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In the Literature

Dual Role of Circulating Angiopoietin-Like 4 (ANGPTL4) in Promoting Hypertriglyceridemia and Lowering Proteinuria in Nephrotic Syndrome

Commentary on Clement LC, Macé C, Avila-Casado C, et al. Circulating angiopoietin-like 4 links proteinuria with hypertriglyceridemia in nephrotic syndrome. Nat Med. 2014;20(1):37-46.

Hyperlipidemia is a hallmark of nephrotic syndrome and is characterized by elevation of plasma cholesterol, triglyceride, low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and lipoprotein(a) levels, as well as altered structure and function of high-density lipoprotein (HDL).[1-3] Recently in *Nature Medicine*, Clement et al[4] published a series of experiments that showed that elevation of circulating angiopoietin-like 4 (Angptl4 in rats; ANGPTL4 in humans) appears to be the missing link that ties hypertriglyceridemia to albuminuria in nephrotic syndrome. Nephrotic hyperlipidemia is due largely to the acquired deficiencies of lipoprotein lipase (LPL),[5-7] hepatic lipase,[8] the VLDL receptor,[5,9] the LDL receptor,[10-12] lecithin-cholesterol acyltransferase (LCAT),[13] and the HDL docking receptor (SRB1; encoded by *SCARB1*)[14,15]; upregulation of cholesteryl-ester transfer protein (CETP),[16,17] hepatic acyl coenzyme A cholesterol acyltransferase (ACAT, encoded by *ACAT1*),[18,19] diglycerol acyltransferase,[20] and lipoprotein(a)[21]; and altered composition of plasma lipoproteins interfering with their receptor binding and clearance.[22]

Triglyceride-rich lipoproteins (VLDL and chylomicrons) deliver fatty acid to various tissues, including myocytes for energy production and adipocytes for energy storage. Nascent VLDL particles are produced in hepatocytes and released in the circulation, where they acquire apolipoproteins E and C (Apo-E and Apo-C) from cholesterol ester–rich HDL subfraction 2 (HDL2). In the capillaries perfusing muscles and adipose tissues, VLDL binds to the endothelial surface by its Apo-E content and activates the adjacent LPL by its Apo-CII content. This leads to hydrolysis of 70% of VLDL's triglyceride cargo by LPL, release of free fatty acid (FFA), and conversion of VLDL to IDL. Nascent chylomicrons are formed in the enterocytes and released in the circulation, where, like VLDL particles, they acquire Apo-E and Apo-C from HDL2. This is followed by LPL-mediated hydrolysis of 70% of their triglyceride contents and their conversion to chylomicron remnants (Fig 1). Two-thirds of the fatty acids released from VLDL and chylomicrons enter the adjacent myocytes or adipocytes, whereas the other FFAs enter the plasma pool bound to albumin and lipoproteins and are transported to distant sites, mainly the liver. After undergoing lipolysis by LPL, the IDL and chylomicron remnants, which contain 30% of their original triglyceride cargos, are released into the circulation. The remaining triglyceride contents of IDL normally are removed by CETP-mediated exchange of triglycerides for cholesterol ester with HDL2 and by hepatic lipase, leading to the formation of LDL, which ultimately is cleared by LDL receptor. The chylomicron remnants are removed by the liver by a large multifunctional receptor known as LDL receptor–related protein (LRP).

As noted, LPL and hepatic lipase play a central part in triglyceride metabolism, and their acquired deficiencies in nephrotic syndrome are largely responsible for the associated hypertriglyceridemia. Until recently, the mechanism by which nephrotic syndrome causes LPL and hepatic lipase deficiencies was unknown. Recently, Clement et al[4] published a series of experiments that demonstrated the role of elevated FFA to albumin ratio as the mediator of elevation of circulating Angptl4, as well as the dual function of Angptl4 in inhibiting LPL and lowering proteinuria in nephrotic animals.

