Systemic and renal lipids in kidney disease development and progression

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Wahl P, Ducasa GM, Fornoni A. Systemic and renal lipids in kidney disease development and progression. Am J Physiol Renal Physiol 310; F433–F445, 2016. First published December 23, 2015; doi:10.1152/ajprenal.00375.2015.—Altered lipid metabolism characterizes proteinuria and chronic kidney diseases. While it is thought that dyslipidemia is a consequence of kidney disease, a large body of clinical and experimental studies support that altered lipid metabolism may contribute to the pathogenesis and progression of kidney disease. In fact, accumulation of renal lipids has been observed in several conditions of genetic and nongenetic origins, linking local fat to the pathogenesis of kidney disease. Statins, which target cholesterol synthesis, have not been proven beneficial to slow the progression of chronic kidney disease. Therefore, other therapeutic strategies to reduce cholesterol accumulation in peripheral organs, such as the kidney, warrant further investigation. Recent advances in the understanding of the biology of high-density lipoprotein (HDL) have revealed that functional HDL, rather than total HDL per se, may protect from both cardiovascular and kidney diseases, strongly supporting a role for altered cholesterol efflux in the pathogenesis of kidney disease. Although the underlying pathophysiological mechanisms responsible for lipid-induced renal damage have yet to be uncovered, several studies suggest novel mechanisms by which cholesterol, free fatty acids, and sphingolipids may affect glomerular and tubular cell function. This review will focus on the clinical and experimental evidence supporting a causative role of lipids in the pathogenesis of proteinuria and kidney disease, with a primary focus on podocytes.

cholesterol; dyslipidemia; kidney disease; lipids; podocytes

HYPERLIPIDEMIA IS A KNOWN risk factor for cardiovascular disease (97). However, the role of hyperlipidemia as a risk factor for the development and progression of chronic kidney disease (CKD) remains controversial, and also controversial remains the role of statins in the prevention of CKD development and progression (2). Although lipid accumulation has been described in the kidneys of patients with kidney disease (55, 63, 64, 66, 88, 89), if and how CKD is a “fatty kidney disease,” the mechanisms leading to glomerular lipid accumulation, and the relative contribution of these lipids to renal injury remain less understood. Here, we will review the clinical and experimental evidence of how systemic and local disorders of cholesterol metabolism may contribute to CKD development and progression, with a primary focus on how cholesterol and other lipids may affect podocyte biology.

Circulating Cholesterol and Lipoproteins and Kidney Disease

Overview of lipid abnormalities in nephrotic syndrome and CKD. Lipid abnormalities can present themselves in the early stages of CKD and may actively participate in the increased cardiovascular morbidity and mortality observed in patients with CKD (65). Concomitant diseases, as well as available therapeutic strategies to reduce proteinuria and CKD progression, may further worsen dyslipidemia in affected patients. In nephrotic syndrome with or without CKD, both total cholesterol and low-density lipoprotein (LDL) levels are elevated (31). In fact, increased glomerular basement permeability is associated with the loss of lipoprotein lipase activators, resulting in hyperlipidemia (89). Nephrotic syndrome is also associated with severe hypertriglyceridemia, and recent discoveries have identified angiopoietin-like 4, and its degree of sialylation has an attractive therapeutic target for proteinuria and hypertriglyceridemia in nephrotic syndrome (80).

CKD is characterized by increased levels of triglycerides, small dense and oxidized LDL (oxLDL), and lower high-density lipoprotein (HDL)-cholesterol (HDL-C) levels (5). Quantitative lipid abnormalities in predialysis CKD patients include hypertriglyceridemia, increased concentrations of triglyceride-rich lipoprotein remnants, reduced HDL-C levels, as well as increased concentrations of lipoprotein (a) (149). Moreover, total and LDL-cholesterol (LDL-C) levels are usually within normal limits or slightly reduced in these individuals (146). CKD can also affect the composition of lipoproteins because it suppresses the activity of enzymes, such as lecithin-cholesterol acyltransferase (LCAT), while activating enzymes such as plasma cholesteryl ester transfer protein (CETP), resulting in the formation of immature HDL (146). The presence of lipoproteins with altered composition, combined with a
Inflammation is also the cause of resistance to statin treatment by the inflammatory stress associated with CKD, where in 1982. This hypothesis was recently updated to include “lipid nephrotoxicity” hypothesis by Moorhead et al. of dyslipidemia on decreased kidney function was first advocated to kidney disease. In analogy to atherosclerosis, the effect of dyslipidemia on decreased kidney function was first advocated as a “lipid nephrotoxicity” hypothesis by Moorhead et al. (89) in 1982. This hypothesis was recently updated to include the modification of lipid homeostasis and tissue lipid accumulation by the inflammatory stress associated with CKD, where inflammation is also the cause of resistance to statin treatment (121).

