage [4]. In this review, we summarize recent advances in the understanding of the lipids in podocyte biological function and the regulatory mechanisms leading to podocyte lipid accumulation in proteinuric kidney disease. Targeting podocyte lipid metabolism will pave the way to new therapeutic approaches in podocyte injury and proteinuric kidney disease.

Lipids in Podocyte Biological Function

Lipids are not only the key structural components of biological membranes but also potent signal transduction molecules, orchestrating intra- and extracellular signal transmission as well as acting as a source of energy [13]. The structure and function of podocytes largely depend on the integrity of slit diaphragm (SD), a lipid raft-like structure. In addition to sphingolipids, lipids especially cholesterol is enriched 5–8-fold in lipid rafts compared with the rest of the plasma membrane and play an important role in assuring proper localization and function of SD proteins including transmembrane proteins (nephrin), integral membrane proteins (podocin), structural proteins (alpha-actinin-4), signaling adapters (CD2-as-

sociated protein), ion channels (transient receptor potential cation channel 6) and other proteins involved in cell signaling. Fatty acids are also essential to form the phospholipid bilayers of the cell membranes and act as phospholipid messengers, transmitting vital intracellular signals. Moreover, large quantities of cholesterol esters and triacylglycerol (esterified fatty acid [FA]) forming a neutral lipid core, surrounded by a phospholipid monolayer, can accumulate in intracellular lipid droplets for storage [8].

Although FAs including saturated FAs (SFAs) and unsaturated FAs, together with glucose and amino acids, are generally used as energy sources for the formation of ATP, the peculiarities of podocyte energy metabolism keep controversy [14]. Regarding this issue, Abe et al. [15] first reported a primary role of mitochondria and, in particular, a high dependency of podocytes on mitochondrial energy transduction and on FAs as a metabolic fuel source in transformed mouse podocyte cell lines, while glycolysis makes a lesser contribution. On the contrary, Ozawa et al. [16] suggested that both glycolytic and oxidative phosphorylation (OXPHOS) pathways contribute to podocyte energy supply, depending on the intracellular sub-localization of mitochondria and on the cell differentiation status. Imasawa et al. [17] further recorded that high-glucose conditions force differentiating human podocyte cell lines to switch from OXPHOS to glycolysis, with consecutive lactic acidosis. Recently, Brinkkoetter et al. [18] provided comprehensive metabolomics data from freshly isolated glomeruli and primary podocytes, showing that under physiologic conditions, podocytes largely rely on metabolizing glucose as fuel to lactate (anaerobic glycolysis) and only to a minor extent on β -oxidation of FAs for ATP production. This baseline glucose preference can shift toward an enhanced FAs β -oxidation for metabolic reprogramming [19]. Yuan et al. [20] indicated that differentiated (mature) murine podocytes can promote both glycolysis and mitochondrial metabolism to meet their augmented energy demands. During the differentiation process, the predominant energy source from anaerobic glycolysis in immature podocytes is shifted to OXPHOS. Chen et al. [21] also showed that high glucose induces marked upregulation of lipogenic gene expression and the decrease of FA oxidation (FAO), which results in the ectopic lipid deposition in podocytes. Collectively, accumulating evidence suggests that the metabolic switch from mitochondrial respiration to glycolysis or vice versa in podocytes is not uniform but rather depends on the cell type and cell environmental context.

Table 1. Lipid and lipid metabolites that contribute to podocyte lipotoxicity

Class	Model	Outcome	Ref.
Saturated FAs			
Palmitic acid (16:0)*	MPC, DKD mice	↑ Podocyte injury and apoptosis, mitochondrial dysfunction, oxidative stress, ER stress, insulin resistance; ↑ albuminuria	[27–32]
Stearic acid (18:0)*	CKD patients	Decreased abundance in plasma associate with declining renal function	[33]
Unsaturated FAs			
Oleic acid (18:1)*	MPC, DKD mice	↓ Podocytes cell death and loss, ER stress, oxidative stress; ↑ proteasome expression; ↓ albuminuria, histologic changes	[28, 34]
Arachidonic acid (20:4)*	HPC	↑ Actin cytoskeleton remodeling, podocyte injury	[35]
Eicosapentaenoic acid (20:5)*	MPC	↓ Podocyte apoptosis, inflammation	[36]
Glycerolipids			
Triglyceride	CKD, metabolic syndrome mice	↑ Podocyte apoptosis; ↑ albuminuria, histologic changes	[37]
Phospholipids			
Phosphatidylcholine	MPC, DKD mice	Associate with podocyte injury and DKD	[38, 39]
Phosphatidylethanolamine	MPC, DKD mice	Associate with podocyte injury and DKD	[38, 39]
Cardiolipin	HPC, DKD mice	↑ Podocyte injury, mitochondrial dysfunction; ↑ albuminuria, histologic changes	[25]
Phosphosphingolipids			
Sphingomyelin	DKD mice	↑ Actin cytoskeleton remodeling, podocyte injury; ↑ albuminuria, histologic changes	[40]
Glycosphingolipids			
Ganglioside	HPC, MPC, DKD mice	↑ Actin cytoskeleton remodeling, podocyte injury; ↑ albuminuria, histologic changes	[41–43]
Other sphingolipids			
Ceramide	DKD mice	↑ Mitochondrial dysfunction, inflammation, insulin resistance, podocyte injury	[42, 44, 45]
Sphingosine	Primary MPC, podocytopathy and associated NS mice (<i>Asah1^{fl/fl}</i> / <i>Podo-Cre</i> mice)	↓ Exosome release of podocytes, podocyte injury; ↓ urinary exosome excretion	[46, 47]
Sphingosine-1-phosphate	CKD mice	↑ Albuminuria, histological changes; ↑ oxidative stress, mitochondrial dysfunction	[46, 48]
Ceramide-1-phosphate	HPC, DKD mice	↓ Inflammation, insulin resistance, podocyte apoptosis; ↓ albuminuria, histologic changes	[25, 40]
Sterol lipid			
Cholesterol	HPC, HN mice	↑ Podocyte apoptosis; ↑ albuminuria, histologic changes	[49, 50]
Cholesterol esters	HPC, DKD mice, AS mice	↑ Podocyte injury; ↑ albuminuria, histologic changes	[51]

