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Biosynthesis and Metabolism of ApoB-Containing Lipoproteins

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Abstract

Recent advances in human genetics, together with a substantial body of epidemiological, preclinical and clinical trial evidence, strongly support a causal relationship between triglyceride-rich lipoproteins (TRLs) and atherosclerotic cardiovascular disease. Consequently, the secretion and metabolism of TRLs have a significant impact on cardiovascular health. This knowledge underscores the importance of understanding the molecular mechanisms and regulation of very-low-density lipoprotein (VLDL) and chylomicron biogenesis. Fortunately, there has been a resurgence of interest in the intracellular assembly, trafficking, degradation, and secretion of VLDL, leading to many ground-breaking molecular insights. Furthermore, the identification of molecular control mechanisms related to triglyceride metabolism has greatly advanced our understanding of the complex metabolism of TRLs. In this review, we explore recent advances in the assembly, secretion, and metabolism of TRLs. We also discuss available treatment strategies for hypertriglyceridemia.

Contents

APOLIPOPROTEIN B AND TRIGLYCERIDE-RICH LIPOPROTEINS

Lipids, which are not soluble in water, are carried in the circulatory system in lipoprotein particles containing a core of nonpolar lipids such as triacylglycerol and cholesteryl esters, surrounded by an amphipathic monolayer of phospholipids and cholesterol (20). Chylomicrons and very-low-density lipoprotein (VLDL) carry most triglycerides in the circulation and are referred to as triglyceride-rich lipoproteins (TRLs) (20). Chylomicrons are secreted by the intestine and transport dietary fat, whereas VLDL transports triglycerides synthesized in the liver (**Figure 1**). TRLs carry various proteins, notably apolipoprotein B (apoB), which is essential for the formation of TRLs and triglyceride secretion (20). ApoB is a large amphipathic protein (i.e., it contains both hydrophilic and hydrophobic sequences), which exists in two forms: apoB100 and apoB48 (20, 46). In humans, apoB100 is expressed in the liver and secreted on VLDL (apoB100-VLDL), whereas apoB48 is expressed in the intestine and present on chylomicrons and VLDL-sized

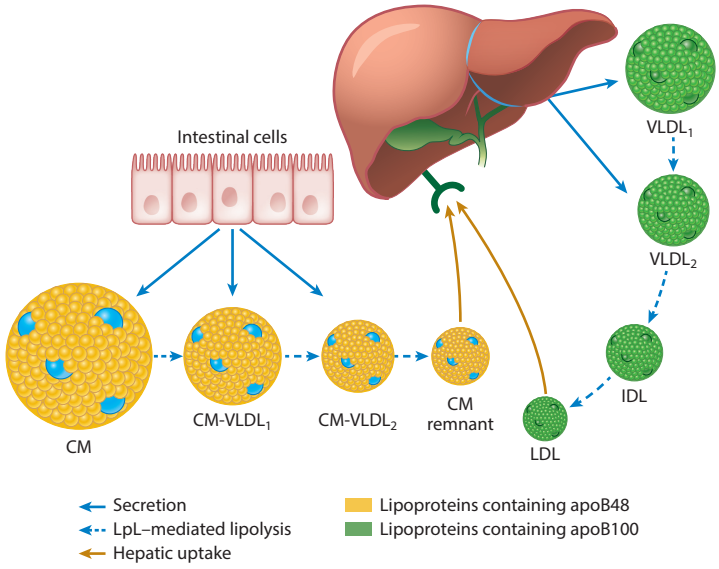

Figure 1

Dietary lipids are packed into apolipoprotein B48 (apoB48)-containing chylomicrons (CMs). The size of these differs; after a meal, the intestine secretes large triglyceride-rich CMs. In the fasting state, it mainly secretes smaller CMs with densities of very-low-density lipoprotein 1 ($VLDL_1$) and $VLDL_2$ particles. Triglycerides in CMs are lipolyzed by lipoprotein lipase (LpL), resulting in smaller CM remnants. The liver secretes apoB100-containing large $VLDL_1$ and smaller $VLDL_2$. LpL converts VLDL to smaller intermediate-density lipoprotein (IDL) and low-density lipoprotein (LDL). The CM remnants and the LDL are cleared from the circulation by the LDL receptor on the liver. Triglyceride-rich lipoprotein remnants are defined as intestinal-derived CM-$VLDL_1$, CM-$VLDL_2$, and CM remnants, as well as liver-derived $VLDL_2$ and IDL.

particles (apoB48-VLDL). Both proteins are coded by the same *APOB* gene and by a single mRNA transcript, approximately 16 kb. ApoB48 is generated when a stop codon (UAA) at residue 2153 is created by RNA editing by the enzyme apobec-1 (99). ApoB differs from other apolipoproteins in that it is nonexchangeable; that is, it cannot equilibrate between different lipoproteins but remains bound to the particle on which it was secreted into plasma. This phenomenon is typically attributed to the existence of antiparallel β sheets that are roughly 30 Å wide. These β sheets create highly robust lipid-binding structures, which have been posited to account for apoB's unique behavior (109). For many years, understanding of the biosynthesis of TRLs has been limited and mainly based on descriptive immunoelectron microscopy studies from the mid-1970s (7). However, we have gained a much better understanding of these processes in recent years, even if much is still unclear.

THE FIRST STEP IN VLDL BIOGENESIS: COTRANSLATIONAL LIPIDATION OR DEGRADATION OF ApoB IN THE ENDOPLASMIC RETICULUM

The nascent apoB polypeptide is synthesized on ribosomes attached to the surface of the endoplasmic reticulum (ER) and translocated through the translocon (i.e., a complex of proteins that transports polypeptides across the ER membranes) to the lumen of the ER. During this process, the nascent apoB polypeptide is cotranslationally lipidated to form a partially lipidated primordial pre-VLDL particle (**Figure 2**). Several chaperone proteins have been shown to facilitate proper

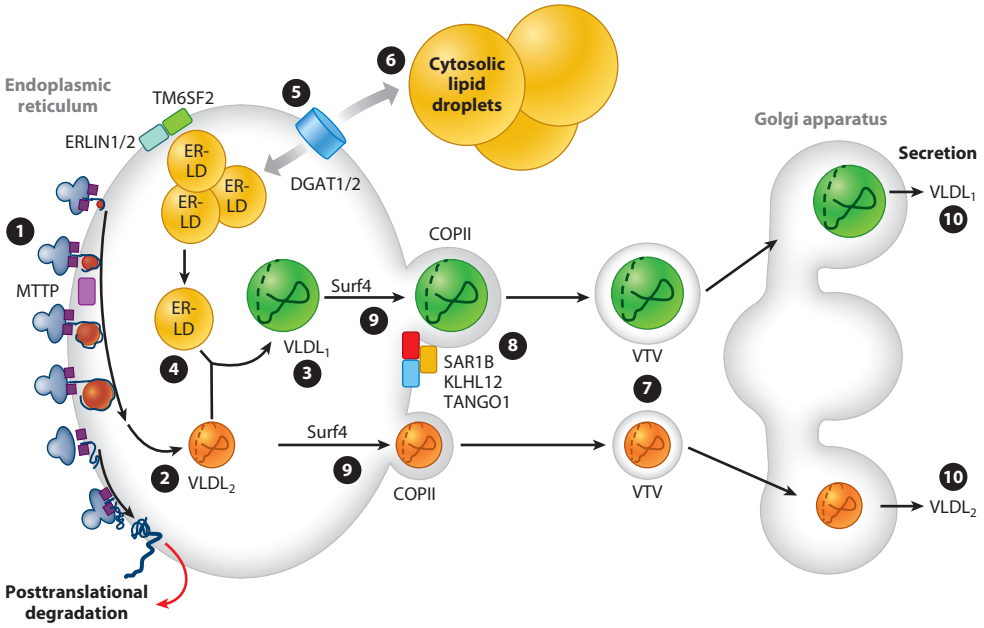

Figure 2

Biosynthesis and secretion of very-low-density lipoprotein (VLDL). Nascent apolipoprotein B100 (apoB100) is cotranslationally lipidated by the action of microsomal triglyceride transfer protein (MTTP) (①) to form a lipid-poor $VLDL_2$ particle (②). ApoB that fails to become lipidated is sorted to posttranslational degradation. The $VLDL_2$ accumulates additional lipids to form a large triglyceride-rich $VLDL_1$ particle (③). How this occurs remains unclear, but it has been proposed to involve fusions with lipid droplets within the endoplasmic reticulum lumen (ER-LD). Transmembrane 6 superfamily member 2 (TM6SF2) seems to facilitate conversion of $VLDL_2$ to $VLDL_1$, but the underlying mechanisms remain to be elucidated. ER lipid raft protein (ERLIN)1/2 have been proposed to stabilize TM6SF2 (④). Diacylglycerol O-acyltransferase (DGAT), localized in the ER membrane, synthesizes triglycerides (⑤), which are stored in cytosolic lipid droplets (⑥). It has been hypothesized that DGAT also catalyzes the formation of the ER-LD (④). VLDL is transported from the ER to the Golgi apparatus in specialized coat complex II (COPII)-coated VLDL transport vesicles (VTVs) formed in the ER exit sites (⑦). The formation of VTVs requires SAR1B (secretion-associated Ras-related GTPase 1B), KLHL12 (Kelch-like protein 12), and TANGO1 (transport and Golgi organization 1) (⑧). Surfeit 4 (Surf4) plays a crucial role in this process by interacting with apoB100 on VLDL and facilitating the incorporation of VLDL into VTVs (⑨). The VLDL is further modified in the Golgi apparatus and transported to the cell membrane, where VLDL is secreted into the circulation (⑩).