**LDL**. CKD leads to the generation of small dense LDL particles, as well as elevation of plasma levels of IDL and chylomicron remnants (147). These lipoproteins are highly prone to oxidation to lipid peroxides and other secondary oxidation products. Accumulation of oxidized LDL, IDL, and chylomicron remnants stimulates monocytes and macrophages to release proinflammatory cytokines and chemokines and accelerates inflammation (44), which, in turn, may promote the progression of CKD. While reduction of LDL with statins has demonstrated a consistent reduction in albuminuria in large meta-analysis, this did not result in preservation of GFR, suggesting that statins, while essential to protect from macrovascular complications, may not affect the progression of CKD (2). In fact, while statins consistently reduce CKD progression in experimental models (106, 144), a minimal but significant effect on GFR was reported only in a subgroup of patient with diabetes and albuminuria (29). These observations strongly suggest that targeting cholesterol synthesis may not protect from CKD progression but do not exclude the possibility that targeting cholesterol uptake and/or efflux from peripheral target organs may be beneficial. In fact, LDL apheresis has been suggested to be an effective measure to reduce proteinuria and podocyte excretion in patients with T2D and DKD (96), as well as in refractory nephrotic syndrome (94), suggesting that cholesterol uptake via LDL and/or inflammatory responses to oxidized LDL may participate in the development of proteinuria and the progression of CKD.

**HDL**. Low HDL has been reported to be an independent risk factor in the development of kidney disease (93, 104, 130). The ADVANCE study is a large prospective analysis of glycemic control and blood pressure lowering in patients with T2D at high risk for vascular events (1, 110). This study specifically analyzed HDL-C levels and risk of microvascular disease in patients with T2D and concluded that HDL-C level was a significant and independent predictor of the development and progression of DKD. Patients in the lower third of baseline HDL-C had a 19% higher risk of nephropathy compared with patients in the highest one-third (91). More recently, a reduction in HDL-C was closely associated with DKD in patients with T2D (125), consistent with the fact that impaired cholesterol efflux in isolated macrophages from patients with T2D correlates with DKD progression (161). Patients with CKD tend to have alterations in both HDL-C and HDL-C quality. HDL-C had a 19% higher risk of nephropathy compared with patients in the lowest one-third (91). More recently, a reduction in HDL-C was closely associated with DKD in patients with T2D (125), consistent with the fact that impaired cholesterol efflux in isolated macrophages from patients with T2D correlates with DKD progression (161). Patients with CKD tend to have alterations in both HDL-C and HDL-C quality. HDL-C had a 19% higher risk of nephropathy compared with patients in the lowest one-third (91).
due, in part, to decreased adipose tissue lipoprotein lipase activity (62). Studies on how these HDL abnormalities may contribute to CKD warrant further research, even more so in light of the fact that HDL has additional anti-inflammatory and antioxidant properties (139), which could influence the development and progression of CKD. However, if impaired cholesterol efflux and/or production of a nonfunctioning HDL is a cause rather than a consequence of CKD remains to be established and is the subject of intensive research studies in our laboratory. In the general population, higher plasma concentrations of HDL-C are associated with atheroprotective properties (2a, 46, 124), yet recent studies have demonstrated that raising the plasma HDL-C concentration does not necessarily reduce cardiovascular risk (9, 18). In addition, genetic variants associated with a higher HDL-C concentration are not associated with a reduced risk of cardiovascular disease (151). The failure to observe cardiovascular protection in multiple trials with CETP inhibitor should not discourage additional research in the field, as CETP inhibitors were selected on the basis of their ability to raise HDL and not on their ability to increase the function of HDL. Therefore, further research is necessary to determine whether agents capable of increasing cholesterol efflux, such as HDL mimetic peptides or recombinant apolipoprotein A-I (ApoA1) may represent new therapeutic strategies for both cardiovascular and kidney diseases.

LCAT. Calabresi et al. (23) reported several abnormalities in HDL particles in predialysis CKD patients; low plasma HDL apolipoprotein levels, low content of LpA-I:A-II particles, and a high content of pre-HDL compared with healthy subjects, which were exacerbated with HD treatment. The abnormal HDL profile is similar of that found in individuals with genetic LCAT deficiency (22). Plasma LCAT concentration and activity were reduced in CKD patients, and there was an elevated plasma unesterified/total cholesterol ratio, which is consistent with a common metabolic defect as a major cause of the low plasma HDL level in patients with genetic LCAT deficiency and in those with CKD (both HD and no HD treatment). There is evidence that LCAT concentration/activity leads to defective cholesterol esterification, impaired pre-HDL maturation, and accelerated catabolism of LpA-I:A-II particles (118). Given the role of a low HDL level in the progression of CKD (8), therapeutic strategies aimed at reducing the acquired LCAT defect could be effective at reversing dyslipidemia and slowing disease progression in patients with CKD.

Triglycerides:HDL-C. Recent studies have stressed the importance of additional lipid targets other than LDL-C to benefit the diabetic population at high residual risk for microvascular disease. In a recent global study, strong evidence for independent associations for high triglycerides and low HDL-C with DKD has been shown (125). Triglycerides and HDL-C were significantly and independently associated with DKD. These associations were similar in magnitude among the sites and among geographic regions. Therefore, current guidelines for lipid treatment give more emphasis than before on the use of triglycerides and HDL-C for treatment thresholds and targets for the prevention of microvascular and macrovascular complications (24). In fact, another study of 124,700 participants in the Japanese Specific Health Check and Guidance System demonstrated that the triglyceride (TG):HDL-C ratio affects the incidence and progression of CKD (143). The study examined the involvement of TG/HDL-C ratio at baseline with 2-yr changes in eGFR and increases in urinary protein excretion in the entire population of participants and with new-onset CKD, and low eGFR and proteinuria among those without CKD. The study also examined the involvement of TG:HDL-C ratio at baseline with a decrease in eGFR and increase in urinary protein excretion in CKD patients. This study revealed significant involvement of TG:HDL-C ratio, and supporting a higher TG:HDL-C ratio is an independent risk factor for the incidence and progression of CKD, suggesting that elevated levels of small dense LDL-C might induce and aggravate CKD. The mechanism of the impact of TG:HDL-C ratio on decline in eGFR and incidence of CKD are considered to involve TG:HDL-C ratio as a marker of LDL particle size. Further long-term studies with hard kidney disease outcomes, such as the development of end-stage renal disease are required to clarify the causative relationship between serum TG:HDL-C ratio and CKD.

