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Nephrotic syndrome results in hyperlipidemia and profound alterations in lipid and lipoprotein metabolism. Serum cholesterol, triglycerides, apolipoprotein B (apoB)–containing lipoproteins (very low-density lipoprotein [VLDL], immediate-density lipoprotein [IDL], and low-density lipoprotein [LDL]), lipoprotein(a) (Lp[a]), and the total cholesterol/high-density lipoprotein (HDL) cholesterol ratio are increased in nephrotic syndrome. This is accompanied by significant changes in the composition of various lipoproteins including their cholesterol-to-triglyceride, free cholesterol–to–cholesterol ester, and phospholipid-to-protein ratios. These abnormalities are mediated by changes in the expression and activities of the key proteins involved in the biosynthesis, transport, remodeling, and catabolism of lipids and lipoproteins including apoproteins A, B, C, and E; 3-hydroxy-3-methylglutaryl-coenzyme A reductase; fatty acid synthase; LDL receptor; lecithin cholesteryl ester acyltransferase; acyl coenzyme A cholesterol acyltransferase; HDL docking receptor (scavenger receptor class B, type 1 [SR-B1]); HDL endocytic receptor; lipoprotein lipase; and hepatic lipase, among others. The disorders of lipid and lipoprotein metabolism in nephrotic syndrome contribute to the development and progression of cardiovascular and kidney disease. In addition, by limiting delivery of lipid fuel to the muscles for generation of energy and to the adipose tissues for storage of energy, changes in lipid metabolism contribute to the reduction of body mass and impaired exercise capacity. This article provides an overview of the mechanisms, consequences, and treatment of lipid disorders in nephrotic syndrome.

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Glomerular proteinuria ≥3.5 g/day in adults or a urine protein/creatinine ratio of 2 to 3 mg/mg creatinine or greater in children results in nephrotic syndrome, which is characterized by the tetrad of proteinuria, hypoalbuminemia, edema, and hyperlipidemia. The magnitude of hyperlipidemia and the associated alteration in lipoprotein metabolism in nephrotic syndrome parallels the severity of proteinuria. Plasma concentrations of cholesterol, triglycerides, apolipoprotein B (apoB)–containing lipoproteins (very low-density lipoprotein [VLDL], immediate-density lipoprotein [IDL], and low-density lipoprotein [LDL]), and lipoprotein(a) (Lp[a]) are elevated in nephrotic syndrome. However, high-density lipoprotein (HDL) cholesterol concentration is usually unchanged or reduced and occasionally elevated and the total cholesterol to HDL cholesterol ratio is generally increased in nephrotic animals and humans.[1,2] In addition to the quantitative changes, nephrotic syndrome markedly alters the composition and function of the lipoproteins. In this context, the cholesterol to triglyceride, free cholesterol to cholesterol ester, and phospholipid to protein ratios in the lipoproteins are altered in nephrotic syndrome. This is accompanied by a significant increase in apoA-I, apoA-IV, apoB, apoC, and apoE levels and the apoC-III to apoC-II ratio. These abnormalities are mediated by profound changes in the pathways involved in the biosynthesis, transport, remodeling, and catabolism of lipids and lipoproteins. The disorders of lipid metabolism in nephrotic syndrome contribute to the development and progression of cardiovascular and kidney disease and impaired delivery of lipid fuel to the muscles for generation of energy and to the adipose tissues for the storage of energy. The abnormalities of serum lipids and lipoproteins in nephrotic syndrome are largely due to their impaired clearance and, to a lesser extent, their altered biosynthesis.[2–8] The underlying mechanisms by which nephrotic syndrome alters lipid and lipoprotein metabolism are summarized in the following.

Triglyceride-rich lipoproteins and their abnormalities in nephrotic syndrome

The triglyceride-rich lipoproteins, that is, VLDL and chylomicrons, serve as vehicles for delivery of fatty acids to various cells/tissues in the body for generation and storage of energy. Nascent VLDL is produced by encasing triglycerides, cholesterol ester, and phospholipids in apoB-100 within the hepatocytes and released in the circulation, where it obtains apoE and apoC from cholesterol ester–rich HDL-2. In the

capillaries perfusing the target tissues, VLDL binds to the endothelial surface via its apoE content and activates lipoprotein lipase (LPL) via its apoCII content. This results in LPL-mediated hydrolysis of 70% of the VLDL triglyceride content, release of free fatty acids and phospholipids, and transformation of VLDL to intermediate density lipoprotein (IDL). Nascent chylomicrons are generated by incorporation of triglycerides in apoB-40 within the enterocytes and released in the lymphatic circulation and eventually the systemic circulation where it acquires apoE and apoC from HDL-2. Like VLDL, LPL mediates hydrolysis of 70% of a chylomicron's triglyceride contents and formation of a chylomicron remnant. Nearly two-thirds of the fatty acids released from VLDL and chylomicrons are taken up by the adjacent myocytes or adipocytes, whereas the remaining free fatty acids bind to albumin and lipoproteins and are carried to distant tissues, mainly the liver. Once formed, IDL and chylomicron remnants, which contain 30% of their original triglyceride cargos, are released in the circulation. The triglyceride and phospholipid contents of IDL are removed by cholesterol ester transfer protein (CETP)–mediated exchange of triglycerides for cholesterol ester from HDL-2 and hydrolysis of the triglyceride and phospholipid contents of IDL by hepatic lipase and their uptake by the liver. These events lead to the formation of low-density lipoprotein (LDL), which is normally cleared by LDL receptor. The chylomicron remnants are cleared by LDL receptor–related protein (LRP), a large multifunctional receptor that is expressed on hepatocytes. In addition to the lipolytic pathway, a small fraction of VLDL is cleared by the VLDL receptor, which is expressed in skeletal muscle, adipose tissue, and myocardium, and as such, its tissue distribution is similar to that of LPL.[9–11]

Nephrotic syndrome results in elevated serum triglyceride, VLDL, and IDL levels; increased triglyceride contents of apoB-containing lipoproteins; and prolonged postprandial lipemia.[2–6] These abnormalities are due to impaired VLDL and chylomicron clearance.[4,7,8] LPL, hepatic lipase, LRP, VLDL receptor, and proper shuttling of lipids and apoproteins between HDL and apoB-containing lipoproteins are essential steps in maturation and clearance of VLDL and chylomicrons and generation of normal LDL. As described in the following, nephrotic syndrome results in deficiencies of LPL, hepatic lipase, and VLDL receptor and upregulation of CETP and LRP. In addition, changes in the structure of these lipoproteins limit their effective binding to the key receptors, their ability to activate lipolytic enzymes, and their proper lipid and apoprotein exchange with HDL. The impact of nephrotic syndrome on the key steps in triglyceride-rich lipoprotein metabolism (Figure 1) is described here.

