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Hepatic ABCA1 and VLDL triglyceride production

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Abstract

Elevated plasma triglyceride (TG) and reduced high density lipoprotein (HDL) concentrations are prominent features of metabolic syndrome (MS) and type 2 diabetes (T2D). Individuals with Tangier disease also have elevated plasma TG concentrations and a near absence of HDL, resulting from mutations in ATP binding cassette transporter A1 (ABCA1), which facilitates the efflux of cellular phospholipid and free cholesterol to assemble with apolipoprotein A-I (apoA-I), forming nascent HDL particles. In this review, we summarize studies focused on the regulation of hepatic very low density lipoprotein (VLDL) TG production, with particular attention on recent evidence connecting hepatic ABCA1 expression to VLDL, LDL, and HDL metabolism. Silencing ABCA1 in McArdle rat hepatoma cells results in diminished assembly of large (>10nm) nascent HDL particles, diminished PI3 kinase activation, and increased secretion of large, TG-enriched VLDL1 particles. Hepatocyte-specific ABCA1 knockout (HSKO) mice have a similar plasma lipid phenotype as Tangier disease subjects, with a twofold elevation of plasma VLDL TG, 50% lower LDL, and 80% reduction in HDL concentrations. This lipid phenotype arises from increased hepatic secretion of VLDL1 particles, increased hepatic uptake of plasma LDL by the LDL receptor, elimination of nascent HDL particle assembly by the liver, and hypercatabolism of apoA-I by the kidney. These studies highlight a novel role for hepatic ABCA1 in the metabolism of all three major classes of plasma lipoproteins and provide a metabolic link between elevated TG and reduced HDL levels that are a common feature of Tangier disease, MS, and T2D.

Keywords

VLDL overproduction; Tangier disease; liver; metabolic syndrome; type 2 diabetes; nascent HDL; PI3 kinase; triglyceride secretion; VLDL1

1. Introduction

Metabolic syndrome (MS) and type 2 diabetes (T2D) are epidemic in Westernized societies [1]. A major feature of these disorders is dyslipidemia, characterized by elevated very low density lipoprotein (VLDL) triglyceride (TG) levels, low high density lipoprotein (HDL) concentrations, and smaller, denser low density lipoprotein (LDL) and HDL particles [2,3].

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This constellation of plasma lipid and lipoprotein changes is, in part, responsible for the increased risk of cardiovascular disease associated with MS and T2D [2]. Elevated plasma VLDL TG levels are due, in large part, to an increase in hepatic overproduction of large, TG-enriched VLDL particles, referred to as VLDL1, with little change in normal-sized VLDL2 particles [4,5].

Recent studies suggest that hepatic ATP binding cassette transporter A1 (ABCA1) expression may partially account for the inverse association between plasma TG and HDL concentrations [6,7]. ABCA1 is a membrane transporter that facilitates efflux of cellular free cholesterol (FC) and phospholipid (PL) to apolipoprotein A-I (apoA-I), resulting in formation of nascent HDL particles [8]. Genetic deficiency of ABCA1 leads to Tangier disease [9], in which subjects have <5% normal plasma HDL concentrations and elevated TG levels, a phenotype similar to that of individuals with MS and T2D. This review summarizes literature on the regulation of hepatic VLDL production, highlighting recent evidence indicating an important role of hepatic ABCA1 in VLDL, LDL, and HDL metabolism.

2. Hepatic VLDL assembly

VLDLs are large lipoproteins (30–80 nm diameter) that contain a core of TG and CE, surrounded by a surface monolayer of PL, FC and apolipoproteins [10]. Each VLDL particle has only one apoB molecule and several other minor apolipoproteins [11]. Human liver secretes apoB100-containing VLDL particles, whereas the intestine secretes apoB48-containing chylomicrons and VLDL [12]. ApoB48 is identical to the N-terminal 48% of the full-length apoB100 and is formed in the intestine by editing of apoB100 mRNA [13,14]. In contrast, mouse liver secretes both apoB100 and apoB48-containing VLDL particles [15]. VLDL particles vary in size and composition and can be separated into two main classes: large, buoyant VLDL1 particles ($S_f > 100$), which contain more TG, and smaller, more dense VLDL2 particles ($S_f 20-100$) [16,17]. VLDL assembly consists of two distinct steps [18,19]. In the first step, apoB100 is co-translationally lipidated in the endoplasmic reticulum (ER) with the help of microsomal TG transfer protein (MTP), forming small pre-VLDL particles. In the second step, pre-VLDL particles acquire bulk lipids [20], forming mature TG-enriched VLDL particles for secretion. Some evidence supports the second step occurring in the ER [20–22], whereas other studies suggest that pre-VLDL undergo further lipidation in post-ER compartments [23–31]. Both steps require MTP for successful assembly of VLDL particles [32].