**Genetic Disorders of Cholesterol Metabolism and Kidney Disease**

**Evidence of cholesterol accumulation in kidney diseases.** Lipid deposition is frequently observed in kidney biopsy specimens and can be mediated by infiltrating macrophages or directly affected by resident glomerular cells. This phenomenon, described as glomerular lipidosis, can occur in several disorders of genetic origin, such as familiar type III hypercholesterolemia (138) and LCAT deficiency, in which renal accumulation of lipoprotein X (LpX) and lipid droplets was found in glomeruli (69, 102). Glomerular lipidosis, however, is also observed in diseases of nongenetic origin, such as focal and segmental glomerulosclerosis (127) and diabetic glomerulosclerosis (55). Although many of such diseases suggest that there may be a link between altered lipid metabolism and kidney disease development and progression, additional experimental studies are needed to determine whether and how altered glomerular cholesterol metabolism may cause proteinuria and glomerulosclerosis. We will review in this paragraph the clinical and experimental evidence supporting a link of causality between lipoproteins/cholesterol metabolism and kidney disease. We will not discuss the contribution of free fatty acids and angiotensin-like 4, as these have been extensively reviewed elsewhere (28, 80).

**APOL1.** The APOL1 gene encodes a secreted high-density lipoprotein, which colocalizes with APOA1 in HDL particles (34), where APOA1 promotes efflux of cholesterol from cells. Two amino acid substitutions in the coding sequence of APOL1, S342G, and I384M (G1), and a two amino acid deletions, N388 and Y389 (G2), were identified. Individuals with one risk allele (G1 or G2) have no or only minimally increased kidney disease risk. However, individuals of African ancestry with two risk alleles have a substantially increased risk of focal segmental glomerulosclerosis (FSGS), HIV-associated nephropathy (HIVAN), arterionephrosclerosis (hyper-tension-attributed kidney disease), and nondiabetic end-stage kidney disease (ESKD). Immunofluorescent staining demonstrated APOL1 expression in normal human kidneys localized to podocytes of the glomerulus, the proximal tubules, and the extraglomerular arterial endothelium. In kidney biopsies from patients with HIVAN and FSGS, a decrease in podocyte APOL1 expression levels and the de novo appearance of...
APOL1 within cells of the arterial medial wall were observed (81). APOL1 expression in normal human podocytes suggests that APOL1 may be contributing to podocyte function under physiological conditions. However, genetic absence of APOL1 is compatible with normal renal function (61), and APOL1-deficient podocytes are normal (70). On the contrary, APOL1 can be dramatically induced by interferons and Toll-like receptor agonists (TLR), and overexpression of any APOL1 risk variants is toxic to human cells (70, 99). It has also been suggested that APOL1 may induce podocyte injury via activation of autophagy (27, 152). More recently, the possibility that APOL1 may influence calcium permeability in oocytes has opened new research opportunities (54). However, the dependency of APOL1 risks variants on cell toxicity remains to be established, as a variant-independent effect was observed in oocytes. Finally, it is also possible that APOL1 is directly involved in cholesterol metabolism in podocytes. As we have demonstrated, the importance of cholesterol efflux pathways as modulators of podocyte function (88) and APOL1 is a component of HDL particles, it is possible that APOL1 modulates cholesterol efflux in podocytes and that this may be a mechanism by which APOL1 contributes to the pathogenesis of glomerular diseases, such as HIVAN and FSGS.

LCAT. LCAT deficiency is an autosomal recessive disorder caused by mutations of the LCAT gene resulting in familial LCAT deficiency. LCAT deficiency is a genetic disease in which esterification of free cholesterol in the plasma is impaired. Patients with familial LCAT deficiency are characterized as having, among other symptoms, nephrotic-range proteinuria with chronic progressive glomerulopathy, resulting in renal failure. An accumulation of lipid deposits in the glomerular basement membrane and in the mesangial region of the kidney has been observed (14, 105). As affected patients have increased lipoprotein-X in the sera, and Lcat-deficient mice on an atherogenic diet are characterized by renal accumulation of LpX in association with lipid droplets and glomerulosclerosis, it remains to be established whether LCAT deficiency per se or LpX excess are responsible for renal damage (69).