folding and lipidation of the nascent apoB polypeptide during this process, including the microsomal triglyceride transfer protein (MTTP) (35). The N-terminal domain of apoB interacts with MTTP, which transfers lipids from the ER bilayer to the nascent apoB polypeptide (48, 49). Loss-of-function (LOF) mutations in the *MTTP* gene cause loss of lipid transfer activity and result in the rare disorder abetalipoproteinemia, characterized by the inability to form apoB-containing lipoproteins and impaired absorption of dietary fat and vitamins (49). Likewise, drugs that inhibit MTTP activity—used for adjunctive treatment of homozygous familial hypercholesterolemia—block the hepatic secretion of VLDL and thus lower low-density lipoprotein (LDL) levels (50).

Even though apoB lacks a typical transmembrane domain, studies have revealed its bitopic orientation, where apoB domains are simultaneously exposed to the ER lumen and the cytosol (39, 43, 135). It has been postulated that this unique topology is caused by pause–transfer sequences in apoB that interrupt its translocation without affecting its translation (31).

In humans, there is a negative association between plasma tissue plasminogen activator (tPA) activity and LDL cholesterol levels, but the specific mechanisms behind this relationship have been unclear. Very recently, Dai et al. (33) demonstrated that tPA binds to the N-terminus of apoB, in part through the lysine-binding site of its Kringle 2 domain. This binding effectively blocks the interaction between apoB and MTTP within hepatocytes, reducing the assembly of VLDL and subsequently lowering LDL cholesterol. Additionally, plasminogen activator inhibitor 1 (PAI-1) sequesters tPA away from apoB, which, in turn, increases VLDL assembly. Interestingly, individuals with PAI-1 deficiency exhibit smaller VLDL particles and reduced plasma levels of LDL cholesterol (33). These findings point to a mechanism that fine-tunes the process of VLDL assembly within hepatocytes through intracellular interactions involving tPA, PAI-1, and apoB.

The lipid-binding proline-rich acidic protein 1 (PRAP1) has also been shown to regulate MTTP-mediated lipid transfer (95). PRAP1 has been observed bound to TRLs in the circulation, and it has been proposed that MTTP recognizes PRAP1-bound triglycerides as cargo and promotes their transfer into nascent VLDL and chylomicrons. PRAP1-deficient mice fed a chow diet manifested an increase in the length of their small intestines, likely to compensate for challenges in absorbing lipids (95).

Pulse–chase studies using radiolabeled amino acids have been used to investigate the assembly and secretion of VLDL in cultured hepatocytes and hepatoma cell lines. Findings from these studies showed that a significant portion of newly synthesized apoB100 failed to form primordial pre-VLDL particles; instead, it was degraded in the cell (23, 24). Elegant studies showed that misfolding or inadequate cotranslational lipidation of apoB results in cotranslational ubiquitination and proteasomal degradation, a type of ER-associated degradation (ERAD) (42). ERAD is highly dependent on lipid availability; thus, apoB100 appears to be continuously synthesized, and apoB100 that is not used for VLDL formation is sorted to posttranslational degradation (23, 90, 147). Fisher & Ginsberg (41) made important contributions to our understanding of this process by demonstrating that apoB100 loops out on the cytosolic side of the ER, interacting with heat shock protein 70 (HSP70) and proteasomes, while its N-terminal sequence remains connected to the ER membrane and translocon. This looping out and subsequent degradation could be overcome by increased triglyceride biosynthesis (41). The triglycerides are synthesized from diacylglycerides by diacylglycerol O-acyltransferase 1 (DGAT1) and DGAT2. DGAT1, predominantly found in the small intestine, uses exogenous fatty acids, while DGAT2 in the liver relies on de novo lipogenesis. It should be noted that the notion that apoB is expressed in excess, and that the surplus is degraded, is based on in vitro studies; in vivo studies—at least partly—contradict this concept, as overexpression of mouse apoB in transgenic mice on a chow diet resulted in increased VLDL secretion (83). However, human immunodeficiency virus protease inhibitors, known to induce hyperlipidemia (28), have been shown to inhibit the proteasomal degradation of apoB (77), indicating that proteasomal degradation is physiologically relevant in humans.

Novel molecular chaperones regulating ERAD of apoB continue to emerge (26), and recent studies demonstrated that hepatic E3 ubiquitin ligase murine double minute 2 (MDM2) targets apoB for proteasomal degradation through direct protein–protein interaction, which leads to reduced VLDL triglyceride secretion in hepatocytes and subsequent hepatic lipid accumulation (74). A marked upregulation of MDM2 is observed in the livers of human and mouse models with hepatic fat accumulation (74). Recent studies have also shown that liver-specific zona pellucida domain-containing protein (LZP) regulates hepatic VLDL synthesis by preventing ubiquitination and degradation of apoB via an E3 ubiquitin ligase specific for apoB (140).

Ginsberg & Fisher (44) demonstrated that posttranslational degradation of apoB100 also occurs in a subcellular compartment separate from the rough ER, referred to as post-ER presecretory proteolysis (PERPP). Through a series of elegant studies, they unveiled the intricate process of

PERPP, which is capable of degrading as much as 90% of newly synthesized apoB. Activation of PERPP prompts the aggregation of apoB-containing lipoproteins, which are subsequently directed toward degradation via the autophagy pathway (44). This phenomenon is stimulated under various common metabolic conditions and may explain why diets rich in polyunsaturated fatty acids lead to reduced plasma VLDL levels (44).

LATER STEPS IN VLDL BIOGENESIS: LIPIDATION OF PRE-VLDL TO FORM MATURE VLDL

The mechanisms for how pre-VLDL particles are converted to lipid-rich VLDL are still not fully elucidated. Initially, the assembly process was considered a two-step model (22), but it is now evident that it is a multistep process.

In the first step, primordial pre-VLDL particles are formed in the ER, as described above. These pre-VLDL particles are either retained and degraded in the cell or converted to smaller triglyceride-poor $VLDL_2$ particles by the addition of triglyceride in a linear lipidation process (116). The conversion of pre-VLDL to $VLDL_2$ likely involves MTTP, explaining why MTTP activity is also needed for the secretion of apoB100 after the translation of the protein is complete (104).

The $VLDL_2$ particles are either released from the cell or converted to large, triglyceride-rich $VLDL_1$, which is then secreted (116). The conversion of $VLDL_2$ to $VLDL_1$ requires ADP-ribosylation factor 1 (ARF-1), a GTPase protein essential for transport from the ER to the ER–Golgi intermediate compartment (ERGIC) (37). This indicates that the process of $VLDL_1$ formation includes the transfer of apoB100 from the ER to the ERGIC compartment, where the VLDL particle becomes fully lipidated (10). Consequently, one would anticipate a time span of approximately 15 min between the synthesis of apoB100 and the significant incorporation of lipids to create $VLDL_1$ particles. Indeed, human turnover studies have shown that VLDL triglycerides entered the circulation within 18 min after their synthesis, whereas VLDL apoB entered the circulation after 33 min (4).

This lipidation step differs from how nascent pre-VLDL and $VLDL_2$ particles are formed, and likely involves a bulk-load uptake of triglycerides (89). A $VLDL_1$ particle contains approximately 43,000 molecules of triglycerides compared with approximately 11,000 in $VLDL_2$. The mechanisms for the rapid uptake of such a load of triglyceride is still unclear but may involve transfer of lipids from a pool within the smooth ER membrane, perhaps via the formation of microsome-associated luminal lipid droplets in the secretory pathway that fuse with the $VLDL_2$ particle (91, 143).

