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PATHOPHYSIOLOGY OF HYPERTRIGLYCERIDEMIA

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CHAPTER 2

ABSTRACT

The importance of triglycerides as risk factor for CVD is currently under debate. The international guidelines do not include TG into their risk calculator despite the recent observations that plasma TG is an independent risk factor for CVD. The understanding of the pathophysiology of triglycerides opens up avenues for development of new drug targets. Hypertriglyceridemia occurs through 1. Abnormalities in hepatic VLDL production, and intestinal chylomicron synthesis 2. Dysfunctional LPL-mediated lipolysis or 3. Impaired remnant clearance. The current review will discuss new aspects in lipolysis by discussing the role of GPIHBP1 and the involvement of apolipoproteins and in the process of hepatic remnant clearance with a focus upon the role of heparin sulfate proteoglycans. Finally we will shortly discuss future perspectives for novel therapies aiming at improving triglyceride homeostasis. This article is part of a Special Issue entitled Triglyceride Metabolism and Disease
INTRODUCTION

Lipoproteins are large macromolecular complexes of hydrophobic lipids and proteins designed to transport water insoluble lipids such as triglycerides and cholesteryl esters in body fluids. Lipoproteins contain a hydrophobic core of triglycerides and cholesterol ester enveloped by a monolayer of phospholipids, unesterified cholesterol and apolipoproteins. Triglyceride-rich lipoproteins (TRLs) originate from the intestine (chylomicrons) or from the liver (very-low-density lipoproteins, VLDL). After ingestion of a meal triglycerides are taken up in the enterocytes and packaged into large particles containing apoB48 as core protein. Upon secretion into the lymphatic system remodelling occurs before chylomicron remnants enter the systemic circulation. Endogenous synthesized TG in the liver is packaged into very low density lipoproteins (VLDL) containing apoB100 as core protein. TRLs play an essential role in delivering fatty acids (FFA) to tissues as source of energy (heart and skeletal muscle) or for storage (adipose tissue). Plasma TG levels are determined by several key metabolic pathways: Intestinal uptake from dietary fat, hepatic production, peripheral lipolysis induced TRL remodelling and hepatic removal of VLDL and chylomicron remnants will be discussed. Abnormalities in TG metabolism are a hallmark of a number of clinical disturbances including type 2 diabetes, familial combined hyperlipidemia, dysbetalipoproteinemia and severe hypertriglyceridemia and are conferred to increased risk for CVD.

Recently, a scientific statement from the American Heart Association was issued to highlight the notion that plasma TG levels display a steady increase that contributes in a large extent to the continuously increasing cardiometabolic risk particularly.

In the present review, recent insight into pathophysiology of hypertriglyceridemia and future developments in triglyceride-lowering therapies are discussed.

TG METABOLISM (FIGURE 1-4)

Dietary fat absorption and formation of chylomicrons in the intestine

Triglycerides derived from dietary sources are hydrolysed in the intestine by pancreatic lipase in 2-monoacylglycerol (2-MG) and fatty acid (FA), which can be absorbed by the enterocytes by diffusion or specific transporters such as FAT/CD36. Within the enterocyte, 2-MG and FA are resynthesized into TGs by the enzyme acyl-CoA:diacylglycerol acyltransferase (DGAT). Subsequently, microsomal triglyceride transfer protein (MTP) in complex with protein disulphite isomerase (PDI) facilitates the lipidation of apolipoprotein B48 (apoB48), as a first step towards chylomicron formation. Epithelial COPII (Coatomere Protein II) transport carriers like SAR1a and SAR1b are essential for the transport of chylomicrons to the Golgi apparatus. Human relevance is underscored by the observation that chylomicron retention disease and Anderson disease are autosomal recessive disorders of severe fat malabsorption with a complete absence of circulating apoB48 particles due to a genetic defect in the COPII machinery. Nascent chylomicron particles are exocytosed from the basolateral membrane...
and enter the lymphatic compartment and eventually the systemic circulation. The intestine harbors the option to synthesize apoC-III and possibly apoA-V. Whether these apolipoproteins are secreted associated with chylomicron particles is still unknown. Upon entering the circulation direct apolipoprotein exchange occurs with HDL particles enriching the chylomicron particles with apoE and apoC-III. In the fasting state chylomicrons are small, whereas ingestion of a meal leads to an increase of chylomicron size rather than chylomicron particle number.