APOE. Lipoprotein glomerulopathy (LGP) is a unique and rare disorder of renal lipodosis that was first reported in a Japanese patient in 1989 (128). The renal manifestation of LPG includes nephrotic syndrome that histologically is accompanied by abnormal lipoprotein deposition in glomerular capillaries and mesangial proliferation. LPG was subsequently found to be due to mutations of the APOE gene (126), which may facilitate the bindings of APOE to glomerular cells (129). Furthermore, APOE gene variants are associated with ESKD (157). The finding of increased glomerular APOE expression in patients with idiopathic nephrotic syndrome is intriguing and may suggest increased acquired binding of APOE to glomeruli (19). However, APOE-deficient glomeruli were reported in focal and segmental glomerulosclerosis (19), and animal studies suggest that APOE deficiency and mutations that affect APOE binding to LDL receptors (LDLR) render renal cells more susceptible to glomerular injury (4, 7, 17, 36, 59, 60, 71, 86, 145, 154). Therefore, the cause-effect relationship between APOE and glomerular injury warrants further investigation. Similarly, while kidneys heavily synthesize APOE (12), the biological importance of ApoE production and or clearance by the kidneys remains to be established. As several different types of glomerular injury have been reported in ApoE-deficient mice, it is likely that APOE deficiency represents a susceptibility factor rather than a specific causative factor in the development of glomerular injury.

Considerations on other apolipoproteins. APOM is a 26-kDa apolipoprotein and is a member of the lipocalin family expressed in the liver and in the kidney (56, 156). In the plasma, APOM is associated with HDL particles (156); in kidney proximal tubular cells, APOM binds to megalin, thus preventing its excretion in the urine by megalin-mediated endocytosis (35). Although APOM is strongly expressed in kidney tubular epithelial cells, mutations, polymorphisms, or allelic variants of the APOM gene have not been associated with any glomerular phenotype and will, therefore, not be discussed further.

Loss of function mutations in APOC3 have been described and are associated with a reduced risk of coronary heart disease (142). However, the association between APOC3 deficiency and kidney disease remains to be established and is suggested by the fact that APOC3 deficiency occurs in patients with T2D is associated with renal insufficiency without albuminuria (158). Whether and how the potential protective effects of APOC3 antagonism are mediated by its ability to interfere with the activity of lipoprotein lipase (42) remains to be established.

Apolipoprotein A-I is the main protein of high-density lipoprotein particles, and is encoded by the APOAI gene. Several APOAI mutations have been found, and they either present with the phenotype of LCAT-deficient patients or with APOA1 amyloidosis, affecting both kidney and liver (48). It is interesting to note that APOA1 mimetic peptides may improve nephropathy in ApoE-deficient mice (148), raising the possibility that APOA1 mimetic peptides may be beneficial in kidney diseases.

Apolipoproteins can also function as autoantigens in proteinuria and kidney disease. In fact, certain lipoproteins, as well as some lipid-related enzymes, are immunogenic, can cause the production of autoantibodies, and can cause glomerular injury and proteinuria. In fact, acquired cases of LCAT deficiency resemble membranous nephropathy (140), and autoantibodies against APOL2 have been associated with recurrent FSGS after transplantation (33). Finally, a form of apolipoprotein AI, named A1B has been suggested as one of the many circulating factors that can cause recurrent proteinuria after transplantation in patients with FSGS (78).

Niemann-Pick proteins. Niemann-Pick disease type C (NPC) is an autosomal recessive lysosomal lipid storage disease associated with impaired intracellular cholesterol trafficking. The majority of NPC patients have a defect in NPC1 (95%), while only 5% of the NPC cases are due to mutations in NPC2. Mutations in this gene(s) lead to the inability to transport cholesterol and other lipids out of late endosomes and lysosomes, leading to unesterified cholesterol accumulation within these compartments (112, 113). NPC1 and NPC2 are both expressed in the kidney (74, 77), and NPC2 expression has been further localized to both the distal and proximal convoluted tubules of the kidney (74). Kidney biopsies from affected patients have demonstrated Niemann-Pick disease-associated renal pathology, which included foamy podocytes, vacuolated tubular epithelial cells, and collections of foam cells in the interstitium (47). Additionally, an association with a phenotype resembling membranoproliferative glomerulonephritis type II has also been documented (114). These obser-
Apolipoprotein L-1: High-density lipoprotein which colocalizes with APOL1 (41) but may (67) or may not prevent the progression of kidney development and progression needs to be established, as the anti-hyperlipidemic drug ezetimibe targets a NPC1-like protein (41) but may (67) or may not prevent the progression of kidney disease (52).

ABCA1: Excessive cholesterol deposition can result from impaired cholesterol efflux due to downregulation of ATP-binding cassette transporter (ABCA1) expression (116, 141). Under normal conditions, ABCA1 mediates the efflux of cholesterol and phospholipids to lipid-poor apolipoproteins, primarily apolipoprotein A-1 (APOA1) but also APOE, which then form nascent HDLs. Tangier disease (also known as familial alpha-lipoprotein deficiency or haploalpha-lipoproteinemia) is a rare autosomal recessive inherited disorder characterized by a severe reduction HDL levels. It is caused by a mutation in the ABCA1 gene on chromosome 9q31, resulting in an accumulation of esterified cholesterol in tissues (13, 16, 92, 123). Clinical features include very large, yellow-orange tonsils, enlarged liver, spleen and lymph nodes, abnormal chylomicron remnants, and peripheral neuropathy in children and adolescents (16). Although rare, the presence of a renal phenotype in Tangier patients has been described (37). Familial HDL deficiency is more common and, like Tangier disease, characterized by low plasma HDL, but without the clinical manifestations. Experimental studies were instrumental to establish that ABCA1 deficiency combined to ABCG1 deficiency causes macrophage inflammation (155) and RAC1 dependent migration (107), resulting in accelerated atherosclerosis. Studies on renal cell-specific Abcal-deficient mice will need to be generated to determine the relative contribution of ABCA1-dependent cholesterol efflux on kidney disease development and progression.