LPL deficiency and dysfunction. LPL is the rate-limiting step in lipolysis of chylomicrons and VLDL. LPL is produced in myocytes, adipocytes, and several other cell types and stored in the Golgi apparatus for either intracellular degradation or release to the cell surface. Once released, LPL binds to the endothelium in the adjacent capillaries where it catalyzes hydrolysis of triglycerides in VLDL and chylomicrons. In the capillaries, LPL binds to the endothelial surface via interaction of its positively charged heparin-binding domains with the negatively charged heparan sulfate proteoglycans.[12] The endothelium-derived glycosylphosphatidylinositol-anchored binding protein 1 (GPIHBP1), plays a central part in the fate and function of LPL by anchoring LPL on the endothelium and serving as the ligand for chylomicrons.[13,14] Because heparin can displace and release LPL from the endothelium, measurement
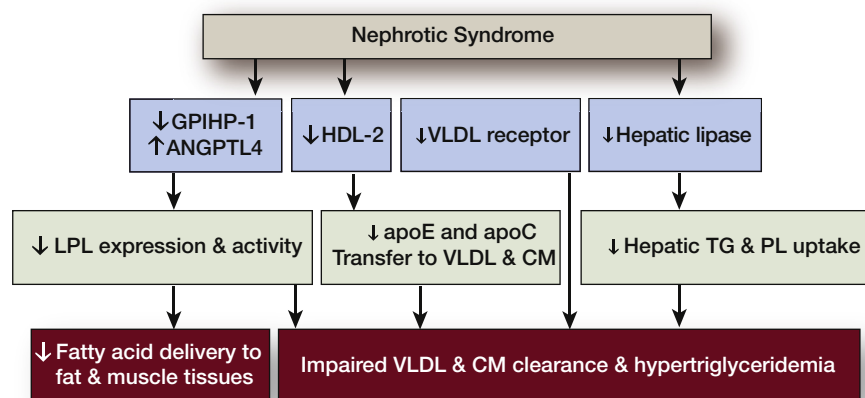
Figure 1 | Via downregulation of the lipoprotein lipase (LPL) adapter molecule GPIHP-1 and upregulation of the LPL inhibitor molecule ANGPTL4, nephrotic syndrome results in a marked decrease in abundance and activity in muscle and adipose tissue. The impact of LPL deficiency is compounded by the scarcity of cholesterol ester–rich high-density lipoprotein (HDL), which is the apoE and apoC donor to the nascent very low-density lipoprotein (VLDL) and chylomicrons (CM) enabling their ability to bind to the endothelial lining and activate LPL. The resulting LPL deficiency and dysfunction limits delivery of lipid fuel to the muscles for generation of energy and to the adipose tissue for storage of energy. In addition, nephrotic syndrome causes hepatic lipase deficiency, which impairs the ability of the liver to extract the triglyceride (TG) and phospholipid (PL) contents of immediate-density lipoprotein (IDL) and HDL. Together, these abnormalities contribute to the development of hypertriglyceridemia, elevation of serum VLDL, and accumulation of atherogenic IDL, CM remnants, and triglyceride (TG)-rich LDL in patients with nephrotic syndrome.

of post–heparin lipolytic activity is conveniently used to assess the endothelium-bound LPL pool in humans, animals, and isolated tissues. Several studies have shown marked reduction of post–heparin lipolytic activity in nephrotic humans and animals,[4,6,15] pointing to the depletion of an endothelium-bound LPL pool. In addition, a series of studies conducted in the author's laboratories have shown marked reductions of heparin-releasable and intracellular LPL, along with a significant reduction of LPL protein abundance despite normal LPL mRNA expression in the adipose tissue, skeletal muscle, and myocardium of Imai rats with spontaneous focal glomerulosclerosis[16] and Sprague-Dawley rats with puromycin-induced nephrotic syndrome.[17] These findings pointed to the posttranscriptional or posttranslational nature of LPL deficiency and dysfunction in nephrotic syndrome. Subsequent studies identified downregulation of GPIHBP1 as the primary cause of the LPL deficiency in nephrotic animals.[18]

Downregulation of LPL and GPIHBP1 is compounded by diminished apoE and apoCII content and increased apoCIII to apoCII ratio in VLDL and chylomicrons. The reductions in apoE (the principal ligand for VLDL and chylomicron binding to endothelium) and the reduction in the ratio of apoCII (LPL activator) to apoCIII (LPL inhibitor) contribute to impaired LPL-mediated lipolysis of triglyceride-rich lipoproteins.[19,20] This is, in part, due to the HDL dysfunction in nephrotic syndrome.[21] In fact, in vivo studies have shown impaired endothelial binding and LPL-mediated lipolysis of VLDL in nephrotic rats and their correction by infusion of HDL from normal animals. Likewise, in vitro studies using cultured rat aortic endothelial cells have shown impaired binding and LPL-mediated lipolysis of VLDL and chylomicrons from nephrotic rats and their restoration by the addition of HDL from normal rats.[8,22] Normally, cholesterol ester–rich HDL-2 lends apoE and apoC to the nascent VLDL and chylomicrons and reclaims them from their remnants after undergoing lipolysis by LPL. Acquisition of apoE and apoC from HDL in exchange for apoA is essential for binding to endothelium and LPL-mediated lypolysis of VLDL and chylomicrons. Due to the paucity of cholesterol ester–rich HDL-2, this process is impaired in nephrotic syndrome and plays an important part in the dysregulation of triglyceride-rich lipoprotein metabolism. These observations illustrate the contribution HDL abnormalities to the associated impairment of triglyceride-rich lipoprotein metabolism in nephrotic syndrome.[21] Given the critical role of LPL and its adapter molecule GPIHBP1 in lipolysis of VLDL and chylomicrons, their acquired deficiency and dysfunction plays a major part in the pathogenesis of hypertriglyceridemia, triglyceride enrichment of VLDL, impaired clearance of chylomicrons, and prolonged postprandial lipemia in nephrotic syndrome.

Hepatic lipase deficiency. Hepatic lipase plays a central role in the metabolism of atherogenic IDL by catalyzing hydrolysis and removal of its triglyceride contents and its ultimate conversion to LDL. In addition, hepatic lipase plays an important role in HDL metabolism by mediating the

unloading of its triglyceride cargo in the liver. Serum IDL and triglyceride content of HDL are markedly elevated in nephrotic syndrome,[1,3,4,15,23] suggesting the presence of hepatic lipase deficiency or dysfunction. In fact, in vitro studies have shown 50% lower heparin-releasable lipase activity in the livers of nephrotic compared with normal control rats.[4] Moreover, studies conducted in the author's laboratories have revealed a marked downregulation of hepatic lipase expression and activity in the liver of rats with nephrotic syndrome.[24,25] Thus, nephrotic syndrome results in hepatic lipase deficiency, which contributes to hypertriglyceridemia, accumulation of atherogenic IDL, and triglyceride enrichment of LDL and HDL.

