TITLE: Systemic and renal lipids in kidney disease development and progression

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RUNNING HEAD: Lipids in kidney disease

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ABSTRACT

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 non-genetic 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 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 pathophysiologic 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.
INTRODUCTION

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 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 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 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) (Lp(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 the formation of immature HDL (146). The presence of lipoproteins with altered composition, combined with a reduction of apolipoproteins apoA01, apoA-II and apoC-II, may contribute to the increased cardiovascular morbidity and mortality of patients with CKD (65). In patients with diabetic kidney disease (DKD), an association between increased total cholesterol and macroalbuminuria was reported (90). A cross sectional study in 732 men with type 2 diabetes (T2D) also demonstrated that the lower quartiles of eGFR were characterized by increased triglyceride and non-HDL-C (75).

In patients on dialysis, analysis of lipoprotein composition suggests underlying defects in the accumulation of triglyceride enriched intermediate and low density lipoproteins (IDL and LDL) that may explain the accelerated atherosclerosis observed in this patient population (98). Since several lipid abnormalities are associated with an
increased risk of renal insufficiency (32, 93, 104, 130) and since is not uncommon for 
CKD patients to have mixed dyslipidemia (51) measurements of ratios of non-HDL-C to 
HDL-C have yielded interesting findings. In a large cohort of patients with normal (or 
near normal) kidney function at baseline, the non-HDLc/HDLc ratio was found to be an 
independent risk factor for the progression of CKD (162). Interestingly the data revealed 
that only female gender was significantly associated with an increased risk of incident 
CKD. These results support NonHDL-C/HDL-C ratio as a potential screening tool to 
identify high-risk CKD patients. A summary of lipid related abnormalities in nephrotic 
syndrome and CKD are shown in Figure 1.

Clinical studies suggesting a causative role of dyslipidemia in the development and 
progression of CKD: a focus on cholesterol efflux

While an association between altered lipid metabolism and proteinuria or CKD 
has been extensively studied, it is unclear if dyslipidemia per se may contribute 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 pro-inflammatory cytokines and chemokines and accelerates inflammation (44), which in turn may promote the progression of CKD. While reduction of LDL with statins have 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 to the development of proteinuria and the progression of CKD.

\textit{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
Lipids in kidney disease

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 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 quantity and HDL quality. Even a mildly impaired GFR is associated with low HDL-C concentrations, which become progressively worse as CKD progresses (6). HDL tends to be smaller and denser with CKD, 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) that could influence the development and progression of CKD. However, if impaired cholesterol efflux and/or production of a non-functioning 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 (46, 58, 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 inhibitor were selected based on their ability to raise HDL and not on their ability to increase the function of HDL. Further research is therefore necessary to determine if agents capable of increasing cholesterol efflux such as HDL mimetic peptides or recombinant ApoA1 may represent new therapeutic strategies for both cardiovascular and kidney diseases.

**LCAT**

Calabrasi 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 preb-HDL compared to 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 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 preb-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.
TG: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 to the use of triglycerides and HDL-C for treatment thresholds and targets for the prevention of micro and macrovascular complications (24). In fact, another study of 124,700 participants in the Japanese Specific Health Check and Guidance System demonstrated that the 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-year changes in eGFR and increase in urinary protein excretion in the entire population of participants and with new-onset CKD, 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 supporting 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 is 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 affect 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 lecithin-
cholesterol acyltransferase (LCAT) deficiency, where renal accumulation of lipoprotein
X (LpX) and lipid droplets was described in glomeruli (69, 102). Glomerular lipidosis,
however, are also observed in diseases of non genetic origin such as focal and
segmental glomerulosclerosis (127) and diabetic glomerulosclerosis (55). While 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 if and how altered glomerular cholesterol metabolism may cause
proteinuria and glomerulosclerosis. We will review in this paragraph the clinical and
experimental evidence strongly supporting a link of causality between
lipoproteins/cholesterol metabolism and kidney disease. We will not discuss the
contribution of free fatty acids and angiopoietin-like 4, as these have been extensively
reviewed elsewhere (28, 80).
The **APOL1** gene encodes a secreted high-density lipoprotein, which co-localizes with apolipoprotein A-I (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 deletion, 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 FSGS, HIVAN, arterionephrosclerosis (hypertension-attributed kidney disease) and non-diabetic ESKD. Immunofluorescent staining demonstrated APOL1 expression in normal human kidneys localized to podocytes of the glomerulus, the promixal 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

Lecithin-cholesterol acyltransferase (LCAT) deficiency is an autosomal recessive
disorder caused by mutations of the \textit{LCAT} gene resulting in familial LCAT deficiency
(FLD). \textit{LCAT} deficiency is a genetic disease in which esterification of free cholesterol in
the plasma is impaired. Patients with familial \textit{LCAT} deficiency are characterized among
other symptoms nephrotic range proteinuria with chronic progressive glomerulopathy
resulting in renal failure. An accumulation of lipid deposits in the glomerular basement
membrane (GBM) 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 if
LCAT deficiency per se or LpX excess are responsible for renal damage (69).