This hypothesis potentially establishes a connection between the development of the VLDL core and the assembly process of a cytosolic lipid droplet. It may also explain why the secretion of $VLDL_1$ particles increases with increasing concentrations of hepatic lipids. Thus, nonalcoholic fatty liver disease (NAFLD) is linked to the overproduction of $VLDL_1$ (5). Likewise, genetic variants leading to impaired VLDL secretion lead to increased hepatic lipid accumulation (84).

While this lipid-transfer hypothesis has merit, there is no conclusive morphological proof supporting the presence of luminal lipid droplets functioning as VLDL precursors. A major reason for this is the difficulty of differentiating between VLDL particles and luminal lipid droplets in the electron microscopy studies. Nevertheless, biochemical studies indicate that lipids from both the cytosolic and the luminal leaflets of the ER membrane can be concentrated and packed into lipid droplets within the luminal compartment of the secretory pathway (143, 146).

Lehner and colleagues (131) isolated luminal lipid droplets from mouse livers and showed that they were relatively poor in triglyceride content compared with cytoplasmic lipid droplets.

Their proteome also differed; they lacked apoB and adipophilin (ADRP/ADPH/PLIN2) but were enriched with triglyceride hydrolase (TGH), carboxylesterase 1 (Ces1), MTTP, and apoE, indicating that TGH is important for mobilizing triglycerides from luminal lipid droplets to nascent VLDL particles (131). These results may explain earlier studies showing that inhibition of intracellular lipolysis markedly reduces VLDL secretion (134). In line with this, impaired intracellular lipolysis and mobilization of triglycerides from lipid droplets for $VLDL_1$ have been proposed to explain why carriers of the palatin-like phospholipase domain-containing protein 3 (PNPLA3) 148M LOF variant do not have increased $VLDL_1$ secretion despite increased liver fat (17).

Recent studies have identified phospholipase A2, group XIIB (*PLA2G12B*) as an important regulator of VLDL triglyceride secretion. The gene was identified when searching for genes that are coexpressed with *APOB* and *MTTP* in humans, and subsequent studies showed that *Pla2g12b*-deficient mice have markedly lower plasma levels of triglycerides (51). Phospholipase A2 (PLA2) hydrolyzes the fatty acid from the sn-2 position of membrane phospholipids, generating a free fatty acid and lysophospholipid, but PLA2G12B lacks this phospholipase activity (86). To elucidate the mechanism for how PLA2G12B affects VLDL plasma levels, Farber and colleagues (124) performed elegant studies in *Pla2g12b*$^{-/-}$ mice and zebrafish and demonstrated that *Pla2g12b*-deficient mice had reduced secretion of VLDL triglycerides and increased hepatic lipid content, indicating that PLA2G12B is important for the formation of triglyceride-rich VLDL. The authors further showed that loss of Pla2g12b in zebrafish larvae caused an accumulation of luminal lipid droplets and small lipoproteins, indicating that PLA2G12B regulated the transfer (or fusion) of triglycerides from luminal lipid droplets to lipid-poor VLDL (124).

Genetic research has played an instrumental role in the discovery of new biological pathways underpinning complex human physiology. Such studies have shown that the transmembrane 6 superfamily member 2 (*TM6SF2*) E167K genetic LOF variant is associated with NAFLD and with reduced plasma triglyceride levels in humans (38). A recent kinetic study employing a novel protocol for postprandial lipid metabolism revealed that the secretion of large triglyceride-rich $VLDL_1$ particles was markedly lower in homozygote *TM6SF2* E167K carriers compared with controls (122). In contrast, the secretion of the smaller, triglyceride-poor $VLDL_2$ was not different between carriers and noncarriers (122). Thus, the *TM6SF2* LOF mutation seems to specifically affect the conversion of $VLDL_2$ to $VLDL_1$. It is still unclear how TM6SF2 facilitates the bulk lipid uptake of triglycerides to $VLDL_2$, but *TM6SF2*—which is located in the ER/ERGIC region close to where $VLDL_2$ is converted to $VLDL_1$—may be important for the formation of luminal lipid droplets that fuse with $VLDL_2$ or for the fusion process per se (122). It has also been shown that carriers of the *TM6SF2* E167K genetic variant have impaired hepatic lipid synthesis from polyunsaturated fatty acids, which leads to a relative deficiency of phosphatidylcholine (PC) (76). Interestingly, expanding lipid droplets recruit CTP:phosphocholine cytidylyltransferase, the rate-limiting enzyme for PC synthesis, and this enzyme is activated when it binds to the surface of the droplets (62). A deficiency in PC may therefore lead to changes in membrane properties, such as fluidity and curvature, which can affect the budding and expansion of lipid droplets from the ER membrane. Thus, a relative deficiency of PC could lead to the impaired ability of hepatocytes to synthesize and secrete large triglyceride-rich $VLDL_1$ (62).

In a recent study, Li et al. (72) revealed that TM6SF2 has a role in enhancing the stability of apoB through its interaction with ER lipid raft protein 1 (ERLIN1) and ERLIN2. These proteins have multiple actions in the cell, including regulating the SREBP (sterol regulatory element-binding protein) signaling pathway, which promotes cellular cholesterol homeostasis. They are also linked to ERAD. While ERLIN1 and ERLIN2 did not have a direct interaction with apoB, they were able to enhance apoB stability by stabilizing TM6SF2. The *TM6SF2* E167K LOF

mutation, which leads to a decrease in TM6SF2 protein expression, may therefore hinder the process of apoB stabilization. The implications of these findings on human pathophysiology are still unclear, and it is still uncertain how a disruption in apoB stabilization specifically impacts the hepatic secretion of $VLDL_1$ but not $VLDL_2$ (122).

INTRACELLULAR TRANSPORT AND SECRETION OF VLDL: ER EXIT OF VLDL

The ER membrane is dynamic and is the source for lipid droplets, VLDL, and transport vesicles. Maintaining its membrane integrity is critical and depends on proteins such as transmembrane protein 41B (TMEM41B) and vacuole membrane protein 1 (VMP1), scramblases that mediate transbilayer shuttling of phospholipids in the membrane (73). Disruption of TMEM41B in cells leads to an imbalance in lipid distribution, resulting in the formation of enlarged lipid droplets. In mice with liver-specific *Tmem41b* deletion, plasma triglyceride levels decrease, lipoprotein trafficking is impaired, and the development of nonalcoholic steatohepatitis is accelerated (53). VMP1, which collaborates with TMEM41B for autophagy, exhibits similar effects when specifically ablated in the liver (56). The overexpression of VMP1 in mice enhances VLDL secretion and reduces hepatic steatosis (56).

Transport of nascent proteins from the ER to the Golgi apparatus is highly regulated and mediated by coat protein complex II (COPII)-coated vesicles. The five core proteins of COPII vesicles (Sar1p, Sec23/24p, and Sec13/31p) act in concert to sculpt the ER membrane into transport vesicles. Extensive biochemical studies have shown that the coat assembly of COPII vesicles starts when Sar1p–GTP is recruited to the membrane, facilitating the association of the Sec23p–Sec24p complex and the recruitment of cargo molecules (60). Following this, the Sec13p–Sec31p complex binds, inducing membrane reshaping. Once the coat assembly reaches completion, the vesicle undergoes budding. Sar1p's GTPase activity is modulated by Sec23p, effectively acting as a timing mechanism that ultimately leads to the deactivation of Sar1p and the removal of the coat. The formed COPII vesicles range between 55 and 70 nm in diameter (82). Because VLDL particles are significantly larger (up to 100 nm in diameter), the mechanism of VLDL transport from the ER to the Golgi apparatus remained unclear until recently.

FORMATION OF VLDL TRANSFER VESICLES AND SECRETION OF VLDL FROM THE ER TO THE GOLGI APPARATUS

It was lately discovered that the transport and Golgi organization 1 (TANGO1) family of proteins can enable COPII vesicles to reach a size that can accommodate large macromolecules (103). These specialized COPII-containing ER-derived vesicles transport VLDL and are referred to as VLDL transport vesicles (VTVs) (114). A TANGO1-like protein called TALI, expressed in the liver and intestine, is required for the formation of VTVs and thus intracellular transport of VLDL and chylomicrons. TANGO1 binds TALI, and both interact with apoB and are necessary for the recruitment of VLDL to ER exit sites for their subsequent export (108). Kelch-like protein 12 (KLHL12), an adaptor protein known to assist COPII-dependent transport of macromolecules, has also been shown to facilitate the ER exit and secretion of VLDL particles (27). The TANGO1 and KLHL12 proteins facilitate vesicle formation by influencing Sec12 recruitment for efficient Sar1b activation or Sec31 ubiquitination (27).

