The physiological and molecular regulation of lipoprotein assembly and secretion

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Triglycerides are insoluble in water and yet are transported at milligram per millilitre concentrations in the bloodstream. This is made possible by the ability of the liver and intestine to assemble lipid–protein emulsions (*i.e.* lipoproteins), which transport hydrophobic molecules. The assembly of triglyceride-rich lipoproteins requires the coordination of protein and lipid synthesis, which occurs on the cytoplasmic surface of the endoplasmic reticulum (ER), and their concerted assembly and translocation into the luminal ER secretory pathway as nascent lipoprotein particles. The availability of lipid substrate for triglyceride production and the machinery for lipoprotein assembly are highly sensitive to nutritional, hormonal, and genetic modulation. Disorders in lipid metabolism or an imbalance between lipogenesis and lipoprotein assembly can lead to hyperlipidemia and/or hepatic steatosis. We selectively review recently-identified machinery, such as transcription factors and nuclear hormone receptors, which provide new clues to the regulation of lipoprotein secretion.

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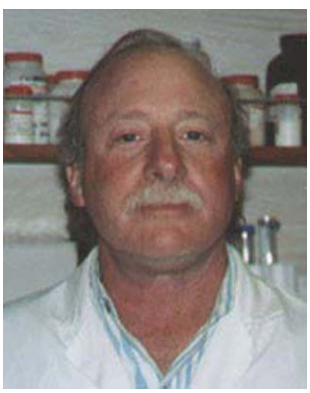

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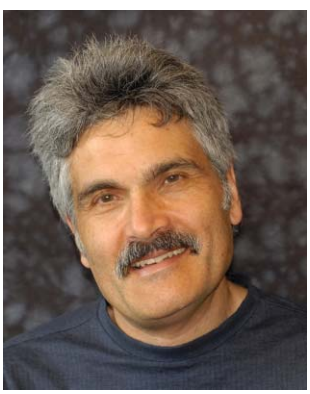
Metabolic context

Animals go through fasting and feeding cycles. In order to maintain a balanced energy supply, they store carbohydrate as liver glycogen and lipid as adipose tissue triglycerides (TG). Because lipid can be stored at much greater densities and is more highly reduced, the caloric value of lipid stores is about 100 times that of carbohydrate stores. However, with the exception of glycerol, which is released from triglycerides, the lipid stores are not able to contribute to net glucose production.

The central nervous system relies on blood glucose for energy and animals can only survive a few minutes of hypoglycemia. Therefore, numerous regulatory systems have evolved to prevent hypoglycemia. One of these is the ability to mobilize fatty acids and ketone bodies as alternative fuel sources to support muscle contraction and spare glucose for the brain.

When glucose levels drop below a particular threshold (~3.5 mM in humans), as occurs during fasting, glucagon is



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secreted by the α-cells of the endocrine pancreas. Glucagon stimulates adipose tissue to hydrolyze its TG and release free fatty acids and glycerol into the bloodstream while stimulating gluconeogenesis in the liver.

Although free fatty acids are an important source of energy for muscle, especially cardiac and slow-twitch skeletal muscle, a large proportion of free fatty acids are cleared from the circulation by the liver. Under fasting conditions, fatty acids undergo β-oxidation in the mitochondria of hepatocytes where they are converted to ketone bodies. Ketone bodies rapidly diffuse into the bloodstream and supply energy to muscle cells, helping to spare blood glucose.

Despite fasting and feeding cycles, very low density lipoprotein (VLDL) production from the liver occurs throughout the day because several free fatty acid sources serve as substrate for VLDL TG. These fatty acid sources include hydrolysis of adipose tissue lipid stores, hepatic *de novo* lipogenesis, and hydrolysis of plasma lipoproteins cleared by the liver. There is evidence that much of the lipid derived from these sources enters a cytosolic TG pool before their mobilization for VLDL assembly.[1–3] In humans, adipose-tissue-derived fatty acids are the largest source of TG for VLDL production.[4] Under fasting conditions, 77% of VLDL TG is derived from recycled adipose tissue fatty acids. With feeding, *de novo* liver lipogenesis still contributes only 8% of the VLDL TG; most of the VLDL TG comes from adipose tissue-derived free fatty acids (43%) and recycling of chylomicron TG cleared from the plasma (15%).[4]

Upon secretion, VLDL circulates and its TG core is a substrate for lipoprotein lipase, an enzyme that resides on the luminal surface of the capillary endothelium (Fig. 1). Hydrolysis of the TG core delivers free fatty acids to muscle and adipose tissue. The resulting TG-depleted particle, the VLDL remnant, also termed intermediate density lipoprotein (IDL), has two competing fates. It can go on to become cholesterol- and cholesterol ester-rich low density lipoprotein (LDL) or be rapidly cleared from the circulation by the liver. The clearance of VLDL remnants from the circulation is primarily dependent upon apoE, a ligand for the LDL receptor (LDLR) and virtually all other members of the LDL receptor family.

It appears that the major functions of hepatic VLDL secretion are to buffer plasma free fatty acid levels through their conversion to VLDL TG, thus providing a readily available alternative lipid fuel source (in the form of hydrolysable TG) in times of need. An important, but minor function is to mobilize the hepatic lipid synthesized from excess glucose after feeding. Through these functions, the ability of the liver to assemble and secrete VLDL particles critically determines steady-state liver and plasma TG levels. Metabolic disorders affecting numerous processes, described below, can result in excess plasma TG (hypertriglyceridemia) and/or excess liver TG (hepatic steatosis).