Upregulation of angiopoietin-like protein 4. As noted previously, nephrotic syndrome results in LPL and hepatic lipase deficiencies. Emerging evidence points to the upregulation of angiopoietin-like protein 4 (ANGPTL4) as another mediator of the LPL deficiency in nephrotic syndrome. ANGPTL4 is a glycoprotein (molecular weight = 45–65 kDa) that is constitutively expressed in the liver, adipose tissue, skeletal muscle, small intestine, and myocardium. High-plasma free fatty acids, fasting, and hypoxia increase production and plasma concentration of ANGPTL4.[26] Fasting and free fatty acids increase ANGPTL4 production via peroxisome proliferator-activated receptors. In addition, ANGPTL4 expression increases during the acute-phase response.[27] Binding of ANGPTL4 to the active LPL dimer leads to its conversion to LPL monomer, which is enzymatically inactive. Therefore, by inactivating LPL, upregulation of ANGPTL4 impairs lipolysis of VLDL and chylomicron and promotes hypertriglyceridemia. Besides LPL, ANGPTL4 inhibits hepatic lipase,[26] which can further increase plasma triglycerides by limiting removal of HDL and IDL triglyceride contents. The inhibitory effect of ANGPTL4 is mitigated by GPIHBP1,[28] which, as noted previously, is markedly reduced in nephrotic syndrome. In contrast to its inhibitory effect on LPL-mediated clearance of circulating triglycerides, ANGPTL4 increases expression of the intracellular hormone-sensitive lipase, which leads to increased lipolysis of intracellular triglycerides in adipose tissues and an increase in the free fatty acid level.[29] Earlier studies have documented a significant increase in the circulating ANGPTL4 and its role in the pathogenesis of hypertriglyceridemia in nephrotic syndrome.[30,31] In a recent study, Clement et al.[32] showed that increased production of ANGPTL4 in nephrotic syndrome is mediated by an increase in the plasma free fatty acid to albumin ratio. Taken together, these observations demonstrated that inhibition of LPL by high circulating ANGPTL4 contributes to the impaired clearance and increase in serum triglycerides in nephrotic syndrome. The LPL-inhibitory effect of ANGPTL4 is mitigated by GPIHBP1, which is the transporter and anchor molecule for LPL.[28] It is of interest that animals with chronic kidney disease and proteinuria show a marked GPIHBP1 deficiency, which heightens the LPL inhibitory effects of ANGPTL4.[18] Interestingly, in a series of in vivo and in vitro experiments, Clement et al.[32] showed that

ANGPTL4 can reduce proteinuria by interacting with the $\alpha v \beta 5$ integrin on glomerular endothelial cells. This assumption was based on experiments that showed that blockade of ANGPTL4 interaction with the $\alpha v \beta 5$ integrin or global knockout of either the ANGPTL4 or $\alpha v \beta 5$ integrin intensify proteinuria in an animal model of minimal change disease. The antiproteinuric effect of ANGPTL4 was confirmed by experiments that showed a significant reduction of the severity and duration of proteinuria without intensification of hypertriglyceridemia in nephrotic animals treated with a recombinant human ANGPTL4 modified at a key LPL-interacting site to obviate its LPL inhibitory property.

It should be noted that patients and animals with advanced chronic kidney disease without nephrosis-range proteinuria commonly exhibit hypertriglyceridemia and elevated plasma and tissue free fatty acid levels, which are associated with LPL, hepatic lipase, and GPIHBP1 deficiencies.[17,18,33,34] These abnormalities resemble those observed in nephrotic humans and animals and as such, could be linked to high levels of circulating ANGPTL4. In fact, plasma ANGPTL4 concentration in maintenance dialysis patients is more than 5-fold higher than in healthy control subjects and correlates positively with the serum free fatty acid level.[35] Therefore, ANGPTL4 seems to be involved in the pathogenesis of LPL deficiency and the associated hypertriglyceridemia in both nephrotic syndrome and advanced chronic kidney disease. Given the central role of LPL-mediated delivery of fatty acid fuel to myocytes for energy production and to adipocytes for energy storage, inhibition of LPL and enhanced lipolysis of intracellular triglycerides in adipocytes by ANGPTL4 can contribute to cachexia, impaired exercise capacity, and reduced body mass in nephrotic and chronic kidney patients.[36]

VLDL receptor deficiency. The VLDL receptor is a member of the LDL receptor family, which binds and internalizes VLDL. It is expressed in skeletal muscle, adipose tissue, and myocardium, and, as such, its tissue distribution is similar to that of LPL.[9–11] In a series of studies, we found a marked reduction of VLDL receptor mRNA and protein abundance in the skeletal muscle, adipose tissues, and myocardium of rats with nephrotic syndrome.[25,37] Given the role of the VLDL receptor in endocytosis and clearance of VLDL, its acquired deficiency may contribute to elevated serum VLDL in nephrotic syndrome.

Role of HDL abnormalities in impaired VLDL and chylomicron metabolism. As described in a later section of this review, nephrotic syndrome results in lecithin cholesteryl ester acyltransferase (LCAT) deficiency and upregulation of CETP, which lead to a decrease in cholesterol ester content and an increase in triglyceride content of HDL and impaired maturation of cholesterol-poor to cholesterol-rich HDL. The scarcity of cholesterol-rich HDL, in turn, limits contribution of apoE and apoC to the nascent VLDL and chylomicrons. Because clearance of the ApoB-containing lipoproteins depends on apoE and apoC for their binding to the endothelium and activation of LPL, HDL abnormalities contribute to the impaired VLDL metabolism in nephrotic syndrome.[21,22]

Upregulation of hepatic LDL receptor–related protein 1. LDL receptor–related protein 1 (LRP-1), also known as α_2-macroglobulin receptor, apoE receptor, or a cluster of differentiation 91, is a large (600 kDa) multifaceted receptor that is a member of the LDL receptor family and is ubiquitously expressed in multiple cell types and tissues including hepatocytes, vascular smooth muscle cells, and neurons, among others.[38] LRP-1 recognizes and binds 40 different ligands including lipoproteins, coagulation factors, proteases, protease inhibitor complexes, extracellular matrix proteins, growth factors, and several other proteins. In addition to serving as the vehicle for endocytosis and degradation of its ligands, LRP-1 serves as a key signaling protein involved in various other biological processes such as cell motility, lipoprotein metabolism, and pathologic conditions including neurodegenerative diseases, atherosclerosis, and cancer.[39,40] In the liver, LRP-1 binds and internalizes chylomicron remnants, IDL, and modified HDL particles. LRP mRNA expression and protein abundance are markedly increased in the liver of nephrotic animals and are partially reversed by statin therapy in these animals.[41] These findings exclude LRP deficiency as the primary cause of increased plasma lipoprotein remnants in nephrotic syndrome. However, structural abnormalities of the IDL and chylomicron remnants appear to limit their binding to and clearance by LRP-1, as reported by Wang *et al.*[20]