Table 1 provides a summary of genes involved in cholesterol metabolism that are linked to kidney disease.

**Renal Cholesterol in Acquired Kidney Disease**

Acute kidney injury. Most of the studies linking altered lipid metabolism to kidney disease have been performed in acute kidney injury (AKI). Hypoxia-induced foam-cell formation and cytokine secretion is a phenomenon that has been described in atherosclerosis and in nonalcoholic steatohepatitis (3, 15, 21, 135). Hypoxia is also a well-recognized pathogenetic mechanism in kidney disease, where hypoxia-inducible factors (HIFs), such as HIF1 and HIF2, are considered to play an important role primarily in AKI. HIF-mediated pathways influence lipid metabolism, erythropoiesis, angiogenesis and vascular tone, cell growth and differentiation, survival and apoptosis, and thus are critical factors in development, physiology and disease (30, 49, 82, 84, 115, 117, 150). There are data supporting HIF1 playing a key role in lipid accumulation by increasing lipid influx and synthesis in hepatocytes through increased LDL and very low-density lipoprotein uptake and increased levels and activity of HMG CoA reductase (HMGCR), respectively (108). More recently, HIF2 has been identified as an important regulator of hepatic lipid metabo-

<table>
<thead>
<tr>
<th>Gene</th>
<th>Molecule</th>
<th>Function</th>
<th>Role in Kidney Disease</th>
</tr>
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<tbody>
<tr>
<td>APOL1</td>
<td>Apolipoprotein L-1</td>
<td>High-density lipoprotein which colocalizes with apolipoprotein A-I in HDL particles and may play a role in lipid exchange and transport</td>
<td>Genetic mutations in individuals of African ancestry have increased risk of certain kidney diseases. May play a role in podocyte injury and podocyte cholesterol metabolism</td>
</tr>
<tr>
<td>LCAT</td>
<td>Lecithin-cholesterol acyltransferase</td>
<td>Central enzyme in the extracellular metabolism of plasma lipoproteins</td>
<td>Lower LCAT prevents HDL maturation. Nephrotic Syndrome leading to ESRD</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein E</td>
<td>Mediates the binding, internalization, and catabolism of lipoprotein particles</td>
<td>APOE deficiency and mutations may increase susceptibility of glomerular injury</td>
</tr>
<tr>
<td>APOM</td>
<td>Apolipoprotein M</td>
<td>Thought to be involved in lipid transport.</td>
<td>Remains to be established</td>
</tr>
<tr>
<td>APOC3</td>
<td>Apolipoprotein C-III</td>
<td>Component of triglyceride-rich very low density lipoproteins (VLDL) and high-density lipoproteins (HDL) in plasma. Plays a multifaceted role in triglyceride homeostasis.</td>
<td>Remains to be established</td>
</tr>
<tr>
<td>APOA1</td>
<td>Apoprotein A-1</td>
<td>Major apoprotein of HDL and promotes cholesterol efflux from cells</td>
<td>Mutations result in LCAT deficiency phenotype or APOA1 amyloidosis in kidney and liver. Can act as autoantigens in proteinuria and kidney disease</td>
</tr>
<tr>
<td>APOL2</td>
<td>Apolipoprotein L-II</td>
<td>May be involved the movement of lipids or the binding of lipids to organelles</td>
<td>Autoantibodies against APOL2 have been associated with recurrent FSGS after transplantation</td>
</tr>
<tr>
<td>APOA1b</td>
<td>Apoprotein A-1b</td>
<td>Circulating factor</td>
<td>Has been indicated as a circulating factor, which could cause proteinuria after transplantation in FSGS patients</td>
</tr>
<tr>
<td>NPC1/2</td>
<td>Niemann-Pick disease, type C1/C2</td>
<td>Mediates intracellular cholesterol trafficking</td>
<td>Mutations in this gene(s) lead to accumulation of unesterified cholesterol, which may contribute to kidney disease development and progression</td>
</tr>
<tr>
<td>ABCA1</td>
<td>ATP-binding cassette subfamily A member 1</td>
<td>Mediates cholesterol efflux in the cellular lipid removal pathway using cholesterol as its substrate</td>
<td>Deficient cholesterol efflux may result in accumulation of unesterified cholesterol and may contribute to progression of kidney disease</td>
</tr>
</tbody>
</table>

HDL, high-density lipoprotein; FSGS, focal segmental glomerulosclerosis.
lism, and its activation results in severe fatty liver disease in mice (119). Furthermore, hypoxia-inducible protein 2 was identified as a novel lipid droplet protein and a specific target gene of HIF1 (43). This finding emphasizes further the important functional role of hypoxia in pathological lipid accumulation. HIF1 expression has been localized to the tubular epithelia in the kidney, whereas HIF2 has been primarily localized in glomerular cells (40). It is plausible that the induction of HIFs or inhibition of HIF degradation through hypoxia may have protective effects in the setting of AKI (68) (83, 153). Chronic hypoxia may also contribute to the pathogenesis of more chronic disorders, such as DKD (134), as genes induced by hypoxia can promote tubulointerstitial injury and renal fibrosis. As renal dysfunction and proteinuria in experimental DKD are associated with increased cholesterol content in kidney cortex (88), novel therapeutic approaches targeting hypoxia-induced transcription factors may prove beneficial in preventing lipid accumulation in kidney diseases other than AKI.