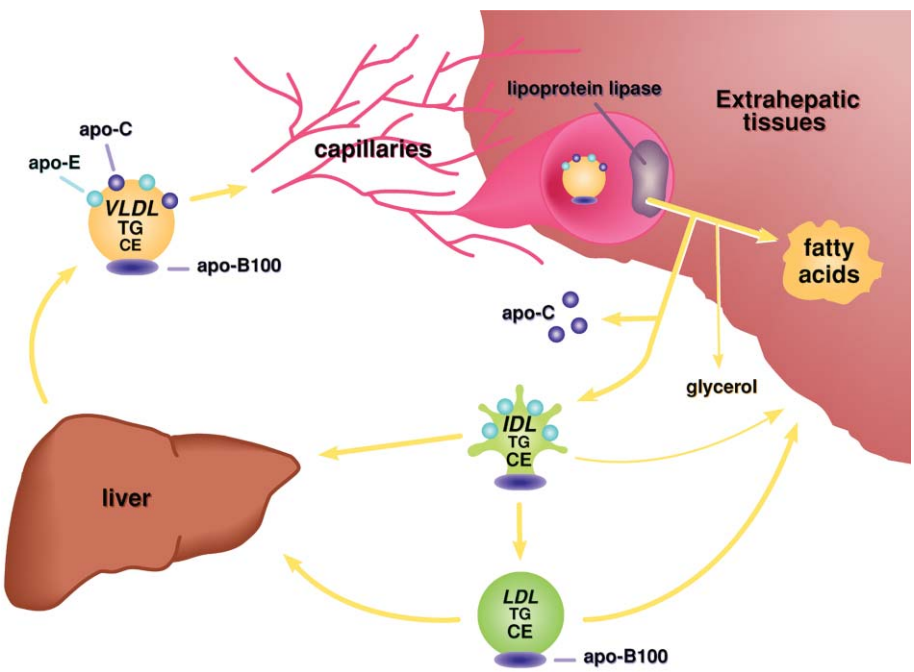

Fig. 1 The VLDL, IDL, LDL pathway. VLDL particles are assembled and secreted from the liver. Each particle carries one molecule of apoB as well as apoE and the C apolipoproteins, apoC1, C2, and C3. The particle carries amphipathic lipids (phospholipid and free cholesterol) on its surface and hydrophobic lipids (cholesterol ester and triglyceride) in its inner core. While in the circulation, the triglycerides are hydrolyzed by lipoprotein lipase, an enzyme residing on the luminal surface of the capillary endothelium in muscle and adipose tissue. This leads to the loss of the C-proteins and the formation of intermediate density lipoprotein (IDL), also known as VLDL remnants. These particles have two competing fates. They can be rapidly cleared by the liver or they can continue to be processed to become LDL. The clearance of IDL from the circulation depends upon the interaction of apoE with the LDL receptor and other members of the LDL receptor family. LDL is a more stable particle than IDL. In humans, about two-thirds of cholesterol is carried on LDL particles. LDL clearance is mediated by the interaction of apoB100 with the LDL receptor, primarily in the liver, but also in virtually all other tissues.

Apolipoprotein B structure

ApoB is a high molecular weight amphipathic protein that serves as the basic scaffolding upon which TG-rich lipoproteins, VLDL and chylomicrons, and cholesterol-rich LDL are assembled. Each lipoprotein particle contains just one apoB molecule.[5] Full-length apoB, apoB100, is synthesized in the liver as a 4536-amino acid polypeptide. Through an RNA editing event that converts the Gln^{2153} codon to a stop codon, a truncated form (apoB48) containing 48% of the protein from the N-terminus is produced from the same RNA.[6] In humans, the RNA editing event occurs in the intestine but not in the liver. Thus, human chylomicrons carry apoB48 whereas VLDL and LDL carry apoB100. In some rodents, the liver produces both forms of apoB.

ApoB100 is a ligand for the LDLR and mediates the binding and receptor mediated endocytosis of LDL. Since the receptor binding domain is C-terminal to Gln^{2153}, apoB48 is unable to bind to the LDLR.[7] Mutations in apoB100 that diminish receptor binding are a cause of hypercholesterolemia.[8] The receptor binding domain of apoB100 includes a cluster of positively-charged residues at amino acids 3359–3367.[9] The cluster resembles the well-characterized receptor binding domain of another LDLR ligand, apoE. Truncations deleting amino acids towards the C-terminus of apoB100 increase the affinity of LDL for the LDLR.[10,11] One model suggests that this segment of the molecule interacts with amino acids near the receptor binding domain and modulates its ability to bind to the LDLR.[12] Since mammalian intestine produces a truncated apoB (apoB48) lacking the LDLR binding domain, chylomicron particles depend upon apoE to bind to the LDLR (and to other members of the LDLR family) to mediate their clearance from the circulation.[13] An interesting evolutionary footnote is that avian species lack apoE and also do not edit apoB; *i.e.* their intestines produce apoB100.[14] Thus, the appearance, during evolution, of a form of apoB unable to bind to the LDLR coincided with the appearance of another LDLR ligand.

A model of apoB100 predicts five distinct secondary structural domains; an N-terminal globular domain, $\beta\alpha_1$, followed by four domains, β_1, α_2, β_2, α_3 (Fig. 2).[15,16] Electron microscopy studies suggest that it is wrapped around the lipoprotein particle with the $\beta\alpha_1$ domain extending away from the particle surface (Fig. 3).[17,18] The N-terminal 20% of apoB is homologous to lipovitellin, an avian egg yolk protein.[19] It contains an unusually large number of cysteine residues, all in disulfide linkages, of which several are essential for lipoprotein assembly.[20,21] Mutations that lead to the production of truncated forms of apoB of insufficient length for assembly of fully-lipidated lipoproteins lead to hypolipidemia.[22] The naturally-occurring truncations of apoB still able to form lipoprotein particles exist down to the smallest 28% of the molecule, suggesting that this is the minimal length required to produce a functional lipoprotein particle.[22] Truncation experiments in cell culture systems also suggest that this is the minimal length of apoB required for lipoprotein assembly.[23–25]

Unlike transmembrane proteins, apoB does not have any canonical amphipathic α-helical domains that are sufficiently long enough to span a membrane bilayer. Rather, hydrophobic β-sheet regions are found throughout the length of the protein. Thus, apoB exhibits the characteristics of an amphipathic protein having hydrophobic and hydrophilic segments capable of forming stable emulsions with lipids. Small angle neutron scattering analysis of solubilized apoB100 suggests a flexible and extended molecule with curvature and a cavity in the middle, consistent with its ability to wrap around a lipoprotein particle and stabilize neutral lipids in the particle core.[18,25–27]

Apolipoprotein B and microsomal triglyceride transfer protein are critical for VLDL assembly

The transcription of apoB is relatively constant. However, a large proportion of newly-synthesized apoB protein is subject to rapid co-translational degradation.[28,29] This co-translational degradation is the principal determinant of the amount of apoB that is ultimately secreted from cells.[28] Kinetic analysis from pulse-chase experiments indicates that the rate-determining step for apoB secretion is the exit from the rough endoplasmic reticulum (ER).[28] Translocation across the ER membrane appears to be slow enough to yield a significant