**Figure 1 - Intestinal chylomicron synthesis.** Dietary lipids are taken up in the enterocytes, incorporated into chylomicron particles that are secreted into the lymphatic system. DGAT, the COPII machinery system and MTP are required for the intracellular processing of chylomicrons.

**Hepatic VLDL production**

TG is synthesized in the liver and packaged into VLDL particles, with apoB100 as the main protein. The required fatty acids are derived from de novo synthesis using glucose as substrate (DNL) or from lipolysis in adipose tissue by the action of hormone sensitive lipase and adipose tissue TG lipase. The two DGAT enzymes are responsible for the generation of TG stored in lipid droplets. MTP is essential for initial lipidation of apoB100, whereas the COPII machinery is responsible for the early translocation of small VLDL particles from the ER to Golgi apparatus where further lipidation occurs. Mature VLDL particles are then excreted by the hepatocytes.
The liver is the major organ for production of apoA-V and apoC-III, two proteins involved in TG homeostasis. In plasma, apoC-III and apoA-V are constituents of apoB-containing lipoprotein particles and HDL depending upon the plasma TG levels and are strongly associated with plasma TG levels. ApoC-III is a very abundant plasma apolipoprotein (4-10 mg/dl), whereas apoA-V levels are extremely low 50 – 400 μg/l. Interestingly, it has been postulated that apoC-III may contribute to VLDL production, at least in mouse models. ApoC-III overexpression coincides with increased VLDL secretion whereas apoC-III deficiency results in the opposite phenotype. ApoC-III has multiple isoforms due to the presence of 0, 1 or 2 sialic acid residues as a result of O-linked glycosylation. In a recent genome-wide association study, polymorphisms in GalNac-T2 transferase (GALNT2) were associated with increased plasma TG levels and decreased HDL cholesterol levels. Liver-specific Galnt2 results in a decrease of HDLc, whereas the knockdown of mouse liver galnt2 results in the opposite phenotype. It is tempting to see whether mutations in GALNT2 will have an impact on TG metabolism through modulation of apoC-III glycosylation.

The role of apoA-V in facilitating VLDL production remains unclear, since in vitro experiments could not establish any involvement of apoA-V. In line, data from studies in different mouse models do not support a direct role of apoA-V in VLDL production. Interestingly, it has recently been shown that apoA-V in the liver is co-localized with lipid droplets and that increased levels of hepatic apoA-V coincides with increased liver TG storage in mice, suggesting a role for apoA-V in mobilization of TG for VLDL production. It remains, however, to be determined how apoA-V finds its way into the plasma compartment.

The availability of TG partly determines the fate of apoB and consequently the secretion rate of VLDL particles. Insulin plays an essential role in this process. On the one hand insulin resistance increases TG lipolysis in adipose tissue, leading to an increased flux of FFA to the liver and thus an increased availability of TG cargo. On the other hand, insulin activates the regulatory machinery required for apoB synthesis. In an insulin resistant state this regulation is lost. As a consequence, VLDL production will increase leading to the generation of TG-rich atherogenic remnant particles, small dense LDL particles and TG-enriched HDL.
Lipoprotein lipase (LPL)-mediated peripheral lipolysis