To elucidate how VLDL can be targeted to the VTV transport vesicles, Wang and colleagues (132) performed proximity-based proteomics with SAR1b. This led to the discoveries that lipoprotein transport is segregated from general secretion upon ER exit and that the cargo receptor surfeit 4 (Surf4) selectively transports lipoproteins from the ER (132). Surf4 is ubiquitously

expressed and localized in the ER membrane. It comprises five presumed transmembrane sections and a cytosolic domain at its tail end that interacts with Sec24 within the COPII system (110). It has been proposed that Surf4 undergoes a conformational change within the transmembrane domains after binding to apoB. This change allows it to engage with COPII proteins such as Sec24, ultimately promoting the inclusion of cargo into COPII vesicles for export from the ER (144). Hepatic deficiency of Surf4 in mice markedly reduced VLDL secretion and plasma lipids, coupled with increased hepatic lipid accumulation.

Siddiqi and colleagues (102) isolated VTVs and characterized their proteome. The results showed that VTVs differ from their counterparts of intestinal origin, the prechylomicron transport vesicles (PCTVs). For example, VTVs contain Sec22b, small valosin-containing protein-interacting protein (SVIP), apolipoprotein C-I (apoC-I), reticulon 3 (RTN3), cell death inducing DFFA like effector B (cideB), and lysophosphatidylcholine acyltransferase 3 (LPCAT3)—none of which are present in PCTVs (102). Knockdown of RTN3, SVIP, or cideB significantly reduces VTV biogenesis, disrupts ER-to-Golgi trafficking of VLDL, and reduces VLDL secretion (113, 125, 126). The underlying mechanisms for how RTN3, SVIP, and cideB each modulate VTV biogenesis remain to be elucidated.

Additional proteins, including various RAB GTPases, in particular Rab23 and Rab1b, have been identified as regulators of hepatic lipoprotein secretion (61). The significance of the RAB GTPase-activating protein Golgi-protein 73 (GP73) in VLDL secretion was demonstrated by Wei and colleagues (96); its expression increases during ER stress or liver damage. Upon overexpression in mouse livers, intracellular apoB levels rise and VLDL triglyceride secretion drops markedly. GP73, situated in the Golgi apparatus, interacts with RAB23, but it is still unclear how RAB GTPases and GP73 regulate VLDL secretion. In addition, small leucine-rich protein 1 (SMLR1) has been shown to regulate VLDL secretion (128). It was identified through coexpression analysis with MTTP/Mttp in human and murine transcriptome datasets. In mice, hepatic deficiency of Smlr1 reduced VLDL secretion by 45% and induced liver fat accumulation (128). SMLR1's presence in the ER membrane and *cis*-Golgi hints at involvement in VLDL transport, though the precise mechanisms remain unclear. Proteins belonging to the ARF family normally play a role in governing the movement of cellular membranes and the organization of organelles. These proteins encompass guanine-nucleotide-binding (G) proteins such as ARF-like (ARL) proteins and SAR1 and have also been shown to be critical for VLDL formation, likely by influencing the transport of VLDL through the secretory pathway (9).

TRANSPORT TO THE GOLGI APPARATUS AND INTRA-GOLGI MODIFICATION OF TRIGLYCERIDE-RICH LIPOPROTEINS

VTVs transport VLDL from the ER to the Golgi apparatus where VLDL undergoes a number of modifications, including glycosylation by the addition of complex sugar moieties and phosphorylation (127). It was previously unclear how transport vesicles were targeted to the *cis*-Golgi membranes until Sudhof & Rothman (118) showed that specific soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins play a pivotal role in directing, securing, and merging transport vesicles with their designated target membranes. These SNARE proteins, categorized as type II integral membrane proteins, are typically found on transport vesicles (v-SNAREs) and the membranes they are destined for (t-SNAREs).

Interestingly, VTVs and PCTVs utilize different sets of SNARE proteins to form distinct SNARE complexes for their fusion with the *cis*-Golgi (111). The PCTVs employ vesicle-associated membrane protein 7 (VAMP7), whereas VTVs (which do not contain VAMP) utilize Sec22b as their v-SNARE (127).

SNARE proteins have also been shown to mediate fusion between cytosolic lipid droplets (25). It is still unclear whether VLDL becomes further lipidated in the Golgi apparatus. Findings to date are conflicting, but results by several research groups, including Yao et al. (143), indicate that the lipidation of VLDL is fully accomplished in the Golgi apparatus (41).

There is still limited information available regarding the post-Golgi transportation of VLDL. However, it has been hypothesized that a distinct post-Golgi vesicle is responsible for conveying VLDL to the plasma membrane (105). One potential scenario is that this secretory post-Golgi vesicle may carry multiple VLDL particles, as electron microscopy data indicate that in the small intestine, post-Golgi vesicles transport several chylomicrons to the basolateral membrane (105).

Rader and colleagues (117) performed genome-wide association studies and identified sortilin1, a lysosomal sorting protein that binds ligands both in the Golgi apparatus and at the plasma membrane and traffics them to the lysosome, and showed that hepatic sortilin reduced hepatic VLDL secretion and increased LDL catabolism. The same research group further showed that loss of sortilin has little effect on hepatic VLDL secretion unless the cells are stressed (32). Thus, when hepatocytes are exposed to lipid overload or experience ER stress, the deficiency of sortilin results in an increased secretion of apoB. This function could potentially serve as a quality control mechanism within the apoB secretion pathway in hepatocytes.

ASSEMBLY AND SECRETION OF CHYLOMICRONS IN THE INTESTINE

Chylomicrons are secreted by enterocytes in the small intestine. These cells play a crucial role in absorbing dietary fats and fat-soluble vitamins from the digestive tract and packaging them into chylomicrons for transport through the lymphatic system and eventually into the bloodstream. In contrast to the biogenesis of VLDL, much less is known about the biogenesis and secretion of chylomicrons, mainly due to technical challenges (67, 142).

Electron microscopy investigations have revealed that following a high-fat meal, the smooth ER within enterocytes accumulates lipid content, and lipoprotein particles can be observed within the rough endoplasmic reticulum, Golgi apparatus, and secretory vesicles located near the basolateral membranes (106). Consequently, multiple indirect lines of evidence suggest that the initiation of chylomicron assembly involves a small primordial prechylomicron particle roughly the size of high-density lipoprotein (HDL), which is subsequently enlarged through the incorporation of neutral lipids into the core of this emerging particle. Nevertheless, direct evidence supporting this hypothesis is still lacking (67, 142). Recent kinetic studies with stable isotopes in humans have demonstrated that the apoB48-containing particles are secreted not only as chylomicrons but also as smaller particles, with sizes and density ranges corresponding to $VLDL_1$ and $VLDL_2$ particles, both in the basal state and during dietary lipid absorption (15, 16). It is likely that large chylomicrons are formed late in the secretory pathway due to the problems transporting such large particles, and it has been proposed that prechylomicrons fuse with lipid droplets in the Golgi to form fully lipidated chylomicrons (29). Thus, the apoB48-containing $VLDL_1$ and $VLDL_2$ particles could correspond to nascent chylomicron particles, which can either be secreted or pick up more lipids (tentatively by fusion with a lipid droplet) to become a fully lipidated chylomicron.

Over the past decades, there have been several unexpected discoveries regarding the control of TRL assembly in enterocytes (64, 142). First, research has revealed that the intestine can retain and store triglycerides, suggesting that the initial source of triglycerides secreted after a meal could originate from previous food intake (30). This hypothesis may clarify the early increase in plasma triglyceride levels following food intake, as intestinal cells can promptly begin secreting stored triglycerides in the form of chylomicrons without having to wait for the absorption of dietary

lipids through the brush border. Second, the release of chylomicrons has been linked to the taste–gut–brain axis (58, 142). This connection might provide an explanation for why chylomicrons are secreted when glucose or fats are merely tasted, even before ingestion occurs (141).

HOW DO THE ASSEMBLIES OF VLDL AND CHYLOMICRONS DIFFER?

The underlying mechanisms for biogenesis of chylomicrons are likely similar to those of VLDL assembly and secretion, but several differences have been described. Mansbach & Siddiqi (80, 115) have extensively elucidated the intracellular transport of chylomicrons and shown that nascent chylomicrons are transported from the ER to the Golgi apparatus in PCTVs. These vesicles are approximately 250 nm in diameter and contain COPII proteins such as Sar1b, Sec23, Sec24, Sec13, and Sec31, as well as proteins such as VAMP7, CD36, and liver-type fatty acid binding protein (L-FABP) (94).