**Diabetic kidney disease.** Lipid droplets in kidney biopsies from patients with DKD were first identified by Kimmelstiel and Wilson (66) and were more recently localized within podocyte foot processes of 34 patients with DKD compared with 12 patients with normal kidneys (55). Accumulation of lipid droplets occurred in association with the modulation of several lipid-related genes involved primarily in cholesterol uptake and in cholesterol efflux (55). Cholesterol uptake receptor expression, including LDL receptors, oxidized LDL receptors, and acetylated LDL receptors, was significantly increased, while there was downregulation of genes effecting cholesterol efflux, including ABCA1, ABCG1, and APOE. There was a highly significant correlation between glomerular filtration rate, inflammation, and lipid metabolism genes, supporting a possible role of abnormal lipid metabolism in the pathogenesis of DKD. Although a strong alteration of genes involved in fatty acid oxidation was also reported (55), and defective fatty acid oxidation is known to be a strong contributor to chronic kidney disease progression (63), accumulation of both triglycerides and cholesterol may contribute to DKD progression, as suggested from experimental animal models of Type 1 diabetes (T1D) (116). Furthermore, an increase in the expression of sterol regulatory element-binding protein 1 (SREBP1) is associated with an increase in lipid deposits in Type 1 diabetes mouse models. Elevated glucose levels result in an increase in the SREBP1 expression in cultured human mesangial cells, suggesting that diabetic patients will have an elevation in SREBP1 expression when they are hyperglycemic (137). Podocytes express genes and proteins that are involved in cellular cholesterol homeostasis (38, 88). We recently demonstrated that glomerular ABCA1 expression is decreased in glomeruli isolated from patients with Type 2 diabetes (T2D) and early DKD, as well as in human podocytes treated with the sera from patients with T1D and DKD in the absence of changes in LDLR and HMGCR expression (88). These observations indicate that lipid accumulation in podocytes due to defective cellular cholesterol efflux may play an important role in glomerular injury in DKD. Whether and how cellular cholesterol handling is altered in other glomerular disorders unrelated to diabetes remains to be established. Furthermore, the factors involved in increased cholesterol uptake and/or decreased cholesterol efflux in kidney cells remains to be established. In nonalcoholic fatty liver disease, oxidative stress and proinflammatory cytokines contribute to hepatocellular injury and liver inflammation (95). Interestingly, the same inflammatory cytokines may contribute to cholesterol accumulation via suppression of cholesterol efflux. In fact, TNF-α or IL-1β significantly reduced intracellular cholesterol efflux by inhibiting peroxisome proliferator-activated receptor, liver X receptor (LXR) and ABCA1 expression and increased LDLR and SREBP-2 expression (79). Therefore, it is conceivable that circulating inflammatory factors, such as TNF-α or IL-1β, play a key role in the progression of DKD by altering lipid metabolism in glomerular cells similarly to what has been described in hepatocytes. In support of this observation, TNF-α and its associated soluble receptors are strong predictors of DKD progression in T1D and T2D patients (45, 100, 101, 111). Moreover, inflammatory cytokines TNF-α or IL-1β were shown to modify cholesterol-mediated LDLR regulation in mesangial cells. These studies suggest that inflammatory cytokines contribute to lipid-mediated renal damage (122, 160). An inflammation-driven accumulation of cellular cholesterol related to impaired efflux was also described in human mesangial cells, where IL-1β downregulates ABCA1 (120). Interestingly, IL-1β treatment of hepatic and mesangial cells also interrupted LDLR feedback regulation, causing statin resistance (26), which might elucidate why statins are not effective in preventing the progression of DKD. The relative contribution of local and systemic inflammatory cytokines to glomerular injury and proteinuria remains to be established. Better understandings of how cellular cholesterol and inflammatory pathways are linked to cause glomerular cell injury also remain to be established.

**Lipid Modulation of Podocyte Function**

**Physiological role of cholesterol in podocytes.** In podocytes, which are specialized cells of the glomerulus, lipid rafts contribute to the spatial organization of the slit diaphragm (SD). The importance of lipid rafts in the spatial organization of glomerular SD proteins was recognized several years ago, when nephrin and podocin were enriched in detergent-resistant fractions in flotation gradients of Triton X-100 extracts (133). Among several components of lipid rafts, cholesterol is required for proper localization and function of slit diaphragm proteins. In particular, podocin binding to cholesterol occurs through prohibitin domains, and such binding influences the lipid membrane composition to allow podocin to associate with the ion-channel transient receptor potential canonical 6 (TRPC6), a step that is necessary for podocin-dependent activation of TRPC6 (57). It is likely that many other proteins able to bind cholesterol regulate the formation and function of large proteins-cholesterol supercomplexes at the plasma membrane. However, excess accumulation of cellular cholesterol may adversely affect cell function, as described for macrophages (11) and as we have recently reported in podocytes (88).