Ref., reference; MPC, mouse podocytes; DKD, diabetic kidney disease; CKD, chronic kidney disease; ER, endoplasmic reticulum; HPC, human podocytes; NS, nephrotic syndrome; KO, knockout; Podo, podocyte; HN, hypertensive nephropathy; AS, Alport syndrome. * Number of carbon atoms: number of C = C double bonds.

Podocyte Lipid Accumulation in Proteinuric Kidney Disease

Podocytes are vulnerable to lipid accumulation. Excessive lipid accumulation in podocytes can lead to lipotoxicity characterized by mitochondrial oxidative stress [22], inflammatory responses [23, 24], actin cytoskeleton remodeling, insulin resistance, and endoplasmic reticulum (ER) stress, which eventually trigger podocyte hypertrophy, autophagy, dedifferentiation, mesenchymal transition, detachment to apoptosis, and death [25]. Podocyte injury is considered as a hallmark of proteinuric kidney diseases such as DKD, FSGS, MN, and MCD.

Normally, lipids are classified into 8 different categories including fatty acyls, glycerolipids, glycerophospholipids (GPs), sphingolipids, sterols, prenol lipids, saccharolipids, and polyketides [26]. Some of them that abnormally accumulate in podocytes mainly contribute to proteinuric kidney disease, including (1) fatty acyls: SFAs, monounsaturated FAs (MUFAs), polyunsaturated FAs (PUFAs), and PUFA-derivatives eicosanoids; (2) glycerolipids: monoacylglycerols, diacylglycerols, and triacylglycerols also termed as triglycerides; (3) GPs: phosphatidylcholine (PC), phosphatidylserine, phosphatidylethanolamine (PE), phosphatidylinositol, cardiolipin (CL), phosphatidylglycerol, and phosphatidic acid; (4) sphingolipids: phosphosphingolipids (sphingomyelin), glyco-

sphingolipids (gangliosides, globosides, and cerebro-sides), sulfatides, ceramide (-1-phosphate) (C1P), sphingosine (-1-phosphate) (S1P); and (5) sterol lipids: cholesterol and its derivatives cholesterol esters (Table 1).

Free Cholesterol and Cholesterol Ester

Cellular cholesterol homeostasis is highly regulated by cholesterol synthesis at the ER membrane, cholesterol uptake, and efflux (Fig. 1), all processes are tightly regulated and adapted to cellular needs. However, excess cholesterol accumulation in podocytes can perturb the SD, adversely affect podocyte function, and induce proteinuric kidney diseases [52–54].

In podocytes, circulating unoxidized or oxidized low-density lipoprotein (LDL) is the major source for cholesterol uptake into cells via the LDL receptor or c-x-c motif ligand 16, a scavenger receptor [55]. LDL and its receptor complexes are internalized by endocytosis and transport to the lysosome for degradation, resulting in the release of free cholesterol. The proteins Niemann-Pick C1 and C2 have major roles in the transport of free cholesterol from lysosomes to the ER and then to the plasma membrane. It is reported that the downregulation of phosphatase and tensin homolog in podocytes causes the inactivation of the actin depolymerizing factor cofilin-1 by increasing its phosphorylation. This response stimulates the formation of filamentous actin and promotes cholesterol endocytosis, resulting in cholesterol accumulation in podocytes [56].

The efflux of free cholesterol to high-density lipoprotein (HDL) acceptors is mediated by ATP-binding cassette (ABC) transporters such as ABC subfamily A member 1 (ABCA1) and subfamily G member 1 or 8 (ABCG1/8) in the presence of extracellular lipid-poor apolipoproteins (APO proteins) [8, 53]. The increase in cellular free cholesterol is converted to cholesterol ester via ER enzyme sterol-O-acetyltransferase-1 (SOAT1) leading to the formation of cholesterol-enriched lipid droplets. Cholesterol ester can be converted back to free cholesterol via neutral cholesterol esterhydrolase 1 (NCEH).

The accumulation of excess free cholesterol has been demonstrated to be toxic to cells. APO1, an essential component of HDL3 and highly expressed in podocytes, is the highest risk genetic variant of kidney disease and the main cause of glomerulosclerosis in the African-American population [57]. Human podocytes treated with the sera from the patients with DKD showed increased cholesterol accumulation in association with a re-

duction of ABCA1 [58]. Pedigo et al. [49] demonstrated that local TNF expression causes free cholesterol-dependent podocyte apoptosis in FSGS and DKD through a dual mechanism that requires a reduction in ABCA1-mediated cholesterol efflux and decreased cholesterol esterification by SOAT1 activity. Consistently, cholesterol accumulation was detected in podocytes from angiotensin II-infused mice, which was associated with ABCG1-mediated cholesterol efflux by epigenetic modification [50] (Table 1). However, some controversial studies suggested that free cholesterol is not cytotoxic to podocytes. They found that in diabetic *ob/ob* mice, podocyte-specific deletion of *Abca1* enhances podocyte injury which is not attributed to an accumulation of free cholesterol but to the mitochondrial membrane phospholipid CL accumulation. That is because an additional genetic deletion of *Soat1* that prevents cholesterol esterification had no further detrimental effect on glomerular injury [25].