APOE

Lipoprotein glomerulopathy (LPG) is a unique and rare disorder of renal lipidosis
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) that 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 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). If and how the potential protective effects of ApoC3 antagonism are mediated by its ability to interfere with the activity of lipoprotein lipase (LPL) (42) remains to be established.

Apolipoprotein A-I is the main protein of high-density lipoprotein particles, and is encoded by the APOA1 gene. Several APOA1 mutations have been found and they either presents 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 is an autosomal recessive lysosomal lipid storage disease associated with impaired intracellular cholesterol trafficking. The majority of NPC patients have a defect in \textit{NPC1} (95%), while only 5% of the NPC cases are due to mutations in \textit{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). \textit{NPC1} and \textit{NPC2} are both expressed in the kidney (74, 77) and \textit{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 observations indicate that cholesterol accumulation in the lysosomal compartments of glomerular or tubular cells contributes to the renal pathogenesis observed in Niemann–Pick disease. It is interesting to note that is the unesterified portion of cholesterol that may contribute to tissue injury, as demonstrated in target liver cells (10). The role of cholesterol accumulation as a cause of the clinical manifestations of Niemann-Pick type C is further supported by the observation that cholesterol sequestration with cyclodextrin (CD) protects from experimental (76) and
possibly clinical disease (http://addiandcassi.com). Further work on the contribution of NPC proteins to kidney disease 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 down-regulation 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 (Apo-A1) but also ApoE, which then form nascent high-density lipoproteins (HDL). Tangier disease (also known as Familial alpha-lipoprotein deficiency or hypoalphalipoproteinemia) 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 (FHA) 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 macrophages inflammation (155) and Rac-1 dependent migration (107), resulting in accelerated atherosclerosis. Studies on renal cell specific
Abca1 deficient mice will need to be generated in order 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.

RENA L 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 non-alcoholic steatohepatitis (NASH) (3, 15, 21, 135). Hypoxia is also a well recognized pathogenetic mechanism in kidney disease, where hypoxia-inducible factors (HIFs), such as HIF-1 and HIF-2 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 is data supporting HIF-1 playing a key role in lipid accumulation by increasing lipid influx and synthesis in hepatocytes through increased LDL and very low-density lipoprotein (VLDL) uptake and increased levels and activity of HMGCR, respectively (108). More recently, activation of HIF-2 has been identified as is important regulator of hepatic lipid metabolism resulting in severe fatty liver disease in mice (119). Furthermore, hypoxia-inducible protein 2 (HIG2) was identified as a novel lipid droplet
protein and a specific target gene of HIF-1 (43). This further underlies the important function role of hypoxia in pathological lipid accumulation. HIF-1 expression has been localized to the tubular epithelia in the kidney, whereas HIF-2 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 when compared to 12 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 down-regulation 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
Lipids in kidney disease

metabolism in the pathogenesis of DKD. Although also a strong alteration of genes
involved in fatty acid oxidation was reported (55), and defective fatty acid oxidation is 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 T1D (116). Furthermore, an increase in the expression
of sterol regulatory element-binding protein 1 (SREBP-1) is associated with an increase
in lipid deposits in type 1 diabetic mouse models. Elevated glucose levels results in an
increase in the SREBP-1 expression in cultured human mesangial cells, suggesting that
diabetic patients characterized by hyperglycemia will have an elevation in SREBP-1
expression (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
type 1 diabetes (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. If 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 non-alcoholic fatty liver disease (NAFLD), 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, tumor necrosis factor alpha
(TNFα) or interleukin-1 beta (IL-1β) significantly reduced intracellular cholesterol efflux by inhibiting PPAR, LXR, and ABCA1 expression and increased LDLR and SREBP-2 expression (79). Therefore, it is conceivable that circulating inflammatory factors, such as TNFα or IL-1β, play a key role in the progression of DKD by altering lipid metabolism in glomerular cells similarly to what was described in hepatocytes. In support of this observation, TNFα and its associated soluble receptors are strong predictors of DKD progression in T1D and T2D (45, 100, 101, 111). Moreover, inflammatory cytokines TNFα or IL-1β were shown to modify cholesterol-mediated LDL receptor regulation in mesangial cells. These studies suggest that inflammatory cytokines contribute to lipid-mediated renal damage (160)(122). 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 (PHB) domains, and such binding influences the lipid membrane composition to allow cholesterol to associate with the ion-channel 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, CXCL16, CD36, SOAT1 and SREBP1, the relative contribution of these proteins to podocyte injury is unknown. A graphic representation of molecules expressed by podocytes and involved in lipid metabolism is shown in Figure 2. We recently demonstrated that treatment of human podocytes with the sera from patients with DKD leads to cholesterol accumulation when compared to human podocytes exposed to the sera of patients with diabetes and no DKD with the same concentration of total cholesterol, HDL-C and LDL
Lipids in kidney disease