Dysregulation of hepatic fatty acid, triglyceride, and phospholipid metabolism. Nephrotic syndrome results in increased expression of the key enzymes involved in fatty acid biosynthesis including acetyl coenzyme A (CoA) carboxylase, fatty acid synthase, elongation of very long chain fatty acids 2 and 6, and downregulation of genes encoding proteins involved in fatty acid catabolism in the liver.[42] In addition, expression of genes encoding the main steps in phospholipid and triglyceride synthesis are upregulated in the liver of animals with nephrotic syndrome.[42] These include glycerol-3-phosphate acyltransferase, which catalyzes the initial and committing step in glycerolipid biosynthesis and plays a pivotal role in the regulation of cellular triglyceride and phospholipid levels; 1-acyl-sn-glycerol-3-phosphate acyltransferase gamma, an acyltransferase that converts lysophosphatidic acid into phosphatidic acid and represents the second step in phospholipid biosynthesis; and acyl-CoA diglycerolacyltransferase, which catalyzes the final step in triglyceride biosynthesis. Expression and activity of diglycerolacyltransferase-1, the dominant isoform of diglycerolacyltransferase in the liver, are significantly increased in nephrotic animals.[43] These abnormalities suggest that, in addition to impaired clearance of triglyceride-rich lipoproteins, increased production of fatty acids, triglycerides, and phospholipids may contribute to the hyperlipidemia in nephrotic syndrome.

apoB biosynthesis. The serum apoB-100 level is elevated in nephrotic syndrome. Elevation of serum apoB-100 in nephrotic syndrome is associated with and in part due to its increased production and impaired clearance.[44]

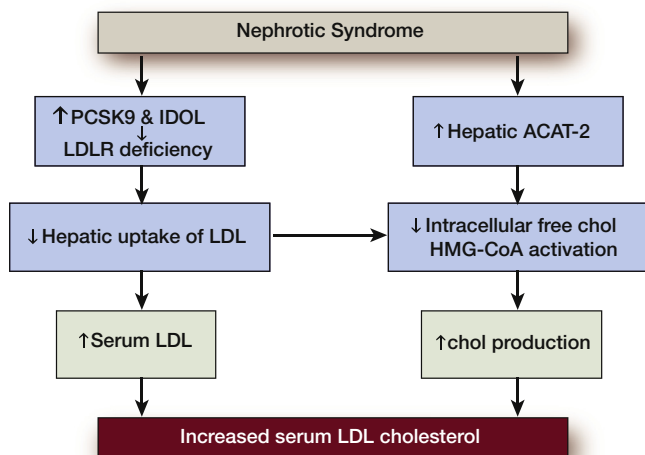**LDL and cholesterol metabolism in nephrotic syndrome**

Nephrotic syndrome results in marked elevation of serum total cholesterol and LDL cholesterol. This is due to a combination of increased production[1] and impaired catabolism/clearance of LDL[3] and apoB-100.[5,45] As described in the following and depicted in Figure 2, several factors contribute to the defective LDL clearance and increased cholesterol biosynthesis in nephrotic syndrome.

Hepatic cholesterol biosynthesis and catabolism. In a series of earlier studies designed to explore the impact of nephrotic syndrome on cholesterol biosynthesis, we determined the mRNA expression and enzymatic activity of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate-limiting step in cholesterol biosynthesis, in rats with puromycin aminonucleoside–induced nephrotic syndrome[46] and in Imai rats with spontaneous focal glomerulosclerosis.[47] The studies showed a marked increase in HMG-CoA reductase in the liver of Imai rats. Longitudinal studies in rats with puromycin-induced nephrosis showed a steady increase in hepatic tissue HMG-CoA reductase expression and activity during the induction of hypercholesterolemia. The study further showed restoration of HMG-CoA reductase expression and activity and

Figure 2 | Via a marked increase in serum proprotein convertase subtilisin kexin type 9 (PCSK9) and the liver tissue inducible degrader of the LDL receptor (IDOL), which are potent degraders of low-density lipoprotein (LDL) receptor (LDLR), nephrotic syndrome results in acquired LDLR deficiency. Acquired LDLR deficiency accounts for the impaired clearance and increased serum LDL in nephrotic patients and animals. In addition, nephrotic syndrome leads to a significant increase in liver acyl-CoA cholesterol acyltransferase-2 (ACAT-2) expression and activity, which results in enhanced esterification of cholesterol and reduction of intracellular free cholesterol. The reduction in cholesterol uptake occasioned by LDLR deficiency and the reduction in intracellular free cholesterol by ACAT-2 work in concert to promote activation of sterol regulatory element-binding protein-2 (SREBP-2) and SREBP-1. Activation of SREBP-2 heightens 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase–mediated cholesterol production, and activation of SREBP-1 increases production of fatty acids, events that contribute to hypercholesterolemia and hypertriglyceridemia in nephrotic syndrome.

nuclear translocation of its master regulator, sterol-responsive element binding protein 2, to baseline after attainment of steady-state hypercholesterolemia in nephrotic animals.[46,48] Taken together, these findings demonstrated the role of increased cholesterol production capacity in the induction of nephrotic hypercholesterolemia. In a subsequent study, we determined the hepatic tissue expression and activity of cholesterol-7 alpha hydroxylase, the rate-limiting enzyme in cholesterol conversion to bile acids for secretion in the bile. The study revealed no change in hepatic tissue cholesterol-7 alpha hydroxylase level during the course of induction and maintenance of nephrotic syndrome and the associated hypercholesterolemia compared with the values obtained at baseline and in the control group.[49] This observation illustrated the role of increased cholesterol biosynthesis as opposed to reduced cholesterol catabolism in the induction of hypercholesterolemia in nephrotic syndrome.

Upregulation of hepatic acyl-CoA:cholesterol acyltransferase-2. Via esterification of free cholesterol, acyl-CoA:cholesterol acyltransferase (ACAT) plays an essential role in packaging cholesterol in apoB-100 in the liver for release in the circulation. In addition, by lowering free cholesterol in hepatocytes, ACAT plays an important part in transcriptional and post-translational regulation of hepatic cholesterol production machinery. In a series of studies, we found marked upregulation of mRNA expression, protein abundance, and enzymatic activity of the liver-specific ACAT-2 in nephrotic animals.[50] In a subsequent study, we found dramatic improvement in serum lipid levels and lipid regulatory machinery with administration of the ACAT inhibitor CI-976 in nephrotic animals.[51] These findings illustrated the critical contribution of upregulation of hepatic ACAT-2 in the pathogenesis of the disorders of lipid metabolism in nephrotic syndrome.