It is known that cholesterol esterification and storage in lipid droplets are essential to maintain proper levels of free cholesterol. However, whether cholesterol ester accumulation is toxic and contributes to renal disease is still unclear [13]. Recent studies showed that blocking cholesterol esterification via SOAT1 inhibition in podocytes attenuates lipotoxicity-induced podocyte injury in DKD and Alport's syndrome in association with ABCA1-mediated cholesterol efflux [51].

Cholesterol synthesis is primarily controlled by the rate-limiting enzyme, 3-hydroxy-3-methyl-glutaryl-CoA reductase. The expression of genes encoding enzymes such as 3-hydroxy-3-methyl-glutaryl-CoA reductase and SOAT1 is regulated by members of the sterol-regulatory element-binding protein (SREBP) family of transcription factors. In addition, other nuclear receptors and transcription factors such as liver X receptor (LXR), farnesoid X receptor (FXR), peroxisome proliferators-activated receptors (PPARs), and nuclear factor of activated T cells are also involved in cholesterol synthesis and metabolism. Recently, our studies found that junctional adhesion molecule (JAM)-like protein (JAML) (Amica1), a novel member of JAM family, mediates podocyte lipid metabolism by regulating SREBP1 and its target genes involved in FAs and cholesterol synthesis. Mechanistically, histone deacetylase-mediated epigenetic processes in the regulation of lipid metabolism have attracted much attention. SIRT1 is one member of SIRT's (class III histone deacetylase) that are highly conserved NAD⁺-dependent deacetylases. SIRT1 not only serves as an important energy status sensor but also protects cells against metabolic stresses. In our studies, we further demonstrated SIRT1 links

(For legend see next page.)

JAML to SREBP1 signaling, which is consistent with the previous studies, showing that SIRT1 can regulate SREBP-1 expression and activity [38].

Saturated Fatty Acids and Triglycerides

Cellular FA homeostasis is regulated by synthesis, uptake, storage, and β -oxidation. Numerous studies have indicated that excessive free FA (FFA) accumulation plays a key role in podocyte injury and proteinuria [59]. FFA and triglyceride syntheses are controlled by SREBP-1c which targets lipogenic enzymes including acetyl-CoA carboxylase (ACC) and FA synthase (FASN). Furthermore, carbohydrate response element-binding protein (ChREBP) is an important transcription factor for cellular fat synthesis. ChREBP deficiency alleviated diabetes-associated renal lipid accumulation by inhibiting mTORC1 activity, suggesting that the reduction of ChREBP is a potential therapeutic strategy to treat DKD

[21]. In addition, other nuclear transcription factor receptors including LXR, FXR, and PPARs are also involved in FA synthesis and metabolism.

Disturbed transport and oxidation of FFAs, paralleled by an impaired antioxidant response, damages podocyte structure and leads to glomerulopathy during the early stage of DKD. FFAs can be transported into cells by CD36 (also known as scavenger receptor B2) [60], FA transport protein (FATP), FA-binding proteins (FABP), or via the assistance of vascular endothelial growth factor B (VEGF-B) [39]. CD36 is the main receptor for FFAs uptake in podocytes. CD36-dependent uptake of palmitic acid led to a dose-dependent increase in the levels of mitochondrial reactive oxygen species (ROS), depolarization of mitochondria, ATP depletion, and apoptosis [27, 60]. Podocyte-specific expression of FABP correlates with proteinuria in diabetic *db/db* mice and enhances FFAs induced podocyte injury [61, 62]. Falkevall et al. [39] suggested that VEGF-B promotes FFA accumulation in the glomeruli of DKD mice via upregulation of FATP4. Inhibiting VEGF-B

Fig. 1. Metabolism of cholesterol and FFAs and their regulatory mechanisms in podocytes. **a** Circulating LDL is the major source for cholesterol uptake via LDLR or CXCL16. LDL, and its receptor complexes are internalized by endocytosis and transport to the lysosome for degradation, resulting in the release of free cholesterol. NPC1/2 transports free cholesterol from lysosomes to the ER and then to the plasma membrane. The efflux of free cholesterol to HDL acceptors is mediated by ABCA1 and ABCG1/8 in the presence of extracellular APO proteins. Also, free cholesterol delivered into ER combines with FFAs to form cholesterol ester via SOAT1. At last, the cholesterol esters will be involved in the lipid droplets for storage. Cholesterol ester can be converted back to free cholesterol via NCEH. Cholesterol synthesis is primarily controlled by HMGCR. During cholesterol deficits, SREBP is transported to the Golgi apparatus and cleaved, allowing its translocation to the nucleus to regulate expression of cholesterol genes. Cholesterol synthesis is also regulated by other nuclear receptors and transcription factors such as LXR, FXR, PPARs, and NFAT. PTEN interferes with the endocytosis of LDL and SIRT6 contributes the export of free cholesterol by ABCG1. SIRT1, which is downregulated by JAML, inhibits the activity of SREBP1 and its target genes. **b** FFAs enter cells by endocytosis and transport via CD36, FATP, and FABP. Cellular FFAs are converted to fatty acyl-CoA which is transported into mitochondria via CPT1 and converted into acetyl-CoA by β -oxidation. In addition, acetyl-CoA is also derived from glucose uptake through GLUT4. Glucose is converted to pyruvate, which enters the mitochondria to form acetyl-CoA. Excess acetyl-CoA entering cytoplasm can be converted to PA by ACC and FASN activity, then extended to form SA in the ER. FFAs are commonly esterified to form TG, PL, and CE. SCD-1 converts SFAs to MUFAs that are incorporated into TG and stored in lipid droplet. DGAT is a microsomal enzyme that catalyzes the final step in TG synthesis. FFAs synthesis is controlled by SREBP-1c. Be-