VLDL and chylomicron particles provide fatty acids to tissues for energy as well as for storage. Lipolysis of TG by lipoprotein lipase (LPL) occurs in small capillaries in tissues that require fatty acids for storage (adipose tissue) or energy (heart and skeletal muscle). Fatty acids are directly taken up by CD36, whereas the liver will eventually clear the remnant particles. LPL is synthesized in parenchymal cells in these tissues and lipase maturation factor 1 (LMF1) is essential for proper folding and assembly of LPL. Subsequently, LPL is transported to the endothelial cell surface where it binds to glycosyl-phosphatidyl-inositol anchored high-density lipoprotein binding protein 1 (GPIHBP1). GPIHBP1 belongs to the family of lymphocyte-6 (Ly6) domain proteins. Human GPIHBP1 contains a heavily negative charged N-terminal domain, the Ly-6 domain consisting of 10
cysteine residues that form 5 double bonds and the gpi-anchor at the C-terminal end of the protein that serves as the attachment site to the extracellular leaflet of the cell membrane. The carboxyl-terminal sequence is removed in the endoplasmic reticulum, where cleavage occurs at one of the predicted sites for attachment of the gpi anchor in human GPIHBP1: residues 159, 153 and 154. The exact cellular localization of GPIHBP1 is still unknown. Based on data of other gpi-anchor proteins it is predicted that GPIHBP1 is localized in the lipid raft domains. GPIHBP1 provides the platform for LPL and TG-rich lipoproteins to come into close proximity, resulting into the hydrolysis of core TG. It remains an intriguing question why GPIHBP1 has so much more potency to bind LPL than the heparin sulfate proteoglycans (HSPGs), a core protein with negatively charged polysaccharide chains, that are abundantly expressed on the cell surface of capillaries. In line, mice with a deficiency in sulfation of HSPGs in endothelial cells, due to a deficiency of GlcNAc N-deacetylase/N-sulfotransferase (Ndst1), exhibit normal plasma TG levels. More importantly, loss of function mutations in GPIHBP1 (so far 8 different mutations have been published) results in a severe hypertriglyceridemic phenotype providing a proof of concept that GPIHBP1 is essential for lipolysis to take place.

Interestingly, GPIHBP1 may have an essential function in the transport of LPL through the endothelial cell layer towards the cell surface of the capillaries. Originally it was suggested that HSPGs were involved in the immobilization of LPL, however recent studies were not able to confirm this. Interestingly, a basement membrane proteoglycan, collagen XVIII, however, may also be involved in LPL translocation as recently shown in col18−/− mice and humans with a mutation in COL18 (Knobloch Syndrome) who develop a mild hypertriglyceridemic phenotype with reduced LPL activity and mass. Although Gpihbp1−/− mice display severe chylomicronemia, the Gpihbp1−/−/Angptl4−/− mice has normal plasma TG levels, illustrating that Angptl4 destabilizes LPL which in the absence of functional GPIHBP1 become an unstable dysfunctional protein. The interesting finding of this model is that the lack of Gpihbp1 can be rescued on the premise that LPL remains in a stable form. It also illustrates that stabilized mouse LPL can perform its action without the presence of functional GPIHBP1.

LPL action is dependent upon various co-factors. ApoC-II is an essential co-factor for LPL activation, whereas apoC-III may inhibits lipolysis. Indeed, low levels of apoC-III, due to a loss of function mutation, results in rapid postprandial clearance due to efficient LPL-mediated hydrolysis. The role of apoAV remains to be elucidated. Based on numerous in vitro experiments and the use of different genetically engineered mouse models, including apoa5 over-expression or deficiency, it has been suggested that apoA-V is required for efficient LPL action. Interestingly, in cell-based assays, apoAV-phospholipids complexes, but not apoE or apoC-III phospholipid particles, avidly bind to the acidic domain of GPIHBP1, although apoA-V could not compete with LPL binding, which additionally requires the Ly-6 domain of GPIHBP1 for binding to take place. Moreover, chylomicron binding requires both the Ly-6 as well as the acidic domain. Thus, chylomicron binding to GPIHBP1 only occurs in the presence of LPL and apo A-V does not seem to play an essential role in this process.
If apoA-V is not directly involved in GPIHBP1-LPL mediated lipolysis, why does one then consistently observe a strong positive correlation with plasma TG levels and why are genetic variations in APOAV consistently a strong marker for plasma TG? These are intriguing questions that will hopefully be answered in the near future and will provide us with more insight into the function of apoA-V.

**Figure 3 - Peripheral Lipolysis.** GPIHBP1 is involved in the transport of LPL through the endothelial cell layer to the cell surface and is required for stabilisation of LPL. At the endothelial cell surface GPIHBP1 forms the platform to allow TG hydrolysis.