Acquired LDL receptor deficiency. By virtue of its capacity to bind and clear LDL from the circulation, LDL receptor plays a pivotal role in LDL and cholesterol metabolism. LDL bound to the LDL receptor is internalized into the clathrin-coated pits and subsequently undergoes lysosomal degradation. The LDL receptor is then returned to the cell membrane to repeat the cycle. Several studies had shown impaired clearance and catabolism of LDL[3] and its principal apoprotein, apoB-100,[5,45] in nephrotic syndrome. However, the underlying mechanism by which nephrotic syndrome impairs LDL clearance was not clear. To address this issue in a series of studies using rats with puromycin-induced nephrotic syndrome and Imai rats with spontaneous focal glomerulosclerosis, we found marked reduction in hepatic tissue LDL receptor protein abundance despite its normal mRNA expression, pointing to the posttranscriptional or posttranslational nature of LDL receptor deficiency.[47,48,52] These observations elucidated a major underlying mechanism for increased serum LDL concentration, impaired LDL clearance, and upregulation of cholesterol production machinery in nephrotic syndrome. In addition, alteration in the composition of LDL may contribute to its impaired clearance via interference with the receptor-binding process.[20]

Upregulation of proprotein convertase subtilisin kexin type 9 and inducible degrader of the LDL receptor. Two major post-translational regulators of the LDL receptor that play a critical part in LDL metabolism have been identified. These include proprotein convertase subtilisin kexin type 9 (PCSK9) and inducible degrader of the LDL receptor (IDOL), which mediate degradation of LDL receptor, and as such, their upregulation leads to impaired clearance and increase in plasma LDL.[53–55] PCSK9 is a serine protease that is produced and released in the circulation by the liver and to a lesser extent by the intestine and kidney. On the surface of hepatocytes PCSK9 binds to the LDL receptor, forming a complex that is internalized and directs the LDL receptor for intracellular degradation.[56] The ability of PCSK9 to promote degradation of the LDL receptor is not related to its enzymatic activity; instead PCSK9 acts as a chaperone that facilitates intracellular degradation of the LDL receptor.[57] By promoting degradation of the LDL receptor, PCSK9 prevents its recycling to the cell membrane, leading to a posttranslational reduction of LDL receptor expression.[54] In fact, loss-of-function mutation of PCSK9 is associated with a marked reduction of plasma LDL-cholesterol and a significant reduction in the risk of coronary heart disease.[58] For this reason, PCSK9 has emerged as a novel therapeutic target for the treatment of hypercholesterolemia.

The other important LDL receptor degrader is IDOL, which is an E3 ubiquitin ligase that mediates ubiquitination and degradation of the LDL receptor. IDOL expression is regulated by the liver X receptor in response to the increase in cellular oxysterols.[59] Given the central role of PCSK9 and IDOL in posttranslational regulation of the LDL receptor, in a recent study, we tested the hypothesis that LDL receptor deficiency, despite its normal gene expression in nephrotic syndrome, may be due to upregulation of hepatic PCSK9 and IDOL. To this end, the LDL receptor, IDOL, PCSK9 expression, and nuclear translocation of the liver X receptor, which regulates IDOL expression, were determined in the liver of nephrotic and control rats. The study revealed a marked increase in serum total and LDL cholesterol and a significant reduction in hepatic LDL receptor protein expression in the nephrotic animals. This was accompanied by a marked upregulation of hepatic PCSK9 and IDOL expression and heightened liver X receptor activation. These findings unraveled the role of upregulation of PCSK9 and IDOL in the pathogenesis of LDL receptor deficiency, hypercholesterolemia, and increased plasma LDL in nephrotic syndrome.[60] In a concurrent study of patients with nephrotic syndrome and healthy control subjects, we found a marked increase in plasma PCSK9 in nephrotic patients in whom plasma PCSK9 showed a direct correlation with the LDL cholesterol level.[61] In addition, plasma PCSK9 was measured in hemodialysis and peritoneal dialysis patients. The study revealed an increase in plasma LDL cholesterol and PCSK9 levels in peritoneal dialysis patients but not hemodialysis patients. Peritoneal dialysis results in significant loss of proteins in peritoneal dialysate effluent, which simulates urinary loss of

protein in nephrotic syndrome. The presence of increased LDL cholesterol and PCSK9 in both nephrotic and functionally anephric peritoneal dialysis patients illustrated the loss of protein as the primary mediator of the altered LDL metabolism.

In addition to its role in degradation of the LDL receptor, recent studies revealed that PCSK9 directly interacts with and targets CD36, also known as scavenger receptor class B type 3 (SR-B3) or fatty acid translocase. CD36 is a member of the scavenger receptor class B family, which includes SR-B1 (the HDL docking receptor that mediates cholesteryl ester uptake) and SR-B2 (a lysosomal integral membrane protein [LIMP2]).[62] CD36 is a multiligand cell surface receptor expressed in several types of cells and tissues, such as macrophages, heart, adipose tissue, and liver. It is a major receptor for oxidized LDL in macrophages and plays a key role in the formation of lipid-laden foam cells and atherosclerosis. In addition, CD36 contributes to lipid use by the muscles, energy storage in adipose tissue, and uptake of fat in tissues with important lipid fluxes via binding long-chain fatty acids and facilitating their transport into the cells.[61] Moreover, CD36 plays an important role in the uptake of fatty acids and storage and secretion of triglycerides by the liver.[63] CD36 is a major mediator of fatty acid uptake by adipocytes and regulation of adipose tissue growth and function. In fact, CD36 null mice are significantly leaner than their wild-type counterparts and exhibit increased plasma levels of triglycerides and fatty acids.[64] Therefore, by promoting CD36 degradation, elevation of PCSK9 must contribute hypertriglyceridemia, elevated serum fatty acid level, and diminished adipose tissue mass in nephrotic patients. Given the recent introduction of PCSK9 antibody for the treatment of hypercholesterolemia,[65–67] therapeutic interventions targeting PCSK9 may be effective in the management of hypercholesterolemia, hypertriglyceridemia, and the associated cardiovascular and other complications of nephrotic syndrome.

Increased plasma lipoprotein(a) in nephrotic syndrome

Lipoprotein(a) (Lp[a]) is an atherogenic and prothrombotic factor that exerts its effects by inhibiting fibrinolysis via competition with plasminogen binding sites,[68] promoting LDL oxidation[69] and facilitating monocyte adhesion.[70] Lp(a) consists of an LDL particle to which the apoA is covalently bound. The Apo(a) molecules exhibit a huge polymorphism that is due to the interindividual differences in the number of kringle IV repeats at the apoA gene locus. This polymorphism determines the plasma level of Lp(a) levels, which vary by more than 1000-fold between different individuals. The plasma Lp(a) level is high in individuals with low molecular weight apoA phenotypes and low in those with high molecular weight Apo(a) phenotypes. The routine methods used to measure LDL cholesterol do not distinguish cholesterol derived from LDL and Lp(a), and as such, the reported result represents the cholesterol contents of both lipoproteins. Consequently, increased Lp(a) can significantly contribute to the measured or calculated LDL cholesterol levels. It is of note

that statins have no effect on Lp(a) level, and its elevated level can diminish the efficacy of the LDL cholesterol–lowering effect of statins in reducing the risk of cardiovascular complications. Nephrotic syndrome results in marked increases in serum Lp(a),[71–73] which is due to its increased production by the liver.[74] Plasma Lp(a) decreases in response to spontaneous remission or antiproteinuric therapies. Increase in Lp(a) in nephrotic syndrome contributes to the prothrombotic and atherogenic diathesis in this population.