sides, ChREBP, LXR, FXR, and PPARs are also important nuclear receptors and transcription factors involving in fat synthesis and metabolism. Furthermore, VEGF-B can induce the expression of FATP, increasing FFAs uptake. SIRT3 deletion aggravates FAO dysfunction. AMPK plays a central role in controlling FFAs metabolism through modulating the downstream ACC and CPT1. JAML deficiency decreases the expression of SREBP1 and its target genes through SIRT1 activation. LDL, low-density lipoprotein; LDLR, low-density lipoprotein receptor; CXCL16, c-x-c motif ligand 16; NPC1/2, Niemann-Pick C1 and C2; ER, endoplasmic reticulum; HDL, high-density lipoprotein; ABCA1, ATP-binding cassette transporters subfamily A member 1; ABCG1/8, ATP-binding cassette transporters subfamily G member 1 or 8; APO, apolipoproteins; FFAs, free fatty acids; SOAT, sterol O-acyltransferase; NCEH, neutral cholesterol esterhydrolase; HMGCR, 3-hydroxy-3-methyl-glutaryl-CoA reductase; SREBP, sterol-regulatory element binding protein; LXR, liver X receptor; FXR, farnesoid X receptor; PPARs, peroxisome proliferators-activated receptors; NFAT, nuclear factor of activated T cells; PTEN, phosphatase and tensin homolog; SIRT, NAD-dependent protein deacetylase sirtuin; JAML, junctional adhesion molecule-like protein; CD36, scavenger receptor B2; FATP, fatty acid transport protein; FABP, fatty acid binding proteins; CPT1, carnitine O-palmitoyltransferase 1; GLUT4, glucose transporter type 4; TCA, tricarboxylic acid; PA, palmitic acid; ACC, acetyl-CoA carboxylase; FASN, fatty acid synthase; SA, stearic acid; PL, phospholipid; TG, triglycerides; CE, cholesterol ester; SCD-1, stearoyl-CoA desaturase 1; SFAs, saturated fatty acids; MUFA, monounsaturated fatty acid; DGAT, diacylglycerol O-acyltransferases; ChREBP, carbohydrate-responsive element-binding protein; VEGF-B, vascular endothelial growth factor B; FAO, fatty acid oxidation; AMPK, adenosine 5'-monophosphate-activated protein kinase.

Fig. 2. Metabolism of sphingolipids and their regulatory mechanisms in podocytes. Ceramide is at the center of sphingolipid metabolism and can be generated through multiple pathways, including de novo synthesis, hydrolysis of sphingomyelin, or the recycling of other complex sphingolipids. First, in the de novo synthesis pathway, ceramide is formed from the condensation of L-serine and palmitoyl-CoA on the surface of the ER. Second, sphingomyelin can be hydrolyzed by sphingomyelinase to generate ceramide. SM synthetase can attach a PC head group onto ceramide to form sphingomyelin. Third, ceramide can be phosphorylated via CK to form ceramide-1-phosphate, another bioactive lipid. Ceramide, in turn, can be generated from ceramide-1-phosphate through the action of C1PPase. Fourth, ceramide can be further catabolized by CDase to sphingosine, which is then phosphorylated to S1P by SK. The efflux of S1P can be mediated by its transporters such as SPNS2, ABCA1, ABCG1, and ABCC1. Circulating S1P can bind to various S1P receptors and modulate MAPK signaling and small GTPase activity. Sphingosine can be recycled back for the generation of ceramide by CS, and S1P can either be dephosphorylated by S1PP or be irreversible cleaved by S1P lyase to form ethanolamine-1-phosphate and C16 fatty aldehyde. Fifth, ceramide can also be glycosylated to form the glucosylceramide or galactosylceramide, which can be converted back to ceramide by

hydrolyzation. Glucosylceramide can be further converted into complex glycosphingolipids, namely gangliosides (GM3), globosides (Gb3), and cerebrosides. Overexpression of SMPDL3b blocks CK activity and the conversion of ceramide to C1P, leading to the decrease in the levels of C1P. SMPDL3b is also a protein with homology to ASMase. Decreased expression of SMPDL3b may lead to decreased ASMase activity and accumulation of sphingomyelin. JAML can enhance high glucose induced the accumulation of ceramides and sphingomyelin. ER, endoplasmic reticulum; SM, sphingomyelin; SMase, sphingomyelinase; PC, phosphatidylcholine; DAG, diacylglycerol; C1PPase, ceramide-1-phosphate phosphatase; CK, ceramide kinase; CDase, ceramidase; SPNS2, Sphingolipid Transporter 2; ABCA1, ATP-binding cassette transporters subfamily A member 1; ABCG1, ATP-binding cassette transporters subfamily G member 1; ABCC1, ATP-binding cassette transporters subfamily C member 1; MAPK, mitogen-activated protein kinase; GTPase, guanosine triphosphate phosphatase; CS, ceramide synthase; SK, sphingosine kinase; S1PP, sphingosine-1-phosphate phosphatase; GCS, glucosylceramide synthase; GCase, glucosylceramidase; GalCS, galactosylceramide synthase; Gal-CDase, galactosylceramidase; SMPDL3b, sphingomyelinase-like phosphodiesterase 3b; JAML, junctional adhesion molecule-like protein; ASMase, acid sphingomyelinase.