**Hepatic remnant clearance**

The process of hepatic remnant clearance is complex. Although the detailed mechanism of the receptor mediated remnant uptake by the liver is still unclear, several endocytic hepatic receptors, present at hepatocyte microvilli, have been studied during the past few decades. It involves interactions between proteins located on the surface of the remnant particle that serve as ligands for hepatic receptors i.e. low density lipoprotein receptor (LDLr), LDL receptor related protein 1 (LRP1) and HSPG. ApoE is essential for hepatic remnant clearance that is illustrated by the observation that apoe<sup>−/−</sup> mice have massive accumulation of remnant particles. ApoE contains positively...
charged residues, which favorably binds to negative charged domains on the hepatic receptors, whereas hepatic lipase (HL) and LPL mediates particle-receptor interaction with the LRP1 and HSPG receptors. 

The LDL receptor was originally reported in to contribute to TRL clearance by the liver. However, as patients with genetic alterations of the LDLR are not characterized by hypertriglyceridemia, the search for other receptors continued. A family member of the LDL receptor involved in remnant clearance is the LDL receptor related protein 1 (LRP1). Predominantly, the binding of apO2-enriched remnants to HSPGs in the space of Disse was shown to depend upon final internalization via LRP1. Since combined LDLr and LRP1 deficiency did not result in hypertriglyceridemia, other pathways had to be present. Evidence from the early nineties already indicated the potential involvement of HSPG in hepatic remnant clearance. In a pioneering study hepatic remnant uptake was enhanced by heparin, a highly sulphated glycosaminoglycan, and inhibited by administration of heparanase, an enzyme that cleaves heparan-sulfate polysaccharides. In line, remnant uptake was abolished in HSPG deficient cells. Recent studies using syndecan-1 null (Sdc1−/−) mice revealed that syndecan-1 is an important HSPG core protein in the liver. Sdc1−/− mice display elevated plasma TG levels and have impaired postprandial remnant clearance which is in agreement with earlier data in syndecan-1 overexpressing cells, indicating that syndecan-1 mediates TRL internalization. Syndecan-1 is synthesized in hepatocytes and undergoes posttranslational modifications that results in specific GAG chain length and sulfation patterns. Liver HSPGs contain an extreme large proportion of highly sulfated heparin-like structures located in the distal part of the GAG chain that allows lipoprotein binding. More than 50 enzymes are involved in the processing and degradation of HSPGs. Thus, chain length and sulfation pattern determine the biological activity of HSPG as was shown in different genetically modified animal models. Addition of sulfate groups by N-deacetylase/N-sulfotransferase 1 (Ndst1) and different O-sulfotransferases (HS2ST1, HS3ST1 and HS6ST1) provide a specific functional signature to HSPG. Mice lacking Ndst1 have a 50% reduction of hepatic HSPG sulfation. Consequently, plasma triglycerides were two-fold increased due to a reduced hepatic clearance of TRL. Mutant mice with defects in hepatic Hs2st1 and Hs6st1 expression also have increased plasma TG levels. Interestingly, in a streptozotocin-induced diabetic mouse model, a reduced hepatic Ndst1 mRNA expression was associated with impaired hepatic remnant clearance thus underscoring the effect of hyperglycemia on HSPG remodelling. Two proteins, heparanase (HSPE) and sulfotransferase 2 (SULF2) are involved in the extracellular remodeling of HSPG. Differences in expression of these proteins may lead to impaired hepatic HSPG-mediated TRL clearance. SULF2 is secreted into the extracellular space where it selectively modifies the 6-O-sulfate esters of HSPG. Interestingly, hepatic Sulf2 expression is highly increased in db/db mice and correlated with elevated plasma TG levels and impaired hepatic TRL clearance. It will be of interest to investigate whether inhibition of hepatic Sulf2 expression leads to an improvement of the dyslipidemic phenotype. Interestingly, transgenic mice overexpressing human heparanase also have increased plasma TG levels, a reduced hepatic TRL clearance and increased formation of fatty
streaks, whereas no difference was found in postheparin LPL activity indicating normal peripheral LPL-mediated lipolysis.\textsuperscript{12}

Collectively, these studies indicate that hepatic remnant clearance is a complicated process involving 3 different receptor-mediated pathways. Studies in genetically engineered mouse models revealed that deficiency of apoE results in the most severe remnant phenotype since all remnant uptake pathways will be partially eliminated. Abnormalities in HSPG result in a mild hypertriglyceridemic phenotype due to delayed clearance of remnant particles.