HDL metabolism and its abnormalities in nephrotic syndrome
Via its powerful anti-inflammatory, antioxidant, and antithrombotic activities and its central role in the reverse cholesterol transport, normal HDL confers protection against cardiovascular disease, oxidative stress, and systemic inflammation.[75] ApoA-I, which is the main apoprotein constituents of HDL, is produced and released in the plasma by the liver. Nascent HDL is formed by partial lipidation of apoA-I with phospholipids and cholesterol via its binding to the adenosine triphosphate (ATP) binding cassette class A type 1 on hepatocytes and enterocytes. Within the interstitial space, nascent HDL binds to the ATP binding cassette class A type 1 on the lipid-laden macrophages and other target cells, triggering activation of cholesterol ester hydrolase, hydrolysis of intracellular cholesterol ester, release of free cholesterol, and its transfer to the surface of HDL. On the surface of HDL, cholesterol is re-esterified by LCAT and stored in the core of HDL. This process leads to transformation of lipid-poor discoid HDL to the spherical cholesterol ester–rich HDL. Once formed, cholesterol ester–rich HDL is released in the interstitial space and transported to the bloodstream through the lymphatic vasculature.[76] In addition to lipid-laden macrophages, HDL receives a substantial quantity of cholesterol from other cell types such as adipocytes, skin fibroblasts, and myocytes.[77,78] In addition, HDL acquires considerable amounts of lipids and apoproteins from the apoB-containing lipoproteins and albumin in the bloodstream. For instance, phospholipid transfer protein transfers phospholipids that are released from lipolysis of VLDL and chylomicrons by lipoprotein lipase in adipose tissue and skeletal muscles to HDL. In addition, cholesterol ester transfer protein (CETP) transfers part of HDL's cholesterol ester content to IDL and LDL in exchange for triglycerides, and albumin transfers its free cholesterol cargo to HDL. Finally, cholesterol ester–rich HDL binds to the HDL docking receptor, SR-B1, on hepatocytes, which accommodates unloading of its cholesterol content and hydrolysis of its triglycerides and phospholipids cargo by hepatic lipase for uptake by the liver. Cholesterol delivered to hepatocytes is in turn converted to bile acids and secreted in the bile. After unloading its lipid cargo, the delipidated HDL is released in the circulation and repeats the cycle.

HDL has numerous important biological functions that are essential for life. These include the following: (i) reverse cholesterol transport, as the primary vehicle for reverse cholesterol transport, normal HDL plays a major role in protection against atherosclerotic cardiovascular disease;

(ii) antioxidant activity, via its constituent antioxidant enzymes paraoxonase and glutathione peroxidase, normal HDL confers protection against oxidative stress; (iii) anti-inflammatory properties, by preventing formation of the proinflammatory oxidized lipids and lipoproteins and by removing oxidized phospholipids and fatty acids from LDL, VLDL, IDL and their disposal in the liver, normal HDL plays an important role in the protection against systemic inflammation. In addition, by removing circulating endotoxin and amyloid A and their disposal in the liver, HDL mitigates inflammation.[79,80] Another important anti-inflammatory property of normal HDL is its ability to inhibit monocyte adhesion to endothelial cells by lowering vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 expression in endothelial cells and inhibiting CD11b expression on monocytes.[81–83] (iv) Via antithrombotic activity, normal HDL lowers the risk of thrombotic complications by reducing formation of oxidized LDL, which promotes platelet aggregation and adhesion by inhibiting tissue factor, P-selectin, E-selectin, platelet activating factor, and thromboxane A2 expression and by upregulating antithrombin-3, protein C, and protein S expression.[84–86] (v) By preservation of endothelial function and viability via Akt-mediated phosphorylation of endothelial nitric oxide synthase, normal HDL promotes activation of endothelial nitric oxide synthase and production of nitric oxide. In addition, via its antioxidant properties, HDL limits the uncoupling (inactivation) of endothelial nitric oxide synthase and oxidation of nitric oxide by reactive oxygen species. Besides enhancing endothelial function, normal HDL facilitates the repair, migration, and proliferation of endothelial cells; increases the number of circulating endothelial progenitor cells; and, by lowering caspase-3 activity, prevents apoptosis of endothelial cell.[87–90]

As described in a recent review,[21] nephrotic syndrome results in profound abnormalities in the structure and function of HDL. These abnormalities are caused by dysregulation of several key proteins involved in HDL structure and loading and unloading of its lipid cargo. The serum HDL cholesterol level is usually within or below the normal limits in patients with nephrotic syndrome. However, HDL cholesterol to total cholesterol ratio is consistently reduced, and maturation of cholesterol ester–poor HDL to cholesterol ester–rich HDL is impaired in nephrotic syndrome.[21,91,92] These abnormalities point to impaired HDL-mediated reverse cholesterol transport, which, in part, account for the atherogenic effects of proteinuria. The underlying mechanisms of the abnormalities of HDL structure and function in nephrotic syndrome are depicted in Figure 3 and briefly described in the following.