signaling in DKD mouse models reduces renal lipotoxicity and re-sensitizes podocytes to insulin signaling.

The SFAs (also called nonesterified FA, NEFA) including palmitic acid and stearic acid, together with the MUFAs such as oleic acid account for 70–80% of plasma FFAs [63]. Podocytes are highly susceptible to damage from SFAs [27–33]. By contrast, MUFAs can attenuate palmitic acid-induced lipotoxicity [28, 34]. Stearoyl-CoA desaturase (SCD)-1, which converts SFAs to MUFAs, is up-

regulated in podocytes in biopsy samples from patients with DKD and ameliorates ER stress and podocyte injury [64]. PUFA such as arachidonic acid, as a major metabolite of phospholipase A2 group IB, regulates the actin bundling remodeling and contributes to the podocyte injury [35]. However, eicosapentaenoic acid and docosahexaenoic acid help lag the progression of CKD [36].

Cellular FFAs are commonly esterified to form 3 main classes of esters: triglycerides, phospholipids, and cholest-

terol esters or transported into mitochondria for β -oxidation and ATP production. Uptake of triglyceride-rich very low-density lipoprotein (VLDL) by podocytes is increased in CKD. Increased triglyceride accumulation leads to podocyte apoptosis and glomerulosclerosis [37]. Diacylglycerol O-acyltransferase (DGAT) is a microsomal enzyme that catalyzes the final step in triglycerides synthesis. Nephrotic syndrome has been reported to cause the upregulation of hepatic DGAT-1 expression and activity, which may lead to associated hypertriglyceridemia [65]. Kampe et al. [29] have found that inhibition of carnitine palmitoyltransferase (CPT1), the rate-limiting enzyme of FAO and downstream target of AMP-activated protein kinase (AMPK), augments palmitic acid-induced podocyte death. Moreover, SIRT3 deletion aggravates FAO dysfunction, resulting in increased apoptosis of kidney tissues and aggravated renal injury [66]. In our studies, we found JAML deficiency has lower neutral lipid deposition in glomeruli from mice with DKD and in high glucose-treated podocytes, which is result from the decrease in the expression of transcriptional factor SREBP1 and its targets, such as FASN, ACC1, and SCD1. In this process, we showed that SIRT1 activation correlates with the increase in AMPK activity and the reduction of SREBP1 [38].

Glycerophospholipids and Sphingolipids

More recently, GPs and sphingolipids have also been implicated in the pathogenesis of glomerular diseases [38, 39, 41, 46, 67] (Table 1). A specific modification of GPs is shown to correlate with kidney deterioration in diabetic subjects and mice [68]. Ducasa et al. [25] found that podocyte-specific *Abca1* deficiency leads to the accumulation of mitochondrial CL, which promotes podocyte injury in DKD. Pharmacologic induction of ABCA1 ameliorated podocyte injury and decreased CL oxidation [25].

Moreover, intracellular accumulation of SLs or metabolites in the form of ceramide, sphingosine, S1P, sphinganine, sphingomyelin, C1P, and glycosphingolipids (gangliosides GM3 and cerebroside) in podocytes may contribute to cellular dysfunction and the progression of proteinuric kidney disease [41, 46, 47] (Fig. 2). Ceramide represents the centerpiece of the sphingolipid metabolic pathway. Ceramide accumulation in podocytes induces mitochondrial damage through ROS production in patients with DKD [44]. Podocyte-specific deletion of the main catalytic subunit of acid ceramidase resulted in ce-

ramide accumulation in glomeruli and development of nephrotic syndrome [45]. Acid sphingomyelinase overexpression also leads to ceramide accumulation and glomerular sclerosis in mice [42].

Ceramide can be further catabolized to sphingosine by ceramidases. Then, sphingosine is phosphorylated to S1P by sphingosine kinase. In addition, ceramide kinase catalyzes the formation of another bioactive lipid, C1P. Studies have showed that increased expression of S1P is associated with increased ROS production and renal injury in CKD [48]. S1P lyase is reported to play a role in the development of proteinuria in mice [69]. Genetic mutations in the gene coding for S1P lyase are associated with severe podocyte injury and nephrotic syndrome in humans [70, 71]. Moreover, diabetic *db/db* mice have less total level of C1P in kidney cortices. Consistently, exogenous administration of C1P protects from DKD progression [40, 72]. Falkevall et al. [39] demonstrated that reducing VEGF-B signaling in *db/db* mice prevents renal lipotoxicity by reducing the accumulation of neutral lipid, GPs, and sphingolipids. Our recent studies have also indicated that JAML not only enhances high glucose-induced the accumulation of FFAs and cholesterol ester but also GPs (such as PC and phosphatidylethanolamine), sphingolipids (such as ceramides), which contributes to podocyte injury by lipidomics analysis. However, the mechanisms by which JAML regulates GPs and sphingolipids need to be further investigated [38].