**Effects of cholesterol accumulation on podocyte function.** Pathological accumulation of cellular lipids occurs also in podocytes exposed to inflammatory cytokines (160) and to puromycin (85), a widely used experimental model of proteinuria. While podocytes have been shown to express ABCA1, LDLR, c-x-c motif ligand 16 (CXCL16), CD36, sterol O-acetyltransferase 1 (SOAT1), and SREBP1, the relative contribution of these proteins to podocyte injury is unknown. A graphic representation of molecules expressed by podocytes
that are involved in lipid metabolism is shown in Fig. 2. We recently demonstrated that treatment of human podocytes with the sera from patients with DKD leads to cholesterol accumulation compared with human podocytes exposed to the sera of patients with diabetes, but no DKD, with the same concentration of total cholesterol, HDL-C, and LDL (88). This was associated with a reduction of ABCA1 and an impairment of cholesterol efflux. The importance of cholesterol accumulation as a negative regulator of podocyte function is supported by additional studies on human podocytes in vitro, which have demonstrated that cholesterol depletion with CD restores insulin signaling through AKT, protects from podocyte apoptosis, and suppresses TLR4 signaling through MYD88 (88). Ongoing studies in our laboratory are being conducted to determine the relative contribution of free and esterified cholesterol to podocyte injury via manipulation of ABCA1 and SOAT1 expression. When administered in vivo to BTBR ob/ob mice, CD treatment restored renal cholesterol content in association with the normalization of proteinuria. These data strongly support that cholesterol accumulation in podocytes is unrelated to the amount of circulating cholesterol, that it is primarily linked to impaired cholesterol efflux, and that it may directly cause podocyte injury. However, podocyte survival and integrity of the actin cytoskeleton are also impaired after exposure to oxidized LDL (20, 50). Although statins were shown to prevent oxidized LDL-induced injury of glomerular podocytes by activating the phosphatidylinositol 3-kinase/AKT-signaling pathway (20), statins were not beneficial in protecting podocytes exposed to the sera of patients with DKD in our studies (88). Interestingly, CXCL16 acts as a scavenger receptor of oxidized LDL in human podocytes (50). This is clinically relevant, as biopsies of patients with membranous nephropathy demonstrated increased glomerular CXCL16 expression accompanied with higher levels of oxLDL. CXCL16 may, therefore, become a new therapeutic target in the treatment of proteinuric glomerular diseases.

**Free fatty acids and podocyte function.** In the setting of DKD, other lipids may contribute to altered podocyte function. Intracellular lipid overload is particularly severe in podocytes of patients with CKD, In this setting, binding and/or uptake of triglyceride-rich LDL by glomerular cells leads to increased endocytic accumulation of triglycerides that could play a role in lipotoxicity (25, 72). In fact, LDL receptor is a major receptor that mediated lipid uptake in podocytes, and high glucose dysregulated the feedback regulation of the LDL receptor pathway. Subsequently, this dysregulation led to lipid accumulation in podocytes, accelerating DKD progression. Saturated free fatty acids (FFAs) involved in the pathogenesis of T2D have been shown to induce endoplasmic reticulum stress and apoptosis of podocytes (73, 131). The loss of podocytes is a hallmark of DKD, and these cells are extremely susceptible to damage from saturated FFAs, yet exposure to monounsaturated FFAs does not render the same effect (131). Amelioration of endoplasmic reticulum stress and podocyte Fig. 2. Cholesterol homeostasis in podocytes. Cholesterol homeostasis is maintained by several mechanisms, and dysregulation of this homeostasis in podocytes may contribute to kidney disease. Cholesterol uptake from circulating oxidized or unoxidized LDL is mediated via the LDL-receptor or CXCL16 and may cause mitochondrial and endoplasmic reticulum stress. Cholesterol synthesis and metabolism are regulated by several nuclear receptors and transcription factors, including SREBP1. Neutral cholesterol accumulates in lipid droplets together with triglycerides that are derived from the uptake and metabolism of free fatty acids primarily via platelet glycoprotein 4 (also known as CD36). These free fatty acids can cause oxidative and endoplasmic reticulum stress based on the degree of saturation. Free cholesterol is transported to the plasma membrane via Niemann-Pick disease, type C1/C2 (NPC1/1/2) for efflux primarily by ATP-binding cassette subfamily A member 1 (ABCA1) or converted by sterol O-acyltransferase 1 (SOAT1) into esterified cholesterol (red pentagons) inside lipid droplets. In conditions of cholesterol deficiency, sterol regulatory element-binding protein 1 (SREBP1) is transported from the endoplasmic reticulum to the Golgi apparatus, where it is cleaved to translocate to the nucleus and initiate cholesterol synthesis. Systemic or locally produced apolipoprotein L-1 (APOL1) might modulate oxidative stress and/or contribute to cholesterol efflux via ABCA1 and ATP-binding cassette subfamily G member 1 (ABCG1) by serving as an HDL acceptor together with apoprotein A-1 (APOA1) and apolipoprotein E (APOE).
apo-poptosis could potentially be caused by the induction of stearoyl-CoA desaturase 1, which is able to convert saturated FFAs to monounsaturated FFAs and has been demonstrated to be upregulated in podocytes in biopsy samples of patients with DKD (132). Dysregulated transport and oxidation of FFAs, coupled with an impaired antioxidant response, leads to structural damage in podocytes, resulting in glomerulopathy during early DKD (103). In podocytes, enhanced FFA uptake is regulated by increased expression of the scavenger receptor platelet glycoprotein 4 (also known as CD36) and decreased fatty acid β-oxidation, resulting in intracellular lipid accumulation. Accumulated FFAs become trapped in the mitochondrial matrix, leading to production of reactive oxygen species, lipid peroxidation, and mitochondrial damage and dysfunction (136). The association between renal accumulation of triglycerides and reduced expression of the ultrasensitive energy sensor AMPKα1 (72) strongly suggests that energy-generating and energy-consuming pathways might link lipid accumulation to podocyte dysfunction in DKD and other disorders that result in CKD. Podocyte-specific expression of fatty acid-binding proteins correlates with proteinuria in patients with obesity-related glomerulopathy, and urinary FABP is an important marker of DKD progression (109), both of which strongly support a causal link between free fatty acid metabolism and kidney diseases. Recent data show that decreased fatty acid oxidation in CKD may contribute to lipid accumulation in the tubular compartment, which, in turn, results in energy depletion followed by apoptosis and dedifferentiation, all contributing to fibrosis and lastly CKD progression (63). Whether and how a similar mechanism operates in podocytes remains to be established.