(88). This was associated with reduction of ABCA-1 and 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, demonstrating 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 Sterol O-acyltransferase 1 (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, is primarily linked to impaired cholesterol efflux and 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 T2DM 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 apoptosis could potentially be by the induction of stearoyl-CoA desaturase 1, which is able to convert saturated FFAs to monounsaturated FFAs and has upregulation in podocytes been demonstrated 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 podocyte 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 a decrease in 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
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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), strongly supporting 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). If 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 inflammasome in podocytes remains to be established. Furthermore, if 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 sphingosin 1 phosphate may represent downstream effector
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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 to 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 modulate SMPDL3b expression in podocytes and which ones are the signaling events linking SMPDL3b deficiency or excess to podocyte injury.

CONCLUSIONS

Several clinical and experimental diseases of genetic and non genetic origin support an important role of lipids, lipoproteins and lipid modifying enzymes in the pathogenesis of kidney diseases. These clinical observations are further supported by novel experimental data demonstrating a causative role of lipids in the pathogenesis of kidney disease development and progression. While 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.

ACKNOWLEDGMENTS
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A.F. is supported by the NIH and NIDDK (grant numbers DK090316, DK104753) and U24DK076169, U54DK083912, UL1TR000460, UM1DK100846, the National Center for Advancing Translational Sciences (grant number 1UL1TR000460), and the Peggy and Harold Katz Family Foundation. G.M.D is also supported the NIH and NIDDK grant number DK104753.

CONFLICT OF INTEREST

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.

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**TABLES**

**Table 1. Overview of Genes Involved in Cholesterol Metabolism and Kidney Disease**
Patients with chronic kidney disease (CKD) exhibit significant alterations in lipoprotein metabolism. LDL and HDL are both lipoproteins involved in the transport of cholesterol. LCAT is a central enzyme in the extracellular metabolism of plasma lipoproteins while TG: HDL-Cholesterol ratio might be useful as a predictive value on the onset and progression of CKD over time.

Figure 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 is 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 NPC1/1/2 for efflux primarily by ABCA1 or converted by SOAT1 into esterified cholesterol (red pentagons) inside lipid droplets. In conditions of cholesterol deficiency, SREBP1 is transported from the endoplasmic
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reticulum to the Golgi apparatus where it is cleaved to translocate to the nucleus and initiate cholesterol synthesis. Systemic or locally produced APOL1 might modulate oxidative stress and/or contribute to cholesterol efflux via ABCA1 and ABCG1 by serving as an HDL acceptor together with APOA1 and APOE.
**Figure 1**

Common circulating Lipid Abnormalities in CKD

- **LDL**
  - **low-density lipoprotein**
  - Cholesterol uptake via LDL and inflammatory response to oxidized LDL may contribute to CKD progression

- **HDL**
  - **high-density lipoprotein**
  - Alteration in both quantity and quality of HDL may contribute to dyslipidemia in CKD

- **LCAT**
  - **Lecithin cholesterol acyltransferase**
  - Reduction may contribute to impaired HDL maturation and HDL dysfunction in CKD

- **TG: HDLC**
  - **Triglyceride: HDL-Cholesterol**
  - Ratio may be a marker of LDL particle size and an independent risk factor for the incidence and progression of CKD
<table>
<thead>
<tr>
<th>GENE</th>
<th>MOLECULE</th>
<th>FUNCTION</th>
<th>ROLE IN KIDNEY DISEASE</th>
</tr>
</thead>
<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</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 maybe 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>APOA-1</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>auto antibodies against APOL2 have been associated with recurrent FSGS after transplantation</td>
</tr>
<tr>
<td>APOA-1b</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>ABCA-1</td>
<td>ATP-Binding Cassette Sub-Family 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 esterified cholesterol and may contribute to progression of kidney disease</td>
</tr>
</tbody>
</table>