The initiation of PCTV formation may be attributed to L-FABP (FABP1), as evidenced by the ability of recombinant FABP1 to independently generate PCTVs (94). It is noteworthy that PCTVs generated by FABP1 lack COPII proteins and do not undergo fusion with the Golgi apparatus (87, 115). Consequently, the formation of PCTVs involves two categories of proteins: those responsible for PCTV formation and cargo selection, and those essential for their fusion with the Golgi apparatus. While proteomic analysis has identified several additional proteins within these vesicles, their specific roles in prechylomicron transport remain to be determined (139).

Interestingly, phosphorylation of Sar1b by protein kinase C zeta (PKCζ) and ATP releases FABP1 from a 75-kDa protein complex in the cytosol, enabling FABP1 to bind to the ER and generate PCTVs. Thus, Sar1b is critical for transport of chylomicrons from the ER to the Golgi apparatus. Indeed, mutations in Sar1b cause Anderson's disease, also known as chylomicron retention disease, an uncommon hereditary malabsorption syndrome characterized by the lack of chylomicrons after eating (112). We still lack full understanding of how Sar1b is regulated, but it has been reported that the expression of Sar1b is increased in insulin-resistant mice and that overexpression of Sar1b induces insulin resistance and intestinal chylomicron overproduction (68).

REGULATORS OF VLDL ASSEMBLY

Fatty Acids Increase VLDL Formation

Elevated delivery of fatty acids results in increased secretion of VLDL from the human liver, as well as from cultured hepatocytes (2, 5, 71). Notably, when examining the relationship between hepatic fat accumulation and apoB production rates for $VLDL_1$, results show that individuals with type 2 diabetes secrete more $VLDL_1$ particles, not larger ones, compared with nondiabetic controls (4, 5). Thus, the quantity of lipid content in $VLDL_1$ remains the same in individuals with type 2 diabetes and those without the condition, but the rate of conversion of $VLDL_2$ to $VLDL_1$ is heightened in individuals with type 2 diabetes.

Insulin Shifts the Balance from $VLDL_1$ to $VLDL_2$

Kinetic studies in humans investigating the acute effect of insulin on VLDL have revealed decreased secretion of VLDL triglycerides and VLDL apoB (6, 71, 79). Furthermore, insulin infusion has a greater effect on VLDL triglyceride secretion than on VLDL apoB (6, 71, 79) and mainly suppresses $VLDL_1$ apoB production, with little effect on $VLDL_2$ apoB100 production (6). Thus, insulin not only reduces the number of overall VLDL particles but also shifts the balance between $VLDL_2$ and $VLDL_1$ to reduce the relative proportion of $VLDL_1$ particles.

Interestingly, insulin effectively reduces $VLDL_1$ secretion in individuals with low liver fat, but it fails to suppress $VLDL_1$ secretion in those with high liver fat, leading to an overproduction of $VLDL_1$ (6). The underlying reason for this lack of effect remains unknown. It is worth noting that insulin reduces the nonesterified fatty acid (NEFA) pool to a similar extent, regardless of liver fat levels. Consequently, the high $VLDL_1$ production in individuals with substantial liver fat may be facilitated either by utilizing a larger portion of systemic NEFA or by tapping into alternative sources of triglycerides, such as hepatic stores.

Insulin's ability to reduce VLDL formation involves at least two mechanisms. First, it regulates the quantity of fatty acids circulating in the bloodstream (70). Second, it directly inhibits the production of $VLDL_1$ in the liver, regardless of the availability of fatty acids (79). The underlying mechanisms for the direct suppression of $VLDL_1$ production remain elusive, and various mechanisms have been proposed. Sparks and colleagues (97) demonstrated that the activation of phosphatidylinositol 3-kinase (PI3-K) is a critical requirement for insulin-induced reduction in apoB secretion from rat hepatocytes. Moreover, insulin suppresses MTTP expression by activating the mitogen-activated protein kinase (MAPK) pathway (8). Another potential mechanism through which insulin may decrease VLDL secretion is by inhibiting the activity of the transcription factor Foxa2 (137). Studies in ob/ob mice have revealed that Foxa2, in conjunction with its coactivator peroxisome proliferator-activated receptor gamma (PPARγ) coactivator β (Pgc-1β), promotes fatty acid oxidation and stimulates MTTP activity in the liver, resulting in increased VLDL secretion (136).

Brain Glucose Controls VLDL Secretion

Rossetti and colleagues (63) reported that glucose-sensing mechanisms in the hypothalamus regulate hepatic triglyceride secretion, but not from the intestine. The brain–liver axis establishes a connection between carbohydrate sensing and the secretion of lipoproteins, achieved by reducing the activity of stearoyl-coenzyme A (CoA) desaturase-1 (SCD1) in the liver and interfering with a late stage in the formation and release of VLDL particles from the liver. These findings align with the concept of a homeostatic loop whereby an increased supply of carbohydrates limits the production of lipids within the body, contributing to the acute downregulation of VLDL secretion following a meal, in conjunction with insulin. It is worth noting that glucose-induced hyperglycemia–hyperinsulinemia results in a 50% reduction in VLDL triglyceride secretion (98), though it is challenging to separate the effects of insulin and glucose in this context.

Independent Regulation of $VLDL_1$ and $VLDL_2$

In addition to the regulation of $VLDL_1$ and $VLDL_2$ production by insulin and PPARα agonists, ethanol stimulates $VLDL_1$ secretion in humans (40). Also, endogenous cholesterol synthesis correlates with $VLDL_2$ apoB, but not $VLDL_1$ apoB, production (100). This finding provides further support for independent regulation of $VLDL_1$ and $VLDL_2$ and may explain why $VLDL_2$ but not $VLDL_1$ is increased in patients with increased plasma cholesterol, as in moderate hypercholesterolemia (100) and familial hypercholesterolemia (55).

Clinical studies have revealed that plasma glucose level, particularly beyond the normal glucose range, is the strongest predictor for $VLDL_1$ overproduction in individuals with type 2 diabetes or insulin resistance (1). Further analysis identified fasting insulin, intra-abdominal fat, liver fat, and homeostatic model assessment of insulin resistance (HOMA-IR) as predictors of $VLDL_1$ apoB and $VLDL_1$ triglyceride production (5). However, in a multiple regression analysis, only liver fat emerged as a significant predictor (5). When it comes to liver fat levels, the key predictors are intra-abdominal fat, adiponectin, and plasma glucose (5).

INTRAVASCULAR METABOLISM OF TRIGLYCERIDE-RICH LIPOPROTEINS

Triglyceride transport between body tissues, from organs involved in de novo synthesis (liver), short-term storage (liver, intestine), and absorption (intestine) to long-term storage depots (adipose tissue) and sites of energy production (skeletal and cardiac muscle), occurs in the aqueous medium of blood plasma. As noted above, triglyceride as a hydrophobic molecule must be carried within the pseudomicellar structure of large lipoproteins and presented to tissues in a form capable of being hydrolyzed in a directed and efficient manner. Chylomicrons and apoB48-VLDL generated in the intestine enter the bloodstream via the thoracic duct after passage though the intestinal lymphatic channels. ApoB100-VLDL is initially secreted by hepatocytes into the space of Disse and then rapidly enters the circulation. Both types of nascent particles require cofactors to mediate their metabolism. These are small proteins—the C apolipoproteins (apoC-I, apoC-II, apoC-III) and apoE—that can exchange between lipoproteins. By contrast, apoB48 and apoB100 are integral to the structure of the particles and remain with the lipoprotein throughout its lifetime in the circulation. HDL acts as a reservoir for these small apolipoproteins and passes them to chylomicrons and VLDL upon entry of the latter into the bloodstream (18, 52) (**Figure 3**).