Human ANGPTL4 is a glycoprotein (molecular weight, 45-65 kDa) that is constitutively expressed in the liver, adipose tissue, skeletal muscle, small intestine, and heart. ANGPTL4 expression is upregulated and its plasma level increases in response to fasting, high FFA levels, and hypoxia.[23] The effects of fasting and FFA on ANGPTL4 are mediated by peroxisome proliferator-activated receptors (PPARs). In addition, as a positive acute-phase protein, ANGPTL4 expression in the liver, heart, muscle, and adipose tissue is increased during the acute-phase response.[24] By binding to the active LPL dimers and converting LPL into inactive monomers, ANGPTL4 can impair clearance of triglyceride contents of VLDL and chylomicrons and cause hypertriglyceridemia. In addition to inhibiting LPL, ANGPTL4 may inhibit hepatic lipase,[23] which can increase plasma triglyceride levels further by impairing clearance of triglyceride contents of circulating IDL and HDL. The LPL-inhibitory effect of ANGPTL4 is mitigated by glycosylphosphatidylinositol-anchored binding protein 1 (GPIHBP1), which is the transporter and

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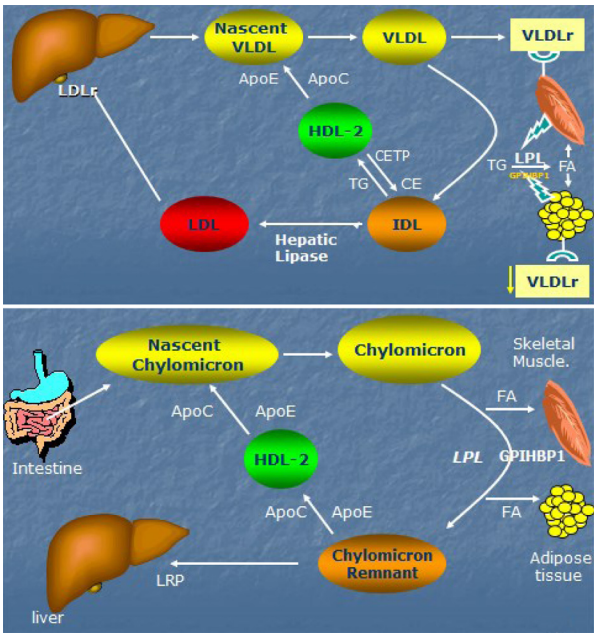

Figure 1. Nascent very low-density lipoprotein (VLDL) particles are produced in hepatocytes (upper panel) and released into the circulation, where they acquire apolipoprotein E (Apo-E) and Apo-C from cholesterol ester (CE)-rich high-density lipoprotein (HDL) subfraction 2 (HDL-2). In capillaries perfusing muscle and adipose tissue, VLDL binds to the endothelial surface by its Apo-E content and activates the adjacent lipoprotein lipase (LPL) by its Apo-CII content. This leads to hydrolysis of VLDL's triglyceride (TG) cargo by LPL, release of free fatty acid (FFA), and conversion of VLDL to intermediate-density lipoprotein (IDL). In addition to the lipolytic pathway, a fraction of circulating VLDL is removed by a VLDL receptor (VLDLr)-mediated pathway. Nascent chylomicrons (lower panel) are formed in enterocytes and released into the circulation, where they acquire Apo-E and Apo-C from HDL-2. This is followed by LPL-mediated hydrolysis of their triglyceride contents and their conversion to chylomicron remnants. Abbreviations: CETP, cholesterylester transfer protein; GPIHBP1, glycosylphosphatidylinositol-anchored binding protein 1; LDLr, low-density lipoprotein receptor.

anchor molecule for LPL.[25] Interestingly, animals with chronic kidney disease and proteinuria show marked GPIHBP1 deficiency,[7] which heightens the LPL-inhibitory effects of ANGPTL4.

In contrast to its inhibitory action on extracellular LPL-mediated clearance of circulating triglycerides, ANGPTL4 increases lipolysis of intracellular triglycerides in adipose tissues by increasing the expression of the intracellular hormone-sensitive lipase.[26] Thus, by simultaneously inhibiting LPL-mediated uptake of fatty acids and enhancing lipolysis of intracellular triglycerides, elevation of circulating ANGPTL4 potentially can contribute to cachexia and reduced body mass in nephrotic patients (Fig 2).