ApoB secretion is predominantly regulated via co- and post-translational turnover pathways [33]. ER-associated degradation targets misfolded or poorly lipidated apoB in the ER for proteasomal degradation, an early step apoB assembly quality control. VLDL secretion can also be controlled by interaction of apoB100 with the LDL receptor, resulting in presecretory post-ER turnover as well as endocytic reuptake of underlipidated apoB-containing lipoproteins [34,35]. A third pathway for apoB degradation is a post-ER pre-secretory proteolysis pathway that is mediated by autophagosomes [36]. This pathway can be induced by reactive oxygen species that trigger oxidant-dependent aggregation of apoB and autophagy-mediated degradation, and by ER stress through activation of protein kinase R-like ER kinase (PERK) [37]. Although the extent to which these different pathways regulate apoB secretion varies between primary hepatocytes and hepatoma cell lines [37], the role of these pathways in regulating apoB secretion in vivo is poorly understood.

3. Regulation of VLDL TG secretion is multifactorial

In the insulin-resistant state, TG-enriched VLDL1 is overproduced by the liver [5,38,39]. Factors that affect VLDL1 production are complex and involve lipid substrate availability, co-factors that function to add lipid to the maturing pre-VLDL particle, and secretory vesicle trafficking.

3.1. Lipid availability regulates VLDL TG secretion

VLDL TG overproduction is positively correlated with hepatic fat content, providing evidence that lipid substrate abundance, particularly TG, is a predominant driver of VLDL1 assembly and secretion [4,5,40,41]. A major source of hepatic fatty acids during insulin resistance stems from dysregulated TG lipolysis in adipose tissue, resulting in increased delivery of fatty acids to the liver [3]. Although fatty acid substrate drives VLDL production, not all fatty acids are equally effective. For instance, the monounsaturated fatty acids stimulate TG synthesis and secretion of VLDL, whereas polyunsaturated fatty acids have the opposite effect [42]. Fatty acids not only provide substrate for TG synthesis, but are also ligands for transcription factors that regulate fatty acid and TG synthesis. Sterol regulatory element binding protein 1 (SREBP1) is a master regulator of fatty acid and TG biosynthesis [43], and polyunsaturated fatty acids, particularly n-3 species, markedly downregulate SREBP1 expression, resulting in reduced VLDL secretion and plasma TG concentrations [44]. Thus, consumption of diets enriched in n-3 polyunsaturated fatty acids decreases plasma TG concentrations and reduces VLDL size primarily through down regulation of hepatic VLDL TG production, and is one of the few effective treatments for elevated plasma TG concentrations [45,46].

PL is a major component of the VLDL particle surface that helps emulsify the core TG and CE. Synthesis of phosphatidylcholine (PC) is required for normal VLDL particle maturation and secretion [47]. Two pathways are involved in hepatic PC synthesis, the Kennedy CTP:phosphocholine cytidyltransferase pathway and the phosphatidylethanolamine N-methyltransferase (PEMT) pathway [48]. The former pathway synthesizes PC from cytidine-diphosphocholine and diacylglycerol (DAG), and is the major pathway for PC synthesis in the liver. The latter pathway uses sequential methylations of phosphatidylethanolamine (PE) to form PC. Loss of either enzyme results in decreased hepatic VLDL secretion, emphasizing the importance of hepatic PC synthesis in providing surface lipid for VLDL particle formation [48]. PC may also serve as a TG precursor, since approximately half of plasma LDL PC delivered to liver is converted to TG through the actions of PC phospholipase C and acyl CoA:diacylglycerol acyltransferase 2 (DGAT2). Hence, plasma PC can serve as substrate for hepatic TG synthesis and perhaps VLDL TG secretion [49]. DAG is a common substrate for PC and TG biosynthesis. Phosphatidate phosphatase-1 (PAP-1) converts phosphatidate to DAG and plays a key role in the biosynthesis of PL and TG. Loss of PAP-1 in McArdle 7777 hepatoma cells (McA cells) decreases VLDL TG and apoB secretion, whereas overexpression of PAP-1 increases VLDL secretion [50].

3.2. Protein factors that affect VLDL production

MTP is an obligatory protein for VLDL secretion. Chemical inhibition or genetic deficiency of MTP inhibits hepatic VLDL and intestinal chylomicron secretion, resulting in abetalipoproteinemia [51–53]. MTP facilitates the co-translational addition of TG and PL into nascent VLDL particles and second-step lipid expansion of maturing VLDL particles in the secretory pathway [42]. In insulin-resistant states, increased MTP expression is linked to hepatic overproduction of VLDL (see below).

Hypertriglyceridemia occurs in some forms of familial hypercholesterolemia involving loss-of-function mutations in the LDL receptor, suggesting a role for the LDL receptor in regulating VLDL secretion. The LDL receptor binds to apoB-containing lipoproteins in the secretory pathway and targets them for degradation, either directly or via rapid endocytic reuptake [35,54]. The LDL receptor-VLDL particle interaction is mediated by apoB or apoE. However, in vivo evidence does not support a major role for the LDL receptor in regulating hepatic VLDL secretion [55].