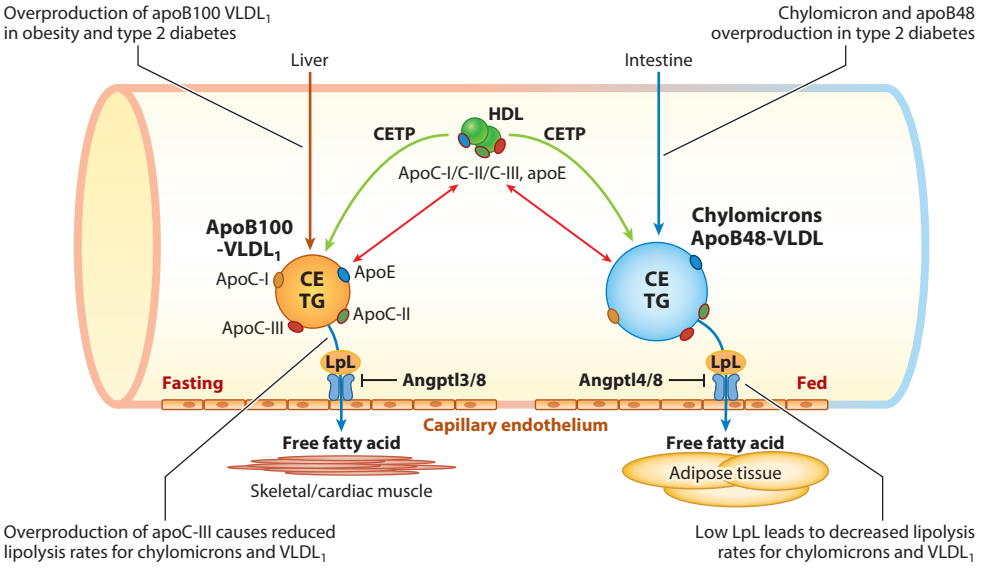

Figure 3

Intravascular processing of triglyceride-rich lipoproteins (TRLs). Chylomicrons [containing apolipoprotein B48 (apoB48) as the major structural protein], released from the intestine in a wave during fat absorption, are lipolyzed rapidly by the action of the major intravascular triglyceride (TG) hydrolyzing enzyme, lipoprotein lipase (LpL). This enzyme is expressed in many tissues but is most abundant in adipose tissue and muscle (skeletal and cardiac), sites that require the TG for storage or energy production. LpL is highly regulated by apolipoproteins and tissue-specific factors. ApoC-II acquired by interparticle exchange from high-density lipoprotein (HDL) is essential for LpL activation, while apoC-III has an inhibitory effect on LpL. Angiopoietin-like (angptl) proteins 3, 4, and 8 act in concert to direct TRL delivery to muscle when angptl4 levels are high and to adipose tissue when angptl3 and angptl8 levels are high. Large very-low-density lipoprotein (VLDL) ($VLDL_1$ containing apoB100 as the major protein) secreted by the liver undergoes a similar lipolysis process and indeed competes with chylomicrons for LpL. During delipidation, cholesteryl ester (CE) is acquired by TRL via the agency of cholesteryl ester transfer protein (CETP). Most CE comes from HDL particles.

The C apolipoproteins vary in size from 7,100 to 11,300 Da and act as regulators of triglyceride hydrolysis. ApoC-II is a positive cofactor that is essential for lipolysis; genetic absence of this protein causes severe hypertriglyceridemia (52). ApoC-II appears to be involved in orienting the particle so that the main lipolytic enzyme—lipoprotein lipase (LpL)—is able to access the triglyceride cargo. ApoC-III is the most abundant C protein and is a major regulator of TRL lipolysis, inhibiting the action of LpL (18, 20). Its absence leads to very rapid triglyceride hydrolysis and very low levels of chylomicrons and VLDL in the circulation (122). When present in excess, apoC-III slows TRL lipolysis. Kinetic studies have revealed a strong positive correlation between plasma apoC-III levels and plasma triglycerides (apoC-III resulting from the negative relationship between apoC-III and chylomicron and VLDL clearance rates) (19) (**Figure 3**). ApoC-I has been less well studied and appears to also be an inhibitor of TRL lipolysis but not a major factor in determining plasma TRL concentrations (52).

Recently, there has been considerable interest in the role of these small apolipoproteins in triglyceride metabolism, in particular apoC-III, due to the evidence from genetic studies that it is associated with cardiovascular disease (CVD) (20). Pharmacologic agents have been developed to lower apoC-III levels with the aim of promoting lipolysis rates and thereby lowering TRL concentrations and the risk of CVD. Similarly, apoC-II mimetics have been generated in an attempt to achieve the same therapeutic goal through a different mechanism (138).

ApoE has a molecular weight of 34,000 Da. Its function is distinct from that of apoC in that it facilitates the interaction of TRL with cell surface receptors, mainly on hepatocytes, and promotes the uptake of particles by cells (**Figure 4**). Like apoB100, apoE contains a high-affinity binding site that is recognized by receptors, especially the LDL receptor, and thus the presence of apoE on the surface of a TRL is thought to direct its fate toward cell uptake rather than further lipolysis (20, 54) (**Figure 4**). Evidence indicates that in addition to inhibiting particle lipolysis, apoC-III can impede the action of apoE and thus increase the residence time of TRL in the bloodstream. Individuals with dysfunctional apoE (carrying the apoE2 variant) fail to clear TRL from the circulation, and particles, termed remnants, accumulate. ApoE appears also to be involved in the processing of TRL particles down the delipidation chain that leads to the formation of LDL (93) (**Figure 4**).

Several specific lipases have been identified as being central to the intravascular lipolysis of TRL. Lipoprotein lipase is a high-capacity triglyceride hydrolase situated on the capillary endothelium of adipose tissue and muscle. It acts on chylomicrons and VLDL to initiate lipolysis and delivery of released fatty acids to tissues (93, 133) (**Figure 3**). Hepatic lipase (HL) in the liver is the enzyme responsible for the lipolysis of smaller triglyceride-containing particles, VLDL remnants, and intermediate-density lipoprotein (IDL) (**Figure 4**). HL, which is capable of hydrolyzing both triglycerides and phospholipids, mediates the conversion of IDL to LDL, and little LDL formation occurs in its absence (93). A third lipase, endothelial lipase, has a less well-defined role in TRL metabolism. It is thought to facilitate the processing of partially lipolyzed TRL, possibly by removing excess phospholipid from the shrinking particle's surface (59) (**Figure 4**). The angiopoietin-like (angptl) proteins 3, 4, and 8 work in concert to regulate lipolysis at the tissue level. Their expression varies markedly between fasting and fed states, and they act as a molecular switch that directs the delivery of triglycerides in TRLs to adipose tissue or skeletal muscle. As a result of genetic studies linking variation in these proteins to CVD risk, they have emerged as possible important targets for drug development (133) (**Figure 3**).

Cholesteryl ester transfer protein (CETP) is an important factor in the metabolism of TRL and other lipoproteins. This circulating protein has a hydrophobic pocket that can sequester a triglyceride or cholesteryl ester molecule. This allows CETP to mediate the bidirectional shuttle of these lipids between particles. An inherited lack of CETP leads to very high levels of HDL cholesterol, low cholesterol content in TRL, and structural abnormalities in LDL (88). CETP

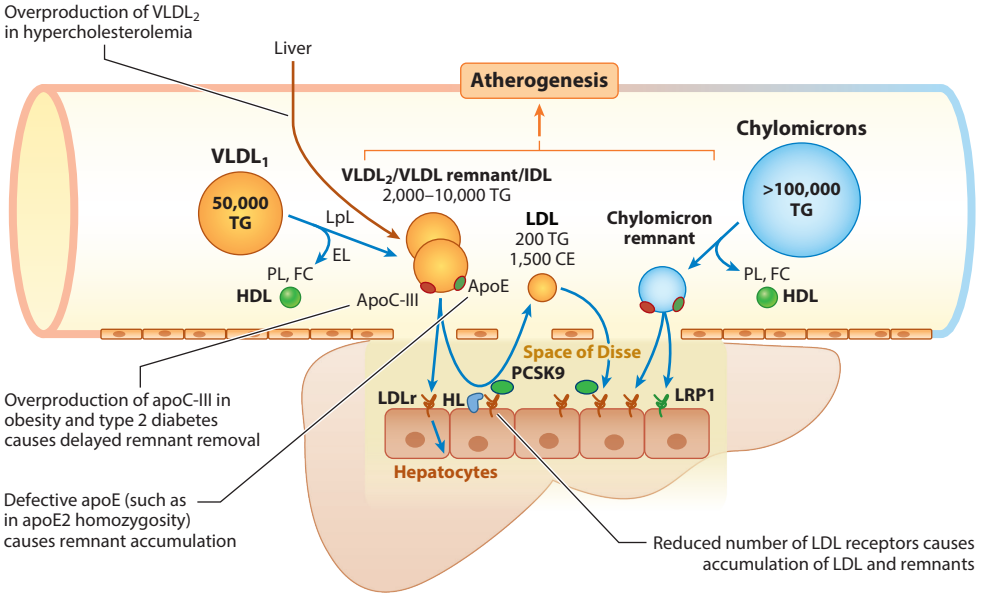

Figure 4

Particle remodeling and clearance. As lipolysis proceeds by the action of lipoprotein lipase (LpL), chylomicrons and very-low-density lipoprotein (VLDL) lose core triglycerides (TG) and so the particle size is reduced considerably. There is a simultaneous process of shedding of excess surface components—phospholipid (PL) and free/unesterified cholesterol (FC)—to high-density lipoprotein (HDL). Endothelial lipase (EL), which has phospholipase activity, may also contribute to this remodeling. Partial triglyceride-rich lipoprotein (TRL) lipolysis products are termed remnants [i.e., VLDL remnants, chylomicron remnants, and intermediate-density lipoprotein (IDL)]. These are depleted in TG and enriched in cholesteryl ester (CE). Chylomicron remnants can enter a liver compartment—the space of Disse—that allows contact with hepatocytes and their cell surface receptors, the low-density lipoprotein (LDL) receptor (LDLr) and LDL receptor-related receptor 1 (LRP1). These receptors mediate cell uptake and digestion of the remnants. Apolipoprotein E (apoE) on chylomicrons and apoE and apoB100 on VLDL act as ligands for these receptors. VLDL remnants/IDL have two fates: one is direct uptake by receptors (LDLr), and the other is further lipolysis by hepatic lipase (HL), an enzyme that removes TG and PL. As a result, LDL is formed and is released into the blood circulation. Proprotein convertase subtilisin kexin 9 (PCSK9) is a major regulator of LDL receptor activity. TRL remnants are believed to be highly atherogenic.