LCAT deficiency. Esterification of free cholesterol by LCAT is critical for efficient extraction of cholesterol from the target cell and maturation of nascent HDL to cholesterol ester–rich HDL. In an earlier study, we found a significant reduction in plasma concentration and enzymatic activity of LCAT despite its normal hepatic mRNA expression in rats with nephrotic syndrome. LCAT deficiency in the nephrotic animals was associated with and primarily due to its heavy losses in the
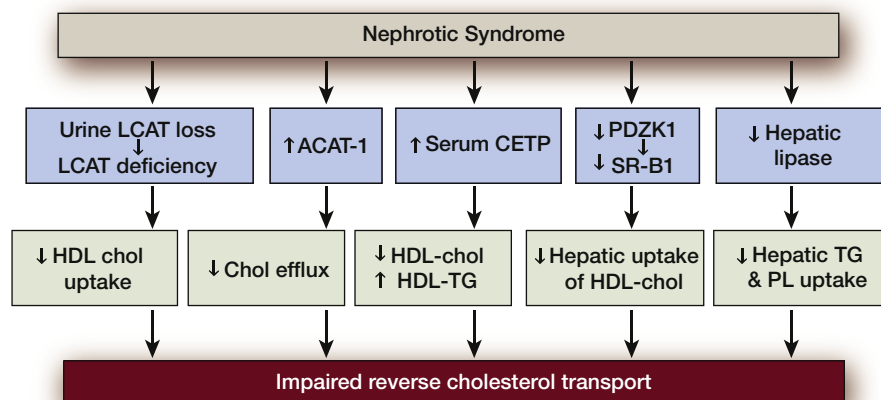
Figure 3 | Nephrotic syndrome results in significant elevation of vascular and renal tissue acyl-CoA cholesterol acyltransferase-1 (ACAT-1) expression and heavy urinary losses and marked reduction of serum lecithin cholesteryl ester acyltransferase (LCAT) level, which work in concert to limit high-density lipoprotein (HDL)–mediated extraction of cholesterol from lipid-laden macrophages and mesangial and other cell types. This is compounded by a marked increase in serum cholesterol ester transfer protein (CETP), which leads to further depletion of HDL cholesterol and enrichment of its triglyceride (TG) cargo. In addition, by inducing downregulation of PDZ-containing kidney protein 1 (PDZK1), nephrotic syndrome results in a marked reduction in liver HDL docking receptor (scavenger receptor class B, type 1 [SR-B1]), which is the gateway for unloading of HDL's cholesterol cargo and hepatic lipase–mediated extraction of its TG and phospholipid (PL) cargos. Together these abnormalities severely impair reverse cholesterol transport and contribute to the atherogenic diathesis in nephrotic syndrome. Chol, cholesterol.

urine.[93] Thus, urinary losses of LCAT result in LCAT deficiency, which plays a major role in the pathogenesis of impaired HDL-mediated reverse cholesterol transport in nephrotic syndrome.

Low serum albumin. In addition to acquiring most of its cholesterol via the ATP binding cassette class A type 1–mediated pathway, HDL receives a significant amount of cholesterol from albumin, which transfers free cholesterol from peripheral tissues to HDL.[94] Therefore, hypoalbuminemia, which is a defining feature of nephrotic syndrome, potentially contributes to the defective cholesterol enrichment of HDL in nephrotic patients.

Elevated plasma CETP. By mediating the transfer of cholesterol esters from HDL to IDL and LDL in exchange for triglycerides, CETP plays a critical role in converting IDL to LDL. Serum CETP is markedly increased in patients with nephrotic syndrome.[95–97] By depleting the cholesterol esters of HDL and increasing its triglyceride cargo, an increase in CETP compounds the effect of LCAT deficiency in preventing transformation of cholesterol ester–poor HDL to cholesterol ester–rich HDL in nephrotic syndrome.

Hepatic HDL docking receptor, SR-B1, deficiency. Reversible binding of cholesterol ester ester–rich HDL to SR-B1, which is expressed on the plasma membrane of hepatocytes, represents the final step in reverse cholesterol transport. Binding to SR-B1 accommodates unloading of HDL's cholesterol cargo in the hepatocyte and hydrolysis of its triglyceride and phospholipid contents by hepatic lipase.[98] In an earlier study, we found a marked reduction in SR-B1 protein abundance despite its normal mRNA expression in the liver of nephrotic animals, indicating a posttranscriptional or posttranslational

mechanism of SR-B1 deficiency.[99] By compromising reverse cholesterol transport, hepatic SR-B1 deficiency contributes to the associated atherogenic diathesis in patients with nephrotic syndrome.

Hepatic PDZ-containing kidney protein 1 deficiency. Binding and stability of SR-B1 in the hepatocyte plasma membrane are dependent on the adapter molecule PDZ-containing kidney protein 1 (PDZK1). PDZK1 is attached to the basolateral plasma membrane of hepatocytes where it interacts with the cytoplasmic C-terminal domain of SR-B1 via its N-terminal PDZ domain.[100] As the main adapter protein for SR-B1, PDZK1 plays a critical role in preventing degradation of SR-B1. In a recent study, we found marked reduction of PDZK1 mRNA and protein expressions in the liver of nephrotic animals.[101] This finding elucidated the mechanism of acquired SR-B1 deficiency in nephrotic syndrome.

In addition to the hepatocytes, the PDZK1-SR-B1 complex is expressed in endothelial cells and mediates the protective and regulatory actions of HDL on endothelial cells.[102] Given the important role of PDZK1 in the HDL-mediated reverse cholesterol transport and its protective effects on the endothelial structure and function, acquired PDZK1 deficiency plays a major role in the HDL abnormalities and their adverse consequences in nephrotic syndrome.

Upregulation of hepatic HDL endocytic receptor. The β chain ATP synthase, which is a main constituent of the mitochondrial inner membrane protein complex, is also present in the hepatocyte plasma membrane where it serves as an apoA-I receptor and mediates endocytosis of apoA-I and lipid-poor HDL. This endocytic process depends on the production of adenosine diphosphate derived from activation of ATPase
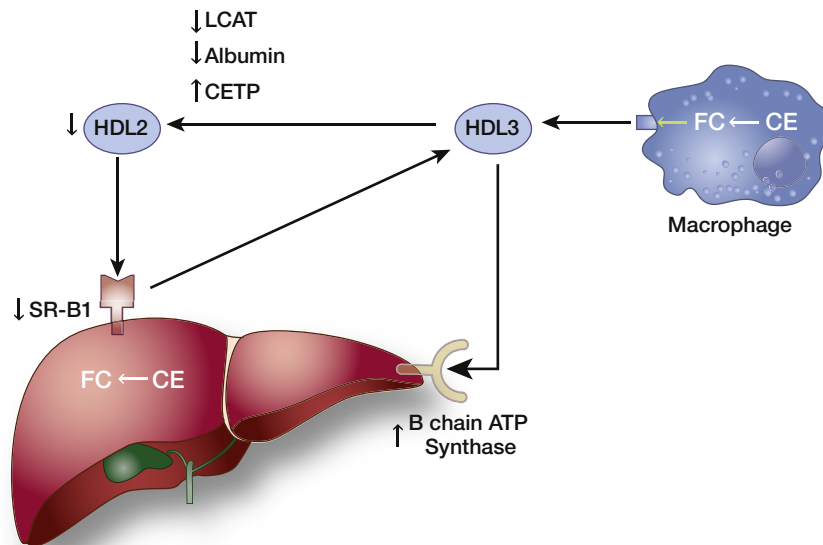
Figure 4 | By causing lecithin cholesteryl ester acyltransferase (LCAT) deficiency and hypoalbuminemia, which limit the transfer of cholesterol from peripheral tissues to high-density lipoprotein (HDL), and increase cholesterol ester transfer protein (CETP), which increases the transfer of HDL cholesterol cargo to immediate-density lipoprotein (IDL)/low-density lipoprotein (LDL), nephrotic syndrome limits cholesterol enrichment of HDL. In addition, downregulation of the hepatic HDL docking receptor SR-B1 (scavenger receptor class B, type 1), occasioned by downregulation of PDZ-containing kidney protein 1, limits HDL's ability to unload its cholesterol cargo in the liver. Together, these abnormalities contribute to profound impairment of reverse cholesterol transport. ATP, adenosine triphosphate; CE, cholesterol ester; FC, free cholesterol.