WHAT DOES THIS IMPORTANT STUDY SHOW?

In their elegant and comprehensive study, Clement et al[4] found marked elevation of serum ANGPTL4/Angptl4 in nephrotic syndrome and demonstrated that its level is correlated directly with serum triglyceride concentration in humans and animals with nephrotic syndrome of diverse causes. They further showed that the increase in production and release of ANGPTL4/Angptl4 by extrarenal organs in nephrotic syndrome is driven by an elevated plasma FFA to albumin ratio. By inhibiting LPL, elevation of the circulating Angptl4 in turn limited clearance of plasma triglycerides and uptake of fatty acids in skeletal muscle, myocardium, and adipose tissue, thereby causing hypertriglyceridemia.

To verify the role of the FFA to albumin ratio as the principal mediator of increased production of Angptl4 in nephrotic syndrome, the authors induced nephrotic syndrome in Nagase rats, which are genetically analbuminemic. Serum triglyceride and Angptl4 levels, both of which are normally elevated in Nagase rats, did not increase further despite the development of heavy proteinuria (without albuminuria); this contrasted with the effects seen in the wild-type rats. The role of the FFA to albumin ratio was supported further by a series of experiments in which Clement et al[4] increased the ratio by intravenous administration of oleic acid (Intralipid; Sigma Chemical Co) in Buffalo Mna rats. This led to a significant increase in plasma FFA to albumin ratio and a significant increase in both Angptl4 messenger RNA expression and circulating Angptl4 levels.

In a series of additional in vivo and in vitro experiments, the authors demonstrated that by interacting with glomerular endothelial $\alpha_v\beta_5$ integrin, circulating Angptl4 can reduce proteinuria. This conclusion was based on the observation that both blockade of the Angptl4–$\alpha_v\beta_5$ integrin interaction and global knockout of either Angptl4 or $\alpha_v\beta_5$ integrin prolonged and intensified proteinuria in an animal model of minimal change disease. The antiproteinuric action of Angptl4 was confirmed further by experiments that showed that administration of a recombinant human ANGPTL4 (modified at a key LPL-interacting site designed to obviate its LPL-inhibitory property) can reduce the severity and duration of proteinuria without increasing triglyceride levels in nephrotic animals. These observations suggest that the increase in circulating ANGPTL4 in nephrotic syndrome may be a biological response designed to attenuate proteinuria.

Because Angptl4 expression is regulated by PPARs and upregulation of Angptl4 by FFAs is mediated by members of the PPAR family,[27] Clement et al[4] determined PPAR expression in the target tissues of the study animals. They found that upregulation of Angptl4 expression was accompanied by a significant increase in PPAR-δ, PPAR-γ, and PPAR-α in peripheral tissues, especially in skeletal muscle and adipose tissue of nephrotic rats. This observation may account for the reported amelioration of proteinuria in