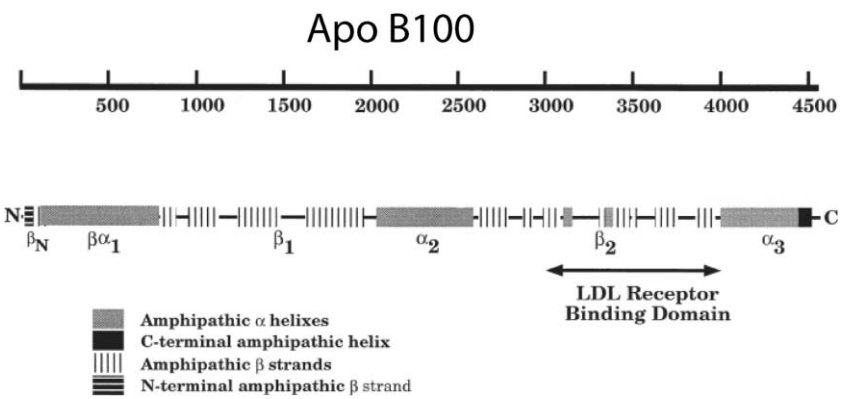

Fig. 2 Pentapartite model of the secondary structural domains of apoB. ApoB consists of five secondary structure domains (bottom line). The $\beta\alpha_1$, α_2, and α_3 domains consist primarily of amphipathic α helixes, and the β_1 and β_2 domains consist primarily of amphipathic β sheets. The $\beta\alpha1$ domain is thought to be a globular domain that serves as a nucleation point for lipid acquisition during lipoprotein assembly. The other four domains also bind lipid and wrap around the periphery of the mature lipoprotein particle. The LDLR binding site resides near the C-terminus in the β_2 domain. The top line shows the amino acid positions of the various domains. Adapted from Segrest *et al.*[15]

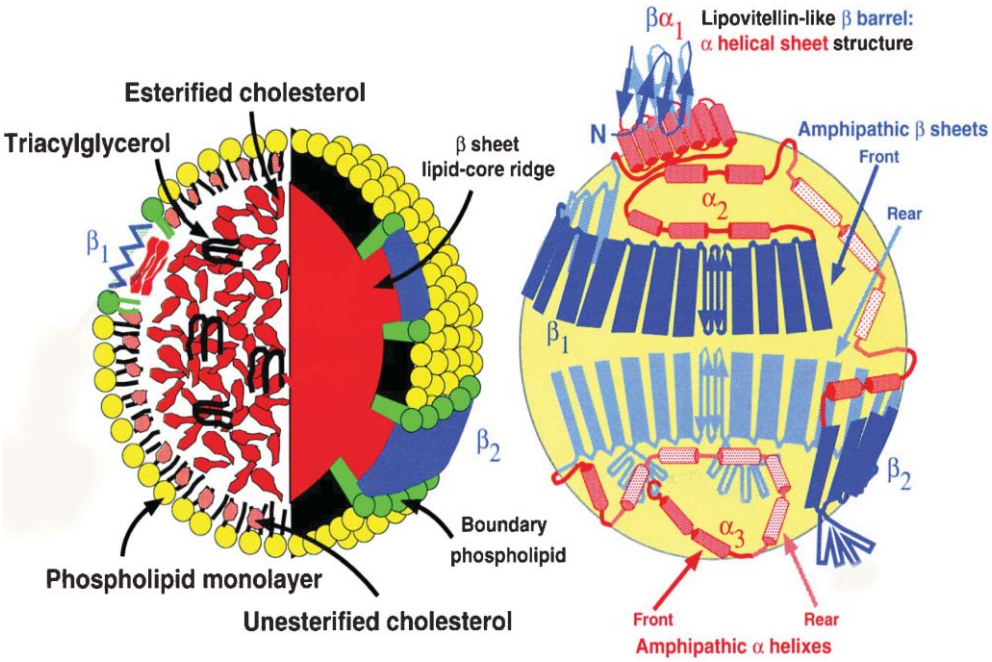

Fig. 3 Three-dimensional model of an LDL particle. Left figure shows the distribution of lipids; amphipathic phospholipids and cholesterol are at the surface while triglycerides and esterified cholesterol are in the interior of the particle. Red, core lipid including amphipathic β sheet-induced lipid-core ridges; green, boundary phospholipid; blue, amphipathic β sheets. The right-hand transparent sphere illustrates the proposed organization of apoB-100 on the LDL particle surface: blue, β structure; yellow, surface phospholipid; red, α-helical structure; darker blue and red, structures on the front of the sphere; lighter blue and red, structures on the back of the sphere. Figure and part of legend adapted from Segrest *et al.*[178]

steady-state pool of membrane-associated apoB.[30–34] Since apoB does not contain protein domains that would confer a transmembrane topology, this pool of apoB reflects transient intermediates in co-translational translocation across the ER membrane. This has been attributed to pause-transfer sequences[35] and to specific β-sheet domains[36] within the apoB polypeptide. This pool is subject to ubiquitination and proteasomal degradation,[37,38] which protects cells from the accumulation of unfolded protein in the ER and activation of the unfolded protein response.[39] The size of the pool subject to degradation is determined by the rate of apoB translocation across the ER membrane.[33]

A major determinant of apoB translocation is the microsomal triglyceride transfer protein (MTP), an ER lumenal protein with lipid transfer activity that exists as a heterodimer with protein disulfide isomerase.[40] Loss of function mutations in the human *Mttp* gene prevent the secretion of apoB-containing lipoproteins, a syndrome termed *abetalipoproteinemia*.[41] It is believed that the MTP protein is located at the site of apoB translocation and facilitates concerted transfer of lipids and folding of apoB as it exits the ribosome and enters the ER lumen.[19] Cells lacking MTP are unable to complete the translocation of full-length apoB100. They degrade apoB and also secrete an 85 kDa N-terminal fragment of the protein. This fragment is also detectable in the plasma of abetalipoproteinemia patients, suggesting a role for MTP in apoB translocation that might be distinct from its role in lipidation of the apolipoprotein.[42] Cells lacking MTP can still secrete triglyceride in HDL-like particles, but not to the extent that they would if they packaged triglycerides with full-length apoB to form VLDL.[43]