function of the receptor, which is triggered by its binding to apoA-I. Unlike SR-B1, which has a high affinity for reversible binding of cholesterol ester–rich HDL, β-chain ATP synthase mediates endocytosis and catabolism of apoA-1 and lipid-poor HDL.[103] In a recent study, we found marked upregulation of this receptor in the liver of nephrotic animals.[104]

Hepatic lipase deficiency. Earlier *in vivo* and *in vitro* studies revealed significant downregulation of hepatic lipase expression and activity in animals with nephrotic syndrome.[24,25] Because binding of HDL to SR-B1 in the liver accommodates hydrolysis and removal of its triglyceride and phospholipid contents by hepatic lipase, acquired hepatic lipase deficiency contributes to the triglyceride enrichment of HDL in nephrotic syndrome.

Taken together, acquired LCAT deficiency and hypoalbuminemia, which limit uptake of cholesterol from peripheral tissues, and increase in CETP, which increases the transfer of HDL's cholesterol ester cargo to IDL/LDL, work in concert to limit cholesterol enrichment of HDL in nephrotic syndrome. In addition, marked reduction of hepatic HDL docking receptor SR-B1, occasioned by downregulation of PDZK1, limits HDL's ability to unload its cholesterol cargo in the liver (Figure 4). These abnormalities result in accumulation of cholesterol ester–poor HDL and severe impairment of HDL-mediated reverse cholesterol transport, thereby contributing to the proatherogenic effect of proteinuria.

Consequences of lipid abnormalities in nephrotic syndrome

The abnormalities of lipid metabolism in nephrotic syndrome described previously can cause serious consequences:

accumulation of atherogenic LDL, IDL, and chylomicron remnants, coupled with impaired HDL-mediated reverse cholesterol transport, contributes to the development and progression of atherosclerosis and cardiovascular disease; impaired delivery of lipid fuel to the skeletal muscles and adipose tissue, occasioned by lipoprotein lipase deficiency and dysfunction, results in the reductions of body mass and diminished exercise capacity[104]; uptake of abnormal lipoproteins by glomerular mesangial cells promotes glomerulosclerosis and reabsorption of the filtered albumin and other lipid-containing proteins leads to accumulation of lipids and cytotoxicity in proximal tubular epithelial cells, events that can result in the loss of nephrons and development and progression of chronic kidney disease[21,105–107]; and an increase in plasma LP(a) in nephrotic patients increases the risk of the thromboembolic and cardiovascular complications.

Treatment of nephrotic dyslipidemia

The conventional and potential novel therapeutic strategies for the management of dyslipidemia in kidney disease were addressed in previous reviews in detail[21,108] and are only briefly described here. Given the central role of proteinuria in the pathogenesis of lipid disorders in nephrotic syndrome, the ideal target of therapeutic intervention is reversal or attenuation of proteinuria.

Statins. Because upregulation of HMG-CoA reductase contributes in part to hypercholesterolemia in nephrotic syndrome, statins are generally effective in attenuating hypercholesterolemia in these patients. However, use of rosuvastatin should be avoided in patients with kidney disease

because it can intensify proteinuria and impair renal function.[108,109]

PCSK9 inhibitors. As mentioned earlier in this review, the underlying mechanism of upregulation of HMG-CoA reductase and impaired clearance of LDL in nephrotic syndrome is PCSK9- and IDOL-mediated degradation of the LDL receptor. Therefore, therapeutic interventions aimed at inhibiting PCSK9 or IDOL can be highly effective in reducing LDL cholesterol in nephrotic patients. A recent clinical trial of the monthly subcutaneous administration of human monoclonal antibody against PCSK9 (Evolocumab, AMG 145) demonstrated a >50% reduction in LDL cholesterol in a large cohort of hypercholesterolemic patients.[110,111] Although the study did not include patients with kidney disease, upregulation of PCSK9 and its central role in the pathogenesis of LDL receptor deficiency and increased LDL cholesterol in patients and animals with nephrotic syndrome[60,61] point to the potential efficacy and specificity of the PCSK9 inhibitors in the nephrotic population. Future studies are needed to explore this possibility.

Acyl coenzyme A:cholesterol acyltransferase inhibitors. Another potential therapeutic target for nephrotic dyslipidemia is acyl coenzyme A:cholesterol acyltransferase (ACAT). ACAT catalyzes conversion of free cholesterol to cholesterol ester for incorporation in VLDL in the liver and formation of chylomicrons in the intestine. Moreover, by modulating the intracellular free cholesterol level and hence activities of sterol-responsive element binding protein-2 and sterol-responsive element binding protein-1 in hepatocytes and other cell types, ACAT regulates the biosynthesis of cholesterol and fatty acids. In addition, by mediating esterification of free cholesterol in macrophages, vascular smooth muscle cells, mesangial cells, and other cell types, ACAT plays a central role in foam-cell formation and confers resistance to HDL-mediated reverse cholesterol transport. Given the well-documented upregulation of hepatic, arterial, and renal tissue ACAT levels in nephrotic syndrome,[50] its pharmacologic inhibition may improve lipid metabolism and lower the risk of cardiovascular disease and glomerulosclerosis in nephrotic syndrome. In fact, in an earlier study, we found marked improvement in proteinuria, renal function, and the plasma lipid profile with administration of a low dose of the ACAT inhibitor avasimibe in nephrotic rats.[51] It should be noted that although contributing to dyslipidemia, atherosclerosis, glomerulosclerosis, and other adverse effects by sequestering cholesterol ester in intracellular vesicles, elevated levels of ACAT protect the cholesterol-laden cells against free cholesterol–mediated disruption of the cell membrane, which, by releasing intracellular proteolytic enzymes, can lead to the rupture of atherosclerotic plaques and acute cardiovascular events. In fact, due to adverse cardiovascular outcomes, a large clinical trial of the ACAT inhibitor avasimibe in patients with coronary atherosclerosis was terminated, and the drug was withdrawn from further consideration.[112] However, treatment with low doses of ACAT inhibitors may potentially confer protection in patients with nephrotic proteinuria and no evidence of atherosclerosis or significant renal insufficiency.

Conclusions

Studies conducted to elucidate the underlying mechanisms of lipid disorders in nephrotic syndrome have unraveled the dysregulation of a vast number of proteins involved in biosynthesis, transport, and disposition of lipids and lipoproteins. These finding have identified new targets for development and clinical trials of novel therapeutic agents that can control nephrotic dyslipidemia and prevent its complications with high specificity and efficacy.

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