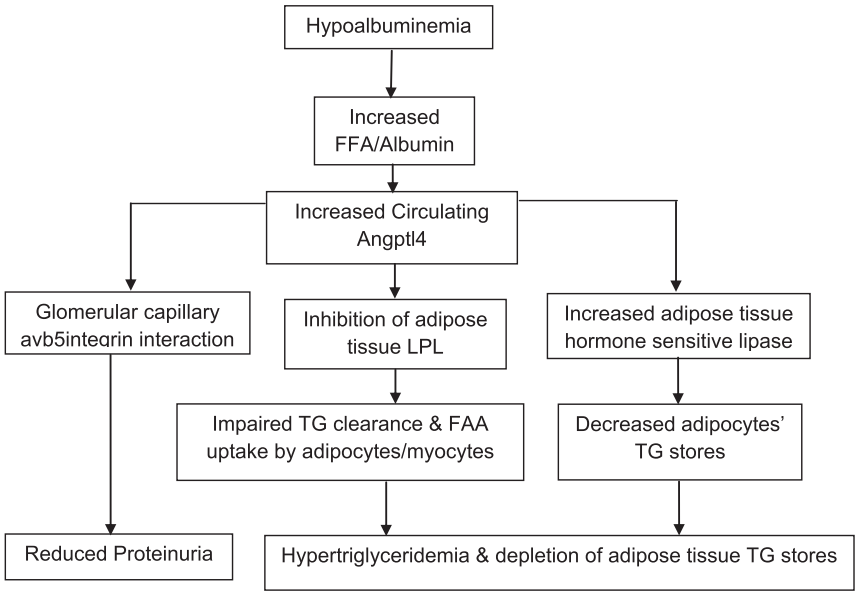

Figure 2. Nephrotic proteinuria results in elevation of plasma free fatty acid (FFA) to albumin ratio, which increases circulating angiopoietin-like 4 (Angptl4) level. By inhibition of lipoprotein lipase (LPL), upregulation of hormone-sensitive lipase, and interaction with glomerular capillary $\alpha_v\beta_5$ integrin, elevation of Angptl4 simultaneously promotes hypertriglyceridemia, depletion of adipose tissue triglyceride (TG) content, and attenuation of proteinuria.

patients and animals treated with PPAR-γ agonists.[28,29] Treatment with PPAR-γ agonists (thiazolidinediones) has been shown to reduce proteinuria, restore synaptopodin, and improve foot-process effacement in rats with puromycin aminonucleoside nephrosis (PAN)-induced nephrotic syndrome and also has been found to reduce apoptosis and restore expression of PPAR and nephrin in PAN-injured cultured podocytes.[28]

HOW DOES THIS STUDY COMPARE WITH PRIOR STUDIES?

The study by Clement et al[4] confirmed and expanded results of earlier studies that had documented the increase in circulating Angptl4 and its causal role in the pathogenesis of hypertriglyceridemia by inhibition of LPL in nephrotic syndrome.[30,31] In addition, they demonstrated that the increase in circulating Angptl4 helps lower proteinuria in nephrotic animals. However, in their earlier studies,[30] they had shown that localized secretion of Angptl4 by podocytes increased proteinuria in the rat model of minimal change disease. Likewise, Villa et al[32] showed the association of podocyte Angptl4 with proteinuria and its decline with attenuation of proteinuria upon administration of an angiotensin I receptor blocker in rats with glomerulonephritis induced by injection of monoclonal antibody targeting the Thy1.1 allele of CD80. On the surface, these observations may contradict the antiproteinuric action of circulating Angptl4 shown in their present study. However, the authors resolved this apparent contradiction by differentiating the effect of the hyposialylated isoform of podocyte Angptl4, which is confined to glomeruli and promotes proteinuria, from circulating Angptl4, which is normosialylated and has antiproteinuric properties.

It should be noted that patients and animals with advanced chronic kidney disease without nephrotic-range proteinuria commonly exhibit hypertriglyceridemia and elevated plasma and tissue FFA levels, which are associated with LPL, hepatic lipase, and GPIHBP1 deficiencies.[6,7,33,34] These abnormalities resemble those observed in nephrotic humans and animals and as such, could be linked to high levels of circulating Angptl4. Baranowski et al[35] demonstrated that plasma ANGPTL4 concentration in maintenance dialysis patients is more than 5-fold higher than in healthy controls and correlates positively with FFA level. Therefore, ANGPTL4 seems to be involved in the pathogenesis of hypertriglyceridemia in both nephrotic syndrome and advanced CKD.

WHAT SHOULD CLINICIANS AND RESEARCHERS DO?

The findings of this important study have revealed the link among plasma FFA to albumin ratio, circulating ANGPTL4, proteinuria, the PPAR system, and hypertriglyceridemia in nephrotic syndrome. The study also demonstrated that a mutant recombinant human ANGPTL4 preparation lacking LPL-inhibitory properties potentially is a novel therapeutic agent for the treatment of refractory nephrotic syndrome. Clinical trials are needed to explore the safety and efficacy of such products in patients with nephrotic syndrome. In addition, the data presented in this study have revealed a novel mechanism of the salutary effects of PPAR agonist in nephrotic patients. Further studies are required to examine the impact of PPAR agonists such as thiazolidinedione on plasma ANGPTL4 and triglyceride levels and urinary protein excretion in patients with nephrotic syndrome.

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