MTP-facilitated translocation, lipidation, and folding of apoB initially produces a nascent HDL-sized particle, which is subsequently modified to form a mature, secretion-competent VLDL in two proposed steps.[44,45] Pulse-chase experiments carried out in the McArdle RH-7777[46,47] or the HepG2[47] hepatoma cell lines show that there is a window of time when lipidation of the particle is sensitive to chemical inhibitors of MTP. If the initial lipidation of the particle is allowed to occur, then the large expansion of the lipid core in the second step is insensitive to the action of an MTP inhibitor. This suggests that MTP functions in the early phases of lipoprotein assembly and is consistent with the observation that MTP binds more avidly to truncated apoB polypeptides than to the full-length protein.[48]

The N-terminus of MTP is homologous to both lipovitellin and to apoB.[49] The region of homology in all three proteins is thought to constitute a "lipid pocket" and allow for sequential transfer of lipids from MTP to apoB.[19,50–53] It was initially proposed that association of MTP with the N-terminus of apoB provided structural components required to form a lipid pocket in apoB.[54] However, recent evidence suggests that motifs within apoB are capable of forming a lipid pocket without a structural requirement for MTP.[50,55] In a revised model,[50] salt bridges within the N-terminus of apoB create a "hairpin-bridge" and form one side of a pyramidal hydrophobic cavity during the initial stages of apoB lipidation. For completion of lipoprotein assembly, the lipid pocket would

open through the dissociation of the salt bridges and separation of lipid-binding β sheets, which would allow lipid to fill and expand the core of the nascent particle.

MTP activity is limiting in the ability of hepatic cells to produce lipoproteins.[56] Thus, changes in any of the three functions of MTP (*i.e.* apoB translocation, lipid transfer, and apoB folding) will affect the rate of VLDL secretion. For example, the discovery of a chemical that blocks the ability of MTP to associate with nascent apoB, has led to the discovery of an effective inhibitor of VLDL secretion.[57] Deleting one MTP allele in mice, reduces hepatic VLDL secretion,[58] whereas overexpression of MTP leads to increased VLDL secretion.[59]

Upon its initial discovery, MTP was considered an attractive drug target for reducing triglyceride levels in hypertriglyceridemic subjects.[60] Gene targeting studies showed that liver-specific deletion of the mouse *Mttp* gene greatly reduced VLDL secretion.[58,61] However, several of the MTP inhibitors that were developed caused the accumulation of TG in livers of experimental animals (hepatic steatosis), leading to the abandonment of MTP as a target.[39,62]

Many abetalipoproteinemic patients do not develop fatty liver and hepatic inflammation,[42] despite a lack of MTP activity. Analysis of a hepatoma cell line that recapitulates this phenotype reveals that transcription of the *Mttp* and the liver fatty acid binding protein (*Fabp1*) genes are coordinated, due to a common DR1 element in their promoters.[63] The coordinated expression is carried out by the peroxisome proliferator-activated receptor α (PPARα)-retinoid X receptor α (RXRα) complex through the DR1 element.[63] The liver fatty acid binding protein (L-FABP) binds to fatty acids and facilitates their uptake from the plasma.[64] Coordinated expression of *Fabp1* with *Mttp* results in delivery of fatty acids for triglyceride synthesis and their incorporation into a nascent VLDL particle,[63] thus explaining how PPARα agonists increase apoB secretion.[65] Blocking the uptake of fatty acids into liver *via* ablation of L-FABP decreases hepatic VLDL secretion.[64] Interestingly, silencing of the *Fabp1* gene also prevents the accumulation of TG caused by loss of *Mttp*.[63] Thus, the development of combined L-FABP and MTP inhibitors may make it possible to target MTP without causing hepatic steatosis.

In addition to the fatty acid uptake carried out by L-FABP and the lipid transfer catalyzed by MTP, hydrolysis of cellular TG constitutes an important step in the mobilization of lipid for VLDL assembly. Up to 70% of VLDL TG is hydrolyzed and re-esterified prior to its packaging in a VLDL particle.[66,67] Triacylglycerol hydrolase (TGH) is an enzyme with TG hydrolase activity[68] that is located in a region of the ER lumen in proximity to mitochondria.[69] This sub-region of the ER is enriched in enzymes, lipids, and apolipoproteins necessary for the assembly of lipoproteins.[70] Stable expression of TGH cDNA in McArdle hepatoma cells depletes intracellular TG stores and increases the secretion of TG and apoB.[71] Conversely, inhibition of TGH in primary hepatocytes decreases secretion of TG and apoB.[72] HepG2 cells are known to be deficient in lipoprotein secretion due to a defect in the mobilization of intracellular lipid stores and thus require an exogenous source of fatty acids for efficient lipoprotein secretion.[73,74] This may be explained by the fact that, unlike primary hepatocytes, they do not express TGH.[75] Despite its ability to promote cytosolic lipid mobilization, the nature of the substrate pool of TGH (*i.e.* luminal *vs.* cytosolic TG) is uncertain due to the lumenal localization of TGH.

Regulation of VLDL secretion by lipid supply

Both *de novo* and extrahepatic sources of fatty acid serve as substrate for TG synthesis in the liver, and, as described above, the relative contribution of each source is highly dependent on nutritional state. *De novo* lipogenesis occurs primarily in the fed state and is controlled by several transcription factors. In general, the expression of lipogenic genes is globally regulated by the sterol regulatory element binding protein (SREBP). Specifically, the SREBP-1c isoform upregulates virtually all enzymes in fatty acid synthesis as well as enzymes that supply acetyl-CoA units and reducing equivalents to the pathway.[76] SREBP-1c is induced by insulin, accounting for the lipogenic effect of chronic hyperinsulinemia.[76,77] The liver X receptor-α (LXRα), a nuclear receptor for oxysterols, regulates lipogenesis through the induction of SREBP-1c expression.[78] A recently discovered transcription factor, the carbohydrate response element binding protein (ChREBP), also upregulates lipogenic gene expression.[79] ChREBP is activated through the formation xylulose-5-phosphate in the pentose shunt following glucose uptake[79] and is also a target gene of LXR.[80]

An increase in liver TG, whether derived from exogenous fatty acids or from *de novo* lipogenesis, can lead to an accumulation of TG as cytoplasmic droplets within the cells or an increase in TG secretion as VLDL. The increased TG secretion can occur through greater loading on individual VLDL particles; *i.e.* larger particle size without an increase in particle number. It can also occur through an increase in VLDL particle number, indicated by an increase in apoB secretion. These outcomes are not consistent among various experimental systems and are likely regulated by a variety of factors.