In addition, other sphingolipids such as glycosphingolipid, especially gangliosides, also contribute to DKD development. Gangliosides GM3, GD3, and O-acetylated disialosyllactosylceramide are the most abundant gangliosides in the kidney. 9-O-acetylated disialosyllactosylceramide is a podocyte-specific ganglioside [41, 42]. In podocytes, GM3 located in lipid raft domains of the SD, binding with the soluble vascular endothelial growth factor receptor, is of very importance to the maintenance of actin cytoskeleton and the prevention of proteinuria [43]. Sphingomyelinase-like phosphodiesterase 3b (SMPDL3b), a lipid raft enzyme, was previously reported to modulate podocyte injury in the context of DKD or FSGS [73]. Studies have indicated that SMPDL3b-positive podocytes are decreased in renal biopsies of patients with recurrence of FSGS. It was found that decreased expression of SMPDL3b leads to decreased acid-sphingomyelinase activity and the accumulation of sphingomyelin, contributing to the pathogenesis of FSGS. In addition, human podocytes treated with the sera from patients with FSGS had decreased SMPDL3b, which was associated with actin cytoskeleton remodeling and apoptosis [74]. However, unlike FSGS, SMPDL3b expres-

Table 2. Therapeutic strategies

Agent	Category	Pathway	Disease model	Outcome	Ref.
Statin	Lipid-lowering drug	HMG-CoA reductase	CKD	↓ Proteinuria, histologic changes, oxidative stress/apoptosis, and podocyte injury	[82, 84]
Fenofibrate	Fibrate	PPARα agonist	HFD	↓ Albuminuria, histologic changes, oxidative stress/fibrosis, and lipid accumulation	[85]
Fenofibrate	Fibrate	PPARα agonist/ AMPK-PGC-1-axis	<i>db/db</i> mice	↓ Albuminuria, histologic changes, inflammation/oxidative stress, apoptosis/fibrosis, and lipid accumulation	[86]
AdipoRon	Adiponectin receptor agonist	AMPK/PPARα pathway	<i>db/db</i> mice	↓ UACR, oxidative stress/apoptosis/fibrosis, and lipid accumulation	[7, 87]
JNJ 39933673	SGLT2 inhibitor	ChREBP-β, SREBP-1	<i>db/db</i> mice	↓ UACR, histological changes, inflammation/fibrosis, lipid accumulation, and podocyte injury	[88]
Dapagliflozin	SGLT2 inhibitor	SREBP-1c	WD-induced obesity mice	↓ UACR, histologic changes, fibrosis, lipid accumulation, and podocyte injury	[88–90]
Obeticholic acid (INT-747)	FXR agonist	Glutathione metabolism pathway	HFD + uninephrectomy	↓ UACR, histologic changes, oxidative stress/apoptosis, and lipid accumulation	[91]
INT-777	Selective TGR5 agonist	TGR5	<i>db/db</i> mice, DIO mice	↓ Proteinuria, histologic changes, inflammation/fibrosis, FA accumulation, podocyte injury	[92]
INT-767	FXR/TGR5 dual agonist	SREBP-1 pathway; TGF-β pathway	<i>db/db</i> mice, STZ-treated mice and DIO mice	↓ Proteinuria, histologic changes, fibrosis, lipid accumulation, and podocyte injury	[93]
Neutralizing monoclonal VEGF-B antibody	VEGF-B antagonism	VEGF-B signaling	Podo- <i>VegfB</i> KO; <i>db/db</i> mice; double KO; HFD or STZ-treated mice	↓ UACR, histologic changes, inflammation, lipid accumulation, and podocyte injury	[39]
Cyclosporin A2/2-hydroxypropyl-β-cyclodextrin	Calcineurin inhibitor/cholesterol chelator	TNF/NFAT/ABCA1/SOAT1 signaling	Podo- <i>Abca1</i> KO, double, triple, and inducible KO mice	↓ UACR, histologic changes, inflammation/oxidative stress/apoptosis, cholesterol accumulation, and podocyte injury	[49, 58]
A30/elamipretide	ABCA1 inductor/cardioprotective peroxidase inhibitor	Mitochondrial dysfunction pathway	Podo- <i>Abca1</i> KO; <i>Soat</i> KO, <i>db/db</i> and <i>BTBR ob/ob</i> mice	↓ UACR, histologic changes, oxidative stress/mitochondrial dysfunction, lipid accumulation, and podocyte injury	[25]
Sandoz 58-035	SOAT1 inhibitor	ABCA1/SOAT1 signaling	<i>db/db Soat</i> KO mice; Col4a3 KO (AS) mice	↓ UACR, histologic changes, cholesterol accumulation, and podocyte injury	[51]
Ceramide-1-phosphate	Lipid supplementation	SMPDL3b/C1P/IR/Cav-1/Akt signaling	Podo- <i>Smpdl3</i> KO, double KO, and <i>db/db</i> mice	↓ UACR, histologic changes, lipid accumulation, podocyte injury	[40]
Rituximab	CD20 monoclonal antibody	SMPDL3b, ASMase	Patients with recurrent FSGS, human podocytes treated with serum from patients	↓ UACR, histologic changes, lipid accumulation, and podocyte injury	[74]
Berberine	Flavonoid	Drp1	<i>db/db</i> mice	↓ UACR, histologic changes, oxidative stress, mitochondrial dysfunction, lipid accumulation, and podocyte injury	[32]