In conclusion, the importance of HSPG for optimal hepatic clearance of TRL is obvious, but in contrast with the LDLr, the relevance of HSPG in human lipid metabolism remains to be established.

\textbf{Figure 4 - Hepatic TRL clearance.} TRL clearance involves 3 hepatic receptors: LDLr, LRP1 and HSPG. Sulf2 is an extracellular protein that modulates HSPG thereby influencing hepatic TRL clearance.
PATHOPHYSIOLOGY OF HUMAN HYPERTRIGLYCERIDEMIA (FIGURE 5)

A mild to severe elevation of plasma TG (2-5 mmol/l) is a common feature in subjects with obesity, the metabolic syndrome or type 2 diabetes mellitus. Lipid abnormalities are a consequence of metabolic dysregulation resulting in mild to severe hypertriglyceridemia due to enhanced VLDL production, a delayed hepatic remnant clearance and mild disturbances in peripheral lipolysis.

More severe elevations of TG (between > 5–10 mmol/l) are observed in individuals diagnosed with familial combined hyperlipidemia (FCH), and familial hypertriglyceridemia (FHTG). FCH is present in 1:300 subjects, which makes FCH the most common genetic lipid disorder associated with increased risk for CVD. The hyperlipidemic phenotype can, however, be mixed including the presence of hypercholesterolemia. Pathophysiological studies have revealed abnormalities in VLDL production as well as hepatic TRL clearance.\textsuperscript{53,54} However, the heterogeneity of the lipid profile makes it difficult to reliably identify true cases, which has severely hampered the elucidation of the underlying metabolic abnormalities.\textsuperscript{54,55}

Figure 5 - A schematic view of the occurrence of elevated plasma TG levels in the population. The higher the plasma TG levels the more impact of the genetic background will be observed.
Dysbetalipoproteinemia or remnant removal disease is characterized by increased levels of remnant particles due to impaired hepatic clearance. Patients are carriers of dysfunctional apoE2. However, the presence of homozygosity for APOE2 alone is not sufficient to explain the pathophysiology of dysbetalipoproteinemia, since only 4% of all homozygous APOE2 carriers will develop severe remnant accumulation.

Severe hyperTG (TG > 10 mmol/l) is a hallmark of rare genetic disorder caused by loss of function of LPL due to mutations in LPL, APOC2, APOA5, GPIHBP1 or LMF1 as has been extensively described.\textsuperscript{14}

TRIGLYCERIDES AND CARDIOVASCULAR DISEASES

Elevated triglyceride levels have been associated with cardiovascular risk in general population as well as in subjects with type 2 diabetes mellitus.\textsuperscript{37} Conversely, hypotriglyceridemia, due to a null mutation in APOC3, has been associated with longevity.\textsuperscript{36} It is difficult, however, to show that TG is a truly independent risk factor since hypertriglyceridemia is often part of the ‘metabolic’ dyslipidemic profile comprising low HDL levels, elevated levels of small dense LDL particles, as well as obesity and insulin resistance. Adjustment for these factors often led to very small effect sizes in association studies with positive hazard ratios. Another complicating factor relates to fasting versus postprandial TGs. Recent studies have suggested that nonfasting plasma TGs may be a superior risk marker for CV-risk.\textsuperscript{38} Indeed, chylomicron and VLDL remnant particles are able to penetrate the vessel wall.\textsuperscript{39}

At the other site of the spectrum, the very large TRL particles as observed in LPL deficiency, do not associate with atherogenesis, but do not lead to an increased risk of pancreatitis.

FUTURE PERSPECTIVES

Elevated levels of triglycerides and TRL remnants are independent risk factors for the development of CVD, which bears direct relevance in view of the pandemic of obesity and type 2 DM. The attention for hyperTG has been limited in view of the absence of effective compounds selectively lowering fasting and postprandial TGs. Novel insight into the TG metabolism has led to several therapeutics being developed which selectively target proteins involved in LPL-mediated lipolysis (ANGPTL4 and apoCIII), interfere with hepatic production (MTP and DGAT1) and/or that increase hepatic TRL clearance (apoCII and SULF2). It will be a challenge to evaluate what the impact of these selective TG lowering strategies is on cardiometabolic risk.
REFERENCE LIST