There is much controversy as to whether increasing TG in the liver, regardless of its source, directly increases TG secretion. Stimulation of lipogenesis through LXR activation,[81] exposure of primary hepatocytes to oleic acid,[82–84] or long-term overexpression of diglyceride acyltransferase 1 (DGAT1)[85] in mice stimulates TG secretion through the production of larger VLDL particles, but does not increase apoB secretion. However, inhibition of β-oxidation,[86] short-term overexpression of both DGAT isoforms (1 and 2),[87] or overexpression SREBP-1a[88] in mice leads to increased liver TG content with no increase in TG secretion. In certain hepatoma cell lines, addition of free fatty acids[89] or overexpression of DGAT1[90] increases apoB secretion at the expense of the post-translational degradation of newly-synthesized apoB. Delivery of high concentrations of fatty acids to mice also increases apoB secretion,[91] and stimulation of lipogenesis through long-term carbohydrate feeding increases apoB production from freshly isolated hepatocytes.[92] Since the ability hepatic TG to stimulate VLDL apoB secretion varies depending on the experimental model, it is likely to involve multiple and perhaps indirect processes.

Fatty acids are ligands for several nuclear receptors that control lipid metabolism and may therefore increase VLDL assembly and secretion through transcriptional activation. Fatty acids bind and activate PPARα,[93] a key regulator of *Mttp*[94] and *Fabp1*.[63] *Mttp* is also a target of hepatocyte nuclear factor-4α (HNF-4α),[95,96] a receptor for fatty acid-derived acyl-CoA thioesters.[97] In addition, oleic acid induces the expression of an MTP reporter in HepG2 cells in a sterol regulatory element-dependent manner,[98] but the transcription factor responsible for this activity is unknown. To complement the upregulation of lipoprotein assembly machinery, fatty acids can also stimulate lipogenesis by upregulating SREBP-1c[99] and LXRα[100] expression and activity.

A recently discovered transcriptional co-activator, peroxisome proliferator activator receptor γ co-activator 1β (PGC-1β), may be the link that orchestrates the various effects that fatty acids have on lipoprotein secretion (Fig. 4). PGC-1β co-activates several transcription factors sensitive to fatty acids, including PPARα, HNF-4α, SREBP-1c, and LXRα, to mediate their transcriptional programs.[101] In addition, saturated fat feeding upregulates the expression of PGC-1β.[99,102] The forced *in vivo* expression of PGC-1β leads to hypertriglyceridemia,[102,103] a consequence of increased apoB secretion.[103] This has been attributed to an induction in lipogenic gene expression through the co-activation of SREBP-1c and LXRα by PGC-1β.[102] Several studies also suggest that PGC-1β induces apoB-dependent VLDL secretion by participating in the transcriptional activation of *Mttp* and other genes implicated in lipoprotein assembly.[63,102,103] One study showed that PGC-1β's co-activation of Foxa2, a transcription factor that regulates lipid and glucose metabolism, increases expression of both *Mttp* and *Dgat*.[103] Co-expression of Foxa2 enhances the stimulating effect of PGC-1β on apoB secretion and hypertriglyceridemia.[103] Other recent findings show that PGC-1β increases VLDL secretion in rat hepatoma cells *via* a PPARα-RXRα-dependent transcriptional activation of *Mttp* and *Fabp1*.[63] Thus, PGC-1β appears to stimulate VLDL particle secretion by co-activating several transcription factors responsible for the expression of *Mttp* (Fig. 4). In addition, PGC-1β can load more lipid onto VLDL particles and increase VLDL TG secretion by co-activating transcription factors for the lipogenesis program.

Insulin resistance and diabetes

The most common lipoprotein disorder in humans is hypertriglyceridemia. In most cases, this involves an increased concentration of VLDL TG, either because each particle carries more TG cargo or because there is an increase in VLDL particle number. Elevated plasma TG is commonly associated with obesity and insulin resistance. Insulin resistance involves an attenuated response to insulin at insulin's target tissues, principally liver, adipose tissue, and muscle. In the liver, insulin normally suppresses glucose output by inhibiting glycogen breakdown and gluconeogenesis. In adipose tissue and muscle, insulin promotes glucose uptake. In addition, insulin inhibits lipolysis of TG in adipose tissue. In liver and adipose tissue, insulin promotes lipogenesis, in large part by

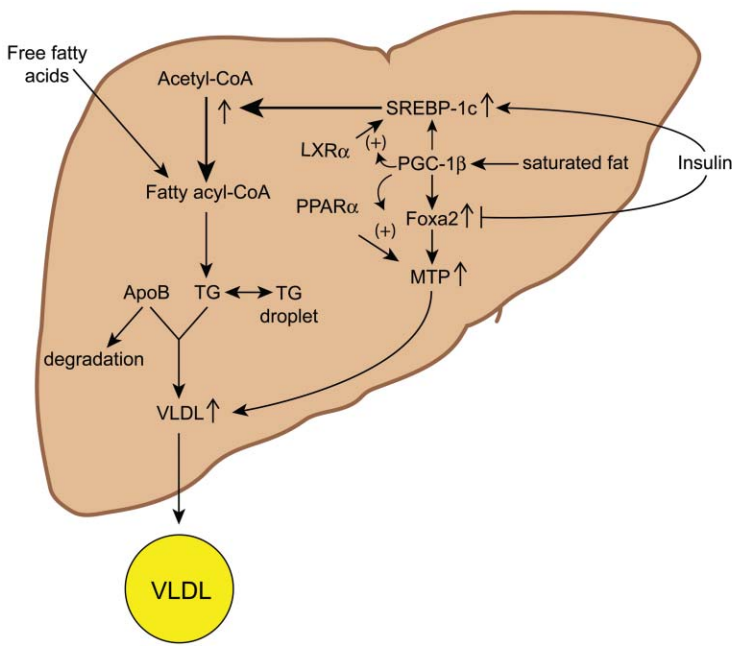

Fig. 4 Effect of fatty acids on hepatic VLDL assembly and secretion. Fatty acids, produced *de novo* or derived from extrahepatic sources have both direct and indirect effects on VLDL production. Direct effects include the ability of fatty acids to increase the availability of glycerolipids for VLDL assembly. The availability within the hepatocyte of TG relative to CE determines the neutral lipid core composition and particle size. Indirect effects of fatty acids occur in response to downstream signal transduction. Fatty acids and their CoA derivatives are ligand activators of several nuclear receptors responsible for controlling the gene expression of lipogenic enzymes. They also indirectly affect the expression of *SREBP*. Fatty acids induce the expression of *Mttp via* PPARα activation and, by affecting the expression of PGC-1β, *via* the co-activation of Foxa2. This may help explain how fatty acids increase the secretion of both TG and apoB in some model systems.

increasing the expression of SREBP-1c. Insulin resistant individuals can remain non-diabetic by compensating for insulin resistance with increased insulin production. Thus, insulin resistant people are almost invariably hyperinsulinemic.