Ref., reference; HMG-CoA, β-hydroxy-β-methylglutaryl-coenzyme A; CKD, chronic kidney disease; PPAR, peroxisome proliferator-activated receptor; HFD, high-fat diet; AMPK, AMP-activated protein kinase; PGC-1, peroxisome proliferator activated receptor-gamma coactivator-1; *db/db*, leptin receptor-deficient; UACR, urinary albumin creatinine ratio; SGLT2, sodium-glucose cotransporter 2; ChREBP, carbohydrate-responsive element-binding protein; SREBP, sterol regulatory element-binding protein; WD, western diet; FXR, farnesoid X receptor; TGR5, G protein-coupled bile acid receptor; DOI mice, diet-induced obesity mice; TGF-β, transforming growth factor beta; STZ, streptozotocin; Podo, podocyte; VEGF-B, vascular endothelial growth factor B; SOAT1, sterol O-acyltransferase 1; KO, knockout; TNF, tumor necrosis factor; NFAT, nuclear factor of activated T-cells; SOAT1, sterol O-acyltransferase 1; ABCA1, ATP binding cassette A1; AS, Alport syndrome; BTBR, black tan and brachyury; *ob/ob*, leptin-deficient; SMPDL3b, sphingomyelin phosphodiesterase acid-like 3b; C1P, ceramide-1-phosphate; IR, insulin receptor; Cav-1, caveolin-1; Akt, protein kinase B; ASMase, acid-sphingomyelinase; FSGS, focal segmental glomerulosclerosis; Drp1, dynamin-related protein 1.

sion was upregulated in glomeruli from patients with DKD and in human podocytes treated with DKD sera. Notably, overexpression of SMPDL3b resulted in decreased C1P levels in human podocytes in vitro and in the kidney cortex of mice with DKD in vivo [40]. Podocyte-specific *Smpdl3b*-deficiency had restored levels of C1P in the kidney cortex and protected from DKD. These results indicate that SMPDL3b may play different roles in the different pathogenesis of kidney disease.

LDs That Store Intracellular Cholesterol Esters and Triglycerides

Lipid droplets (LDs) are storage organelles consisting of a neutral lipid core (cholesterol ester and triglyceride) surrounded by a phospholipid monolayer and a set of LD-specific proteins. Whether LDs exert cytoprotective or cytotoxic effects in podocytes remains unknown. They are commonly considered a cytoprotective mechanism [13]. Some studies showed that they directly contact other organelles such as mitochondria, endosomes, Golgi complex, and peroxisomes, which are critical to buffer the levels of toxic lipid species [75]. In addition, LDs can act as a protective reservoir for unfolded proteins and toxins by preventing interactions with other cellular compartments [76]. Also, they have the potential to reduce podocyte toxicity, autophagic flux, and cell death by scavenging and storing the disease-associated APOL1 risk variants G1 and G2 [77]. Furthermore, LDs protect mitochondria by sequestering FAs and thus prevent an abnormal flux of FAs into acylcarnitine, which at high levels is toxic to mitochondria [78]. Recently, they have been shown to be innate immune hubs that integrate cell metabolism and host defense, highlighting a positive role of intracellular lipids as key regulators of cell function and survival [79]. However, some studies observed that LDs accumulate in glomeruli of renal biopsy samples from diabetic patients, which coincides with the presence of oxidative stress markers [80]. Therefore, LDs are very active scavenging organelles that can coordinate the function of other organelles in response to stress by modulating storage and lipolysis across different subcellular compartments [81].

Therapeutic Strategies

Statins

Statins are a group of drugs (such as lovastatin and simvastatin) that inhibit the synthesis of cholesterol and

promote the production of LDL-binding receptors in the liver resulting in a usually marked decrease in the level of LDL and a modest increase in the level of HDL circulating in blood plasma. A recent meta-analysis demonstrated that although statins reduces proteinuria and mortality, this effect was not sufficient to slow the clinical progression of non-end-stage CKD [82]. However, all available clinical guides suggest that controlling LDL cholesterol is a part of the multi-target approach in the treatment of DKD [83, 84]. Therefore, other therapies are needed to lower cellular toxic lipid levels (Table 2).

PPAR Agonists

PPAR α/γ agonists are recognized as a potential therapy to treat renal lipotoxicity and DKD [94]. For example, fenofibrate, a potent PPAR α agonist, improves albuminuria, inhibits intrarenal lipid accumulation, and prevents apoptosis and oxidative stress [86]. In mice fed with high-fat diet, fenofibrate can reduce oxidative stress and lipid accumulation in glomeruli and prevent the development of albuminuria and glomerular injury [85].

Adiponectin Receptor Agonists

Adiponectin exerts favorable effects in diabetes mellitus and metabolic syndrome through its anti-inflammatory, antifibrotic, and antioxidant effects. It mediates FA metabolism by inducing AMPK phosphorylation and increasing PPAR α expression through binding to its receptors, adipoR1, and adipoR2, respectively, which in turn activates PPAR γ coactivator 1 α . Moreover, adiponectin potently stimulates ceramidase activity associated with its 2 receptors and enhances ceramide catabolism and the formation of its antiapoptotic metabolite, S1P [95]. Recent studies have shown adipoRon, an orally active synthetic adiponectin receptor agonist, is able to reduce lipotoxicity and podocyte injury by activating the Ca²⁺/liver kinase B1-AMPK/PPAR α pathway in type 2 diabetes-associated DKD [7]. In addition, adipoRon can lower cellular ceramide levels by activation of acid ceramidase, which hydrolyzes ceramide to form sphingosine leading to an increase in S1P, and finally decrease renal pototoxicity, inflammation, and insulin resistance in diabetic mice [87].