has been the subject of intensive investigation as a therapeutic target, mainly because inhibiting the protein significantly elevated HDL. However, this action did not translate into a clinically meaningful outcome, and questions remain as to the net effect of this factor on CVD risk. Further investigations are being undertaken on a CETP inhibitor that significantly lowers LDL levels, in the hope that this may translate into a CVD risk reduction (88).

REGULATION OF THE DELIPIDATION, REMODELING, AND CLEARANCE OF TRIGLYCERIDE-RICH LIPOPROTEINS

While a great deal of attention has been paid to the assembly and secretion of TRLs and the removal of the end products of lipolysis, the processes by which TRLs are remodeled as delipidation progresses is still somewhat of a mystery (**Figure 4**). Lipolysis reduces the core size of TRLs, decreasing the number of triglyceride molecules by 80%–90%. Consequently, the diameter of a chylomicron falls from ≫1,000 nm to <700 nm. Similarly, a newly secreted large VLDL particle in the $VLDL_1$ range, with a diameter of 50–70 nm, will become a $VLDL_2$-sized remnant particle

of 30–35 nm. Particle volume changes are therefore considerable, and extensive remodeling of the surface and core is required to maintain a stable micellar structure. As this remodeling takes place, the small exchangeable apolipoproteins are lost (to HDL). The fate of excess surface cholesterol and phospholipids is less clear, as this has been difficult to study in vivo. It is believed that the majority is transferred into the HDL density interval where it becomes integrated into existing HDL, also causing the remodeling of this lipoprotein class (52) (**Figure 4**).

After the initial lipolytic steps have taken place, a chylomicron or VLDL remnant forms as a partially delipidated intermediate product. Compared with their nascent parent lipoproteins, these remnants are depleted in triglyceride, enriched in cholesteryl ester, and have an altered small apolipoprotein composition, retaining apoC-III and with relative enrichment of apoE (46). Due to these physicochemical properties, it is believed that the remnants are highly atherogenic and can contribute to CVD risk in individuals where they accumulate, such as in those with hypertriglyceridemia and especially type III hyperlipidemia (also known as remnant hyperlipidemia). Analogous to LDL, remnant lipoproteins can enter the artery wall, bind to the extracellular matrix, and deliver their contained cholesterol to resident cells, thereby promoting the progression of atherosclerotic plaque. The extent of remnant formation is a balance between the rates of particle lipolysis and cholesteryl ester acquisition. In individuals with low plasma triglycerides, rapid delipidation leads to the generation of remnants mainly in the IDL size range. In those with higher triglyceride levels, the remnants that form are larger and exist in the small $VLDL_2$ size range (46) (**Figure 4**).

Remnant particles and IDL have two potential metabolic fates: further lipolysis to LDL or direct clearance from the circulation by the action of lipoprotein receptors, primarily in the liver (46, 93) (**Figure 4**). It is unclear what specific features of a remnant's composition and structure determine their further processing. Kinetic studies in humans and animal models indicate that a number of factors appear to be important, including the relative content of apoE and apoC-III on the particle surface. Where the ratio of apoE to apoC-III is high, particles tend to undergo catabolism rather than further delipidation (46). It has also been shown that approximately half of VLDL particles are converted to LDL and the other half are cleared directly from the circulation (93). Studies in individuals with dyslipidemia or those treated with lipid-lowering drugs indicate that apoE and proprotein convertase subtilisin kexin 9 (PCSK9) are major determinants of the IDL to LDL conversion process; subjects with no LDL receptors have a very slow IDL to LDL transfer; and in apoE2 homozygous subjects, little LDL is formed from IDL (93). Treatment with a PCSK9 inhibitor reduces the amount of IDL converted to LDL, which contributes to the LDL-lowering action of these drugs (92, 120).

DEFECTIVE REGULATION OF TRIGLYCERIDE METABOLISM IN DYSLIPIDEMIA

Pathways of lipid transport evolved in humans to make optimal use of the relatively scarce resource of animal fat—both its triglyceride and cholesterol constituents. It is more efficient to use cholesterol from the diet than to synthesize it de novo in body tissues. Likewise, triglyceride is a valuable source of energy that can be stored compactly in adipocytes and released as needed.

Thus, the metabolic pathways depicted in **Figures 3** and **4** work best, and with no disease consequences, when intake of fat is low and episodic. In most people, pathogenic sequelae are a result of surfeit, as these highly regulated pathways are overwhelmed by excess intake. The lipoprotein assembly mechanisms described above not only permit plasma lipid transport to occur but also allow tissues (especially the liver) to export triglyceride and cholesterol when they accumulate above levels that are conducive to cellular health, as high levels of cholesterol and triglyceride are toxic to cells.

Plasma triglyceride levels in the population vary widely, from optimal (<1.2 mmol/L) through borderline (1.2–1.7 mmol/L) and moderately elevated (1.7−5.7 mmol/L) concentrations to severe (5.7−10.0 mmol/L) and extreme (>10.0 mmol/L) degrees of hypertriglyceridemia (46).

In borderline and moderate hypertriglyceridemia, the elevation of plasma triglycerides is primarily due to the accumulation of $VLDL_1$, caused by a combination of overproduction of $VLDL_1$ by the liver and reduced TRL lipolysis. In more severe conditions, there is a marked increase in the plasma levels of both chylomicrons and VLDL, principally due to very slow lipolysis rates (46). In individuals with optimal plasma triglycerides, the liver releases approximately 500 to 1,000 mg of $VLDL_1$-apoB100 each day, but in those with elevated plasma triglycerides, this production rate rises to approximately 1,500 mg/day (21, 93) (**Figure 4**). A major underlying cause of increased $VLDL_1$ assembly and the secretion of supranormal amounts of $VLDL_1$ is obesity and the accompanying increase in liver fat content, as noted above (3, 21). High intracellular triglyceride stores prompt hepatocytes to upregulate the $VLDL_1$ assembly pathway as a means of exporting the lipid to the plasma compartment.

Since the liver has the capability to make apoB-containing lipoproteins that differ widely in size and composition (**Figure 2**), the pathways of assembly and secretion can be tuned to the changing metabolic status of hepatocytes, in particular their lipid content. Increased production of $VLDL_1$ in hypertriglyceridemia may be driven by insulin resistance in type 2 diabetes or, in overweight and obese individuals, by the need for the liver to export excess intracellular fat to a safer compartment (blood plasma). Overproduction of $VLDL_2$ occurs in individuals with hypercholesterolemia, probably in response to stimulated cholesterol synthesis in the liver or as a mechanism to allow the hepatocytes to get rid of excess cholesterol. The amount of directly secreted $VLDL_2$-apoB100 is normally 200–300 mg/day, but this increases to approximately 500 mg/day in those with high plasma cholesterol levels, which contributes to elevated circulating levels of small VLDL, IDL, and LDL. However, in most people, the primary cause of high cholesterol is reduced LDL receptor activity and defective clearance of VLDL remnants, IDL, and LDL particles (20) (**Figure 4**).

It is now recognized that the intestine plays a major role in regulating plasma triglyceride levels in the bloodstream, not just while fat absorption is occurring but also between meals where retained triglycerides in enterocytes are released continuously in the form of apoB48-containing VLDL particles (20, 129). When presented with a meal rich in fat, the intestine will greatly upregulate the synthesis of both very large chylomicrons and particles in the VLDL size range (**Figure 3**). The rate of production is a function of the amount of fat to be absorbed, but it is also increasingly clear that it is subject to hormonal regulation by insulin and incretins (20, 129). Kinetic studies have revealed that when insulin resistance is present, the production of apoB48-containing chylomicrons and VLDL is supranormal (20, 121, 129). Again, comparable to VLDL production in the liver, the degree of TRL overproduction by the intestine appears to be linked to glycemic status (20, 129). This metabolic consequence of insulin resistance has only recently been understood, and it may help explain the considerable lipoprotein-associated residual risk despite statin therapy that is observed in individuals with type 2 diabetes. While statin treatment reduces the plasma levels of VLDL and LDL in hyperlipidemic patients, the overproduction of chylomicrons and apoB48-VLDL by the intestine continues, and this is a potential target for further intervention strategies (123).