Despite insulin's induction of lipogenesis in the liver, it acutely inhibits hepatic VLDL production.[104–107] This effect has been attributed to the reduction in availability of free fatty acids from adipocyte lipolysis.[104] However, studies in cultured cells suggest that insulin directly inhibits apoB secretion, independent of exogenous fatty acid supply.[105,106] The exact mechanism by which insulin exerts this acute, direct regulation has been elusive. The effect is partially dependent on the activity of phosphatidylinositol 3-kinase (PI3K)[108–111] but not on one of its downstream targets, Akt1.[112] It is also dependent on the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway.[113] The importance of the MAPK/ERK pathway in regulating VLDL secretion was illustrated recently in HepG2 cells.[114] HepG2 cells have constitutively overactive MAPK/ERK signaling and secrete LDL-sized particles instead of the larger VLDL-sized particles. Inhibition of the MAPK/ERK pathway in these cells induces the secretion of VLDL-sized particles.

Insulin inhibits the transcription of *Mttp*.[115] This occurs at least partially through the MAPK/ERK signaling pathway.[116] Insulin also inhibits the ability of Foxa2 to upregulate *Mttp* (Fig. 4) and reverses the Foxa2-dependent increase in apoB secretion.[103] Interestingly, the grapefruit flavonoid, naringenin, like insulin, reduces *Mttp* expression and apoB secretion by signaling through both the MAPK/ERK and PI3K pathways, but it does so in an IRS-1/2 independent manner.[110,113] Despite the multiple insulin-signaling pathways converging on the repression of *Mttp* expression, this mechanism is unlikely to account for the acute effect of insulin on VLDL production due to the long half-life of the MTP protein (4.4 days).[115] Thus, the acute effect of insulin on VLDL secretion remains elusive.

In contrast to the effects of acute insulin doses, chronic hyperinsulinemia caused by insulin resistance is associated with increased VLDL TG and apoB secretion.[117,118] This results from a reduction in the post-translational degradation of apoB. [117,118] MTP is increased in insulin resistance,[117,119] which may contribute to the rescue of apoB from degradation. Interestingly, insulin resistance also leads to a loss of the acute insulin-mediated inhibition of apoB secretion.[118,120] There is evidence that this effect is a result of an attenuation of signaling through the PI3K pathway; administration of the PI3K inhibitor, wortmannin, increases VLDL apoB secretion to levels observed in mice with induced insulin resistance.[111]

Insulin resistance is selective for some of insulin's actions, which underlies the basis for the metabolic characteristics associated with hyperinsulinemia. For example, in animal models of insulin resistance, insulin fails to suppress hepatic glucose and apoB production, but still promotes lipogenesis.[117,118,121] The loss of insulin-dependent regulation of gluconeogenesis may result from insulin's ability to inhibit expression of the insulin receptor substrate 2 gene (*Irs2*).[122] However, suppression of this gene in insulin resistance does not interfere with the insulin-mediated increase in SREBP-1c expression.[118,121] Further evidence for the independence of the pathways is that the suppression of glucose output is more sensitive to insulin than is the suppression of VLDL TG output.[123] In addition to the increase in lipogenesis in insulin resistance, an increase in TG lipolysis in adipose tissue and free fatty acid levels provides yet another source of lipid to the liver. The increase in *Mttp* expression in insulin resistance may not be enough to compensate for the increase in liver lipid load, which leads to a build-up of TG in the liver. All of these factors combine to promote hyperglycemia, hyperlipidemia, and hepatic steatosis in insulin resistant states.

The role of the LDL receptor in apoB secretion

Patients with mutations in the LDLR, in addition to having defective LDL clearance, overproduce VLDL.[124–126] Several lines of evidence point to a direct effect of the LDLR on apoB secretion. First, antibodies against the LDLR increase the net secretion of VLDL from cultured HepG2 cells.[127] Second, hepatocytes from mice lacking a functional LDLR secrete more apoB100 than do wild type hepatocytes.[128,129] Third, adenovirus-mediated overexpression of the LDLR greatly reduces apoB100 secretion.[128] Fourth, in wild type mice overexpressing SREBP-1a, there is a large increase in lipogenesis and in hepatic TG content, but essentially no increase in plasma TG.[88] However, in *Ldlr*$^{-/-}$ mice overexpressing SREBP-1a, instead of an increase in liver TG, there is a dramatic increase in plasma TG due to increased lipoprotein production.[88] Fifth, liver-specific MTP-null mice display a severe deficiency in hepatic apoB secretion due to the loss of the MTP-dependent lipid transfer activity.[130,131] Deletion of the LDLR in these mice partially restores secretion of apoB as LDL-sized lipoproteins.[132] However, the LDLR is not wholly responsible for the decrease in apoB secretion with loss of MTP activity, as MTP inhibitors still lower VLDL secretion in LDLR-null mice.[39]

VLDL production has been estimated *in vivo* in mice using inhibitors of lipoprotein lipase to prevent the clearance of VLDL; the rate of VLDL production is estimated as the increment in VLDL TG or apoB after administration of the inhibitor. With this method, one group failed to detect an increase in VLDL apoB production in *Ldlr*$^{-/-}$ mice,[133] whereas another group did detect an increase.[129] Yet, the former group observed an increase in apoB production in human subjects with defective LDLR.[125] One possible reason for the varying results with the lipase inhibition experiments is that these agents actually stimulate apoB secretion in an LDLR-dependent fashion (Attie & Horton laboratories, unpublished observations).

How does the LDLR modulate apoB secretion? Kinetic modeling suggests that the LDLR promotes the post-translational degradation of apoB through reuptake of newly-secreted lipoproteins at the cell surface and also through a mechanism affecting the intracellular presecretory pool of apoB.[128] These results were corroborated in a study using PLTP-deficient hepatocytes. Loss of PLTP confers a decrease in apoB secretion, an effect not observed in PLTP/LDLR-null hepatocytes.[134] Importantly, heparin, which acts on the cell surface to release apoB from the LDLR, fails to rescue the PLTP-dependent loss in apoB secretion, indicating an intracellular role of the LDLR in this model system.