Sphingolipids and podocyte function. The role of lipids as a major regulator of danger signaling from the circulation to glomerular cell is supported by our studies demonstrating that sphingolipid-related enzymes, such as SMPDL3b localize to lipid raft domain, where they determine the fate of podocytes exposed to inflammatory stimuli (159). Interestingly, as SMPDL3b acts as major modulator of TLR4 signaling in macrophages (53), the role of this sphingolipid-related enzyme in the activation of the inflammosome in podocytes remains to be established. Furthermore, whether and how the effect of SMPDL3b on podocyte function is mediated by the SMPDL3b enzymatic activity remains to be established. In fact, both ceramide 1 phosphate and sphingosine 1 phosphate may represent downstream effectors of SMPDL3b function (87). While SMPDL3b deficiency or excess in podocytes does not result in podocyte injury per se (39), SMPDL3b deficiency is likely to represent a predisposing factor for the recurrence of FSGS after transplantation. On the contrary, upregulation of SMPDL3b may occur in podocytes in DKD in association with increased RhoA activity and induction of apoptosis (159). Further studies are needed to determine what modulates SMPDL3b expression in podocytes and which podocytes are involved in the signaling events linking SMPDL3b deficiency or excess to podocyte injury.

Conclusions

Several clinical and experimental diseases of genetic and nongenetic origin suggest an important role of lipids, lipoproteins, and lipid-modifying enzymes in the pathogenesis of kidney diseases. These clinical observations are supported by novel experimental data demonstrating a causative role of lipids in the pathogenesis of kidney disease development and progression. Although cholesterol is a major physiological modulator of lipid raft function in podocytes, excessive accumulation of cholesterol and or triglycerides causes podocyte injury and proteinuria. Moreover, SMPDL3b is a lipid raft sphingomyelinase that modifies the plasma lipid composition and modulates intracellular inflammatory pathways, as well as the ability of circulating factors to affect podocyte function and survival. Therefore, we suggest that targeting lipid dysmetabolism in kidney disease may increase the opportunity for successful drug discovery in the field of proteinuric kidney diseases.

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DISCLOSURES

A.F. is an inventor on pending or issued patents aimed to diagnose or treat proteinuric renal diseases and stands to gain royalties from future commercialization. A.F. is also a consultant for Hoffman-La Roche, Genentech, Mesoblast, Abbvie, Boehringer Ingelheim, Alexion on subject matters that are unrelated to this publication.

AUTHOR CONTRIBUTIONS

Author contributions: P.W. and A.F. drafted manuscript; P.W., G.M.D., and A.F. edited and revised manuscript; P.W. and A.F. approved final version of manuscript.

REFERENCES

8. Baragetti A, Norata GD, Sarccina C, Rastelli F, Grigore L, Carl- 


21. Cramer T, Yamanishi Y, Clausen BE, Forster I, Pavlinski R, Makk- 


25. Douchateau PN, Pullinger CR, Orellana RE, Kunitake ST, Naya- 


Review

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Rankin EB, Rha J, Selak MA, Unger TL, Keith B, Liu Q, Haase VH. 

Rye KA, Barter PJ. 

Schaeffner ES, Kurth T, Curhan GC, Glynn RJ, Rexrode KM, Saito T, Matsunaga A, Oikawa S. 


Saito T, Osatuka T, Sato H, Furuta T, Sato T, Soma J, Abe K, Yoshinaga K. 


Schaffner ES, Kurth T, Curhan GC, Glynn RJ, Rexrode KM, Bainget C, Buring JE, Gaziano JM. 

Sieber J, Lindenmeyer MT, Kampe K, Campbell KN, Cohen CD, Hopfer H, Mundel P, Jehle AW. 


Singh DK, Winarczik P, Fourney R. 


Sun L, Halaibel N, Zhang W, Rogers T, Levi M. 


Tabet F, Rye KA. 


Tang C, Kanter JE, Bornfeldt KE, Leboeuf RC, Oram JF. 


Vaziri ND. 


Vaziri ND, Kondo M, Asahi K, Kurahashi I, Ohashi Y, Watanabe T. 


Vaziri ND, Kim HJ, Moradi H. 

Vaziri ND, Kim HJ, Moradi H. 


Yoshinaga K, Sakaguchi H. 

Zoppini G, Carey VJ. 

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