While overproduction of TRLs by the liver and intestine is a significant factor in the etiology of hypertriglyceridemia, the overall lipolysis rate is the major determinant of plasma triglyceride levels, especially in individuals with more marked forms of hypertriglyceridemia characterized by the accumulation of both VLDL and chylomicrons (46). Metabolic investigations reveal that the fractional clearance rate (a measure of lipolytic efficiency and particle clearance) for $VLDL_1$

varies widely, from approximately 25 pools/day in those with low/optimal triglycerides to less than 5 pools/day when triglycerides are >3.0 mmol/L (93). The net rate of lipolysis is controlled by many factors (**Figure 3**), and genetic studies in populations have shown that variants in the genes for LpL, apoC-III, and angptl3, -4, and -8 are all linked to altered plasma triglycerides (45, 107). However, the dominant regulatory factor appears to be apoC-III. Its concentration exhibits a strong negative association with the fractional clearance rate of both $VLDL_1$-apoB100 and apoB48-containing chylomicron particles (19). ApoC-III appears to have a physiological role as a counterregulatory factor to LpL in tissues, acting as a moderating influence to ensure that chylomicrons and $VLDL_1$ deliver their contained triglycerides to multiple sites rather than having all of the particle's core triglycerides hydrolyzed in a single tissue bed. It is noteworthy that apoC-III may have an integrating role in the metabolism of carbohydrates and triglycerides, as its synthesis in the liver is influenced strongly by glycemic status (19).

The intermediate products of lipolysis, remnant particles, accumulate in the circulation due to enhanced formation during retarded lipolysis or because of slow clearance through receptor-mediated pathways (**Figure 4**). Increasing the number of cell membrane LDL receptors with drugs such as statins or PCSK9 inhibitors lowers not only LDL but also circulating levels of VLDL remnants and IDL (20, 145). This removal of potentially atherogenic lipoproteins from the bloodstream makes an as-yet-unquantified contribution to the reduced CVD risk seen in clinical trials of these agents. In this regard, it is worth mentioning that genetic studies of TRL and LDL particles' relationship to CVD risk show that the former appears much more atherogenic than previously thought (14) (**Figure 4**).

HYPERTRIGLYCERIDEMIA THERAPY

As discussed above, mild-to-moderate hypertriglyceridemia is common, and its prevalence is increasing rapidly due to societal and environmental pressures promoting increased energy intake and reduced physical activity. The diagnosis of hypertriglyceridemia should be based on the analysis of samples on at least two occasions after an overnight fast because isolated elevated triglyceride values can occur, and the exclusion of secondary causes of triglyceride elevations is mandatory. Despite consistent and wide-ranging evidence that high triglycerides and remnant particles are strongly and causally associated with CVD risk, the current guidelines do not provide a definition for high triglycerides (66, 78) [though the recent European Atherosclerosis Society (EAS) consensus statement proposes a classification based on perceived risk of disease (46)]. EAS guidelines define threshold values for three categories of plasma triglycerides to flag potential therapeutic actions (78). These alert values are given for plasma non-HDL cholesterol, total apoB, and remnant cholesterol, allowing for a more personalized, patient-centered approach to treatment (36, 57). It should be noted that postprandially, approximately one-third of plasma total cholesterol measured by nuclear magnetic resonance resides in the remnant particles, including VLDL and IDL subclasses (11). The potential genetic causes for triglyceride elevation are uncommon but should be excluded by utilizing clinical history and genetic testing. Severe hypertriglyceridemia, defined as triglyceride concentration >10 mmol/L (>885 mg/dL), always signals the need for immediate action and fibrate therapy due to the increased risk of acute pancreatitis (65).

MANAGEMENT OF HYPERTRIGLYCERIDEMIA

Lifestyle Interventions

Overall, the guidelines strongly recommend lifestyle interventions, emphasizing dietary changes as the backbone of the management of elevated triglycerides (46, 69). It is agreed that adopting an optimal diet can serve as an effective treatment. As the majority of hypertriglyceridemic subjects

are overweight with abdominal obesity, the management of excess body fat has become one of the greatest health challenges of our era.

Weight loss requires hypoenergetic diets, but appropriately modifying the composition of the diet is also crucial, beyond the focus on weight reduction. High-carbohydrate diets are known to increase both VLDL triglyceride and VLDL apoB100 production rates, which are hallmarks of hypertriglyceridemia (93). Substantial evidence indicates that fructose, ingested in sugar-sweetened beverages, is lipogenic, as it stimulates hepatic de novo lipogenesis and also causes liver fat accumulation (119). Briefly, dietary guidelines recommend avoiding added sugars, sugary beverages, and excess alcohol while increasing the intake of whole grains and fiber (46, 78). Recommendations further highlight the need to individualize dietary changes according to the severity of hypertriglyceridemia (101). Other features of a healthy diet include decreasing the intake of saturated fat from animal sources in favor of mono- and polyunsaturated fats. These dietary changes are fundamental components of the widely accepted model of a healthy diet, the Mediterranean diet (75).

One key component of dietary interventions is the content and quality of omega-3 fatty acids (n3-FAs). Studies have consistently confirmed that dietary intake of n3-FAs is associated with a reduction in plasma triglycerides, but whether this reduction has an impact on CVD events is disputed (78, 130). It should be noted that n3-FAs include two different fatty acids: eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). A high dose (4 g) of EPA was used in the REDUCE trial, where the atherosclerotic CVD (ASCVD) risk was reduced by 25% and plasma triglycerides were reduced by approximately 20%; however, this change was not directly related to ASCVD risk reduction (13). On the basis of demonstrated beneficial ASCVD outcomes, icosapent ethyl is recommended for patients with elevated triglyceride levels and ASCVD, as an alternative to EPA/DHA combinations (81). However, n3-FAs may increase atrial fibrillation, and this potential negative effect should be considered before beginning therapy (47).

Pharmacotherapy for Hypertriglyceridemia

If plasma triglycerides remain elevated (>2.0 mmol/L, >175 mg/dL) after lifestyle interventions, a careful cardiovascular risk assessment should be performed and the overall risk level estimated. For individuals at high or very high risk, statin therapy should be initiated, as it reduces plasma triglyceride by 10% to 20%, on average. Therefore, statin therapy is the first step and the backbone of pharmacological therapies to manage hypertriglyceridemia (78). Available data suggest that statins can enhance TRL clearance (93). If triglyceride levels remain elevated on statin therapy, there is a persisting residual risk of CVD (78). The remaining option is to then further lower triglyceride levels, and potentially reduce the residual risk, with drugs including n3-FAs, fibrates, or niacin. Fibrates such as PPARα agonists are known to effectively reduce plasma triglycerides (85). However, the reduction of plasma triglyceride levels using these agents has not been associated with the reduction of CVD outcomes in recent clinical trials (12, 78). The PROMINENT study used pemafibrate, a novel selective PPARα modulator. The study included 10,497 patients with type 2 diabetes on statin therapy (34). Triglyceride levels were reduced by approximately 26% with concomitant significant reductions in remnant cholesterol. Notably, apoC-III levels, a critical regulator of TRL lipolysis, were also reduced by approximately 26%. Despite these changes, no significant reduction was reported in the composite primary end point, CVD events. Notably, there was a small increase of 4.8% in plasma apoB concentration in patients on pemafibrate. This finding indicates that the actual number of apoB particles did not decrease in the actively treated arm compared with placebo. It is possible that this rise in apoB reflects enhanced generation of TRL remnant or LDL particles (20, 36, 46). In this context, it is worth noting that recent data

strongly suggest that these remnant particles are in fact more atherogenic than LDL particles per se (14). The actual role of TRL remnant particles in atherogenesis has become a hot topic (20, 46); a key question revolves around how we can reduce the number of remnant particles in clinical practice. Another ongoing problem is the urgent need for a specific assay of these TRL remnant particles.

The development of interventions for hypertriglyceridemia is crucial for reducing the risk of CVD, improving public health, and promoting overall well-being. This may involve a combination of lifestyle modifications, pharmacological treatments, and targeted approaches to address the specific causes of elevated triglyceride levels in individuals.

DISCLOSURE STATEMENT

The authors report no conflicts of interest and are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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