The studies in primary hepatocytes indicate that the LDLR interacts with apoB within the secretory pathway, targeting apoB for degradation. This predicts that a mutant form of the LDLR retained in the secretory pathway would maintain the ability to decrease apoB secretion. This prediction is supported by studies of a naturally-occurring mutation in the WHHL rabbit, a model of familial hypercholesterolemia that carries a mutation causing the LDLR to stall in the ER.[135] VLDL secretion measured in perfused liver or in isolated hepatocytes from WHHL rabbits is not increased relative to that in wild type rabbit liver.[136,137] The intracellular role of the LDLR was specifically tested in mouse hepatocytes using two LDLR constructs that are retained in the ER, a naturally-occurring misfolding mutant and a soluble form of the receptor with the KDEL ER retention sequence appended to its C-terminus.[138] When introduced into *Ldlr*$^{-/-}$ primary hepatocytes, both ER-retained mutant forms of the LDLR reduce apoB100 secretion to the same extent as do the wild-type receptor. Furthermore, an ER-retained LDLR containing a mutation that abolishes apoB binding is unable to reduce apoB secretion, suggesting that binding of the LDLR to apoB mediates the effect.

The VLDL particles that are secreted in patients lacking functional LDLR or in mice lacking the LDLR are relatively small.[125,129,132,139,140] This implies that the LDLR preferentially targets apoB that is poorly lipidated for degradation. Perhaps the LDLR binds to apoB during VLDL assembly only until it has acquired a threshold level of neutral lipid, thus insuring the secretion of more fully-lipidated VLDL particles and the degradation of apoB that is not sufficiently lipidated.

The role of apoE in VLDL secretion

Hepatic expression of apoE positively correlates with VLDL TG secretion. Deletion of apoE in mice reduces VLDL TG secretion.[141] Conversely, expression of the predominant human apoE isoform, apoE3, either in replacement or in addition to endogenous apoE, increases secretion of VLDL TG in several *in vivo* models.[142–145] The increase in secretion correlates with the level of apoE3 expression.[143,144] Hepatic expression of apoE is required, as transplantation of bone marrow from WT mice into apoE-null mice is insufficient to restore the VLDL TG secretion defect, despite correcting for apoE-related defects in lipoprotein clearance.[141]

Expression of a rare apoE3 variant with reduced receptor-binding affinity, apoE3-Leiden, fails to restore the defect in VLDL TG secretion in apoE-null mice.[146] However, apoE2, another isoform with reduced LDLR affinity,[147] promotes VLDL TG secretion to the same extent as the other apoE isoforms.[142,145] In addition, loss of apoE still reduces VLDL secretion in mice lacking the LDLR, thus ruling out the LDLR as a mediator of the effect of apoE.[148]

The apoE-dependent changes on TG secretion result from a modulation of the number of VLDL particles secreted, as apoB production correlates with TG secretion in the apoE expression experiments.[144,146,149] How apoE promotes VLDL apoB and TG secretion is unclear but may involve a lipoprotein assembly step early in the hepatic secretory pathway; in apoE-null hepatocytes, an accumulation of lipid droplets were observed by electron microscopy in small membrane-bound vesicles thought to be ER-derived.[150] Other mechanistic studies indicate that the carboxyl-terminal 203-299 residues of apoE are required for its function in promoting VLDL TG secretion.[151]

Regulation of ApoB secretion by bile acids

Bile acids are detergents that facilitate dietary lipid absorption in the intestine. A substantial proportion of these lipids eventually reach the liver on chylomicron remnants where they can be re-secreted on VLDL particles. Recently, it has become apparent that bile acids are ligand activators of nuclear receptors responsible for regulating the transcription of genes whose products control several aspects of lipid metabolism (reviewed in Lee *et al.*).[152]

An early indication that bile acids are directly involved in regulating lipoprotein production was the observation that the rate of bile acid synthesis correlates with several forms of hyperlipidemia.[153] Agents that block the absorption of bile acids within the intestinal tract (*e.g.* cholestyramine) enhance hepatic VLDL secretion.[153] These agents have markedly different effects on plasma lipid levels in different patients. In patients with high LDL cholesterol, cholestyramine treatment decreases the hypercholesterolemia, presumably by reducing hepatic cholesterol levels and upregulating the expression of the LDLR.[154,155] In hypertriglyceridemic patients having abnormally high rates of VLDL production, cholestyramine increases plasma TG levels by further increasing VLDL production.[156]

Bile acids are the major ligands responsible for activating the nuclear receptor farnesoid X receptor (FXR).[157] Several lines of research have demonstrated a negative regulation of VLDL TG secretion by bile acids through activation of FXR. FXR induces the expression of the short heterodimer partner (SHP), a protein that dimerizes with LXRα and with liver receptor homolog 1 (LRH1), making them unable to activate target genes, including SREBP-1c (Fig. 5).[158] Through this mechanism, bile acids reduce lipogenesis, TG secretion,[158] and plasma TG levels[158,159] in animal models of hypertriglyceridemia. Conversely, a reduction in hepatic bile acid levels in mice through loss of *Cyp27*, a gene involved in the acidic pathway of bile acid biosynthesis, increases plasma TG *via* an elevation in SREBP activity.[160,161] Interestingly, bile acid feeding normalizes hepatic fatty acid synthesis and plasma TG levels in these mice. In addition to interfering with lipogenesis, bile acids also antagonize the HNF4α-mediated expression of *Mttp*. This effect is partially attributable to the induction of SHP and inhibition of the transcriptional activity of HNF-4α.[95]

FXR target genes also control plasma VLDL triglyceride clearance and utilization. FXR induces the expression of apoCII,[162] an activator of lipoprotein lipase. FXR also increases the expression of the VLDL receptor.[152] As one might predict from the effects of FXR on VLDL production and turnover, targeted deletion of the *Fxr* gene causes hypertriglyceridemia.[163]

In addition to their effects through FXR, bile acids exert FXR-independent effects on lipid metabolism. They do so by controlling the expression of cholesterol 7α hydroxylase (Cyp7A1), the rate limiting enzyme in the production of bile