Sodium-Glucose Cotransporter-2 Inhibitors

The sodium-glucose cotransporters (SGLTs) are a family of glucose transporters that contribute to renal glucose reabsorption. Studies from SGLT1 and SGLT2 knockout mice have indicated that 97% of proximal tubular glucose transport is mediated via SGLT2 and only

3% by SGLT1 [96]. SGLT2 inhibitors are a new class of antidiabetic drugs with promising effects on the treatment of patients with DKD [97] or nondiabetic CKD [89]. Through glucosuria, SGLT2 inhibitors reduce body weight and body fat and shift substrate utilization from carbohydrates to lipids and, possibly, ketone bodies. It has been reported that in *db/db* mice, JNJ 39933673, a selective SGLT2 inhibitor prevented the development of DKD and modulated renal lipid metabolism, at least in part through inhibition of the transcriptional factor ChREBP- β and SREBP1. Subsequently, their target genes pyruvate kinase, SCD-1, and DGAT1 expression were decreased [88]. Moreover, dapagliflozin, a highly selective inhibitor of renal SGLT2, prevented podocyte injury, glomerular pathology, and renal fibrosis in the kidney from Western diet-fed mice by reducing renal lipid accumulation [90].

Bile Acid Receptors Agonists

Bile acids, as signaling molecules, can also regulate glucose and lipid homeostasis as well as energy expenditure by activating bile acids receptors. Two major receptors for bile acids have been identified as follows: the nuclear receptor FXR and the membrane-bound, bile acid-activated G protein-coupled receptor TGR5 (GPBAR1 or GPR131). Previous studies indicated that FXR agonist obeticholic acid (INT-747) attenuated renal injury, renal lipid accumulation, and lipid peroxidation in uninephrectomized obese mice [91]. Moreover, TGR5 was downregulated by high glucose and/or FAs in ORG and DKD. A selective TGR5 agonist INT-777 reduced proteinuria, podocyte injury, mesangial expansion, fibrosis, and CD68 macrophage infiltration in the kidney. INT-777 also induced mitochondrial biogenesis and prevented oxidative stress and lipid accumulation, thus establishing a protective role of TGR5 in the inhibition of ORG and DKD [92]. Recent results showed that the dual FXR/TGR5 agonist INT-767 combined effects of both singular activation of FXR and TGR5, improved proteinuria and prevented podocyte injury, inhibited the progression of DKD and ORG [93].

VEGF-B Signaling Inhibition

VEGF-B, through binding with receptors such as VEGFR1 and neuropilin-1, induces the expression of the FA transport proteins FATP3 and FATP4, increasing lipid accumulation. Reducing of VEGF-B signaling or administration of neutralizing VEGF-B antibodies decreases lipid accumulation in podocytes and has renoprotective effects in type 1 and type 2 diabetes mice [39, 98].

Others

2-Hydroxypropyl- β -cyclodextrin has shown the protective effects on podocytes by preventing cholesterol accumulation mediated by ABCA1 upregulation [49, 58]. ABCA1 deficiency was associated with the selective accumulation of CL and subsequent podocyte injury in DKD. Cardiolipin peroxidase inhibitor, elamipretide, reversed DKD progression, with improvements in podocyte number, mesangial expansion, and mitochondrial morphology [25]. Sandoz 58-035, an SOAT1 inhibitor, prevented cholesterol ester accumulation in podocytes by increasing ABCA1 expression and may treat renal disease associated with DKD and Alport syndrome [51]. Exogenous C1P replacement was sufficient to restore the insulin-mediated prosurvival signaling pathway in human podocytes and to normalize urinary albumin levels in mice with DKD [40]. Rituximab, a monoclonal antibody directed against CD20 on B lymphocytes, also prevented recurrent FSGS by modulating podocyte function in an SMP-DL-3b-dependent manner [74]. Moreover, berberine, a kind of isoquinoline alkaloid present in Chinese herbal medicines, protected glomerular podocytes via inhibiting dynamin-related protein-1-mediated mitochondrial fission and dysfunction [32]. Besides aforementioned promising treatments, there are many other therapeutic targets have been evaluated in different preclinical models, such as LXRA agonists, pan-TGF β neutralizing antibodies, NF- κ B inhibitors, and fibroblast growth factor-21 therapy [94].

Conclusion

In this review, we summarize recent advances in the understanding of lipids in podocyte biological function and the regulatory mechanisms leading to podocyte lipid accumulation in proteinuric kidney disease. We also provide evidence showing the therapeutic effects of rebalancing the podocyte lipid metabolism on podocyte injury and proteinuric kidney disease. Collectively, pharmacologic targeting of podocyte lipid metabolism will pave the way to new therapeutic approaches in podocyte injury and for the treatment of DKD and other proteinuric kidney diseases.

Conflict of Interest Statement

Dr. Fan Yi is an associate editor of *Kidney Diseases*.

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Author Contributions

Y.S. wrote the manuscript; S.C. and Y.H. prepared the figures and the tables; and F.Y. decided on the topics and wrote the manuscript.

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