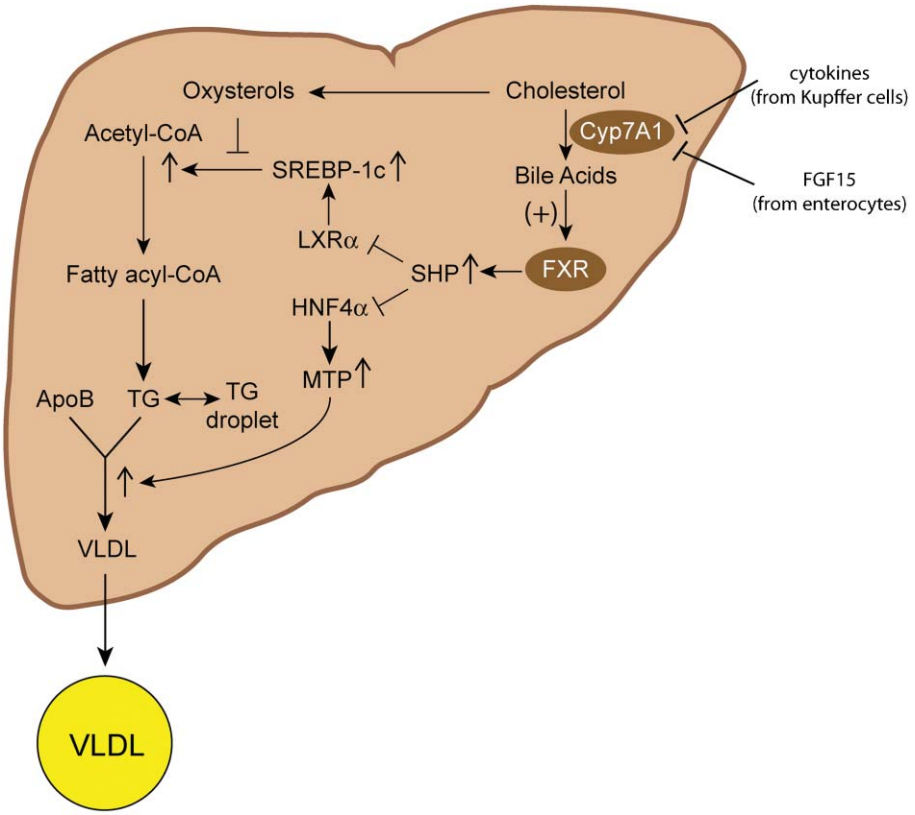

Fig. 5 Relationship between bile acid metabolism and VLDL secretion. Bile acids affect VLDL secretion by FXR-dependent and FXR-independent pathways. FXR-dependent pathways include their ability to repress SREBP-mediated lipogenesis and *Mttp* expression by induction of SHP. FXR-independent pathways include the repression of *Cyp7A1* and, hence, bile acid synthesis through the induction of inflammatory cytokine secretion by Kupffer cells and FGF15 secretion by enterocytes. The decrease in bile acid synthesis increases cellular sterol levels, which inhibit SREBP activation and lipogenesis.

acids from cholesterol. Since sterols inhibit activation of SREBP-1c and SREBP-2, the upregulation of Cyp7A1 results in the de-repression of SREBP activation. Consequently, activation of bile acid synthesis through overexpression of *Cyp7A1* results in increased SREBP-mediated lipogenesis and increased secretion of VLDL.[164] The induction of *Cyp7A1*[165] is likely the mechanism by which bile acid binding resins stimulate VLDL production. Bile acids exert negative feedback regulation on their own production. In one mechanism, bile acids stimulate inflammatory cytokine secretion from Kupffer cells, hepatic resident macrophages.[166] These cytokines activate a signaling pathway in hepatocytes leading to the repression of *Cyp7A1* and bile acid synthesis (Fig. 5). Inflammatory cytokines also block *Mttp* expression.[167] Another signaling molecule able to repress *Cyp7A1* is fibroblast growth factor 15 (FGF15), which is produced in the enterocytes of the intestine in response to bile acids (Fig. 5).[168] Thus, through the repression of *Mttp* and *Cyp7A1*, bile acids can modulate VLDL secretion through FXR-independent mechanisms.

Independent of transcriptional mechanisms, bile acids may directly inhibit VLDL secretion by disrupting lipoprotein assembly. Exposure of primary human and rat hepatocytes to physiological concentrations of bile acids (10 μM–200 μM) inhibits the secretion of VLDL[169–171] and apoB.[170,171] The effect of bile acids on VLDL secretion occurs within 15–30 min of bile acid exposure,[169] and is accompanied by either no change[171] or an increase [169,170] in intracellular TG levels. These data suggest that through this direct mechanism, bile acids can also disrupt the lipoprotein assembly process in addition to affecting cellular lipid availability. Consistent with such a mechanism, taurocholate stimulates the degradation of lipidated apoB100 as well as an N-terminal non-lipidated but secreted fragment.[172]

Polyunsaturated fatty acids

Apart from the regulation of *Mttp* and lipogenesis, some fatty acids may affect the fate of apoB.[173] Fatty acids with an n-3 double bond are associated with reduced rates of apoB secretion.[174,175] Because antioxidants reverse this effect, it has been proposed that lipid peroxidation induced by polyunsaturated fats either directly leads to oxidative damage of apoB or indirectly leads to stimulation of its post-translational degradation.[176] This proposal is consistent with the observation that in mice deficient in the superoxide dismutase 1 and 2 genes, where there is an increase in oxidative stress to the liver, there is a dramatic defect in VLDL secretion.[177]

Remaining questions

While significant progress has been achieved in regard to understanding the mechanisms responsible for the assembly

and secretion of hepatic VLDL, there is still no non-toxic and efficacious therapeutic regimen capable of reducing hepatic VLDL secretion without causing fatty liver and enhancing the development of hepatitis. Although MTP inhibitors are effective in reducing plasma levels of apoB, cholesterol and triglyceride, their use is associated with hepatosteatosis.[63] Thus, inhibitors to one of the choice targets for ameliorating hyperlipidemia (especially hypertriglyceridemia) has remained elusive. Gaining insights regarding how lipid can be diverted from hepatic VLDL production without causing its retention in the liver may provide an effective therapeutic intervention for ameliorating both hyperlipidemia and obesity.

The goal of this review is to provide a concise update on the processes controlling hepatic production of apoB-containing lipoproteins. It reflects our selection of current topics. This necessitated the omission of a vast literature reflecting the outstanding contributions of many investigators to whom we apologize for our inability to cover the entire field.

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