Phospholipid transfer protein (PLTP) is a plasma protein that transfers PL between VLDL and HDL particles. A PLTP single nucleotide polymorphism (rs7679) in humans is associated with higher PLTP transcript levels, higher plasma HDL, and lower plasma TG concentrations [56]. PLTP deficiency leads to decreased hepatic apoB and triglyceride production in wild type, human apoB transgenic, and apoE knockout mice compared with their respective controls, indicating a role for PLTP in hepatic VLDL secretion [57]. However, PLTP-deficient mice in the LDL receptor knockout background had normal apoB production, suggesting the LDL receptor initiates binding and degradation of immature apoB-containing lipoproteins in the secretory pathway of PLTP-deficient mice [57]. Liver X receptor (LXR) activates a program of genes that result in cholesterol efflux in extrahepatic tissues and increased hepatic TG synthesis, VLDL secretion, and hypertriglyceridemia [58]. Both PLTP and SREBP1c are LXR-responsive genes and provide a concerted mechanism whereby SREBP1c-mediated hepatic TG synthesis is coupled to increased PLTP expression and VLDL assembly and secretion [59]. Taken together, these data suggest an important role for PLTP in providing PL for VLDL particle assembly.

Triacylglycerol hydrolase (TGH; also known as carboxylesterase 3 in mice and carboxylesterase 1 in humans) is an ER-localized enzyme that hydrolyzes preformed TG during VLDL particle assembly [60]. Overexpression of TGH in McA cells results in increased TG and apoB secretion [60,61], whereas chemical inhibition of TGH had the opposite effect [62]. Targeted deletion of TGH in mice also reduced hepatocyte VLDL secretion [63], suggesting a critical function of TGH-mediated hydrolysis of hepatic TG in VLDL particle assembly and secretion.

ADP-ribosylation factor 1 (Arf1) is a member of the Ras superfamily of small GTP-binding proteins that functions in the formation of coatamer I (COPI) secretory vesicles involved in vesicular trafficking between the ER to Golgi cellular compartments [64]. Dominant negative mutation of Arf1 decreases vesicular trafficking of VLDL2 from the ER to the Golgi cellular compartments, reducing VLDL secretion [65]. Arf1 also activates phospholipase D, increasing phosphatidic acid production and VLDL secretion.

Apolipoproteins also regulate VLDL secretion. Hepatic apoE expression promotes VLDL secretion [66]. ApoCIII simulates VLDL assembly and secretion in addition to inhibiting VLDL lipolysis by LPL, both of which contribute to hypertriglyceridemia [67–71]. ApoAIV promotes particle expansion and TG secretion in transfected porcine intestinal epithelial cells [72]. Hepatic apoAV expression reduces TG secretion and VLDL particle size in oleate-stimulated McA cells [73] and in mice with liver apoAV overexpression [74]. In addition, liver overexpression of ApoAV stimulates lipoprotein lipase-mediated VLDL-TG hydrolysis, resulting in lower plasma TG concentrations [74].

3.3. Insulin regulation of VLDL secretion

Insulin signaling is a well-studied hormonal pathway regulating VLDL secretion. Insulin interacts with its receptor, stimulating phosphorylation of insulin receptor (IR) and insulin receptor substrate (IRS). This, in turn, activates phosphoinositide-3 kinases (PI3 kinases) and Akt, initiating downstream effects on glucose and lipid metabolism and inhibiting

VLDL secretion. Liver overproduction of VLDL is thought to result in part from loss of insulin-mediated inhibition of VLDL secretion. Compared with healthy controls, diabetic men have increased VLDL TG secretion and a less pronounced decrease in VLDL TG secretion in response to insulin administration [75].

Physiologically, insulin modulates VLDL secretion in several ways. In adipose tissue, insulin acts as an anti-lipolytic hormone, decreasing TG lipolysis [76]. Therefore, free fatty acid flux to the liver and VLDL secretion is decreased. In the case of insulin resistance, free fatty acid flux from adipose tissue increases, providing hepatic substrate for TG synthesis and promoting VLDL secretion.

Although insulin acutely promotes hepatic lipogenesis, it inhibits VLDL secretion. Acute insulin treatment decreases VLDL apoB and TG secretion in immortalized and primary hepatocytes, isolated liver perfusion, and in mice and human subjects [77–83]. Insulin decreases VLDL secretion in a PI3 kinase-dependent pathway, promoting apoB degradation and inhibiting second-step bulk lipid addition to pre-VLDL particles in the secretory pathway, resulting in secretion of smaller VLDL particles (i.e., VLDL2) [82,84]. Activation of PI3 kinase is associated with its translocation to the ER membrane, suggesting that translocated PI3 kinase and its product, PIP₃, may alter the secretory vesicle microenvironment in a way that is unfavorable for VLDL second-step assembly [84].

Forkhead box O1 (FoxO1) is a transcription factor that stimulates hepatic MTP expression, increasing VLDL production by the liver [85]. Insulin signaling leads to PI3 kinase and Akt-mediated phosphorylation of FoxO1, resulting in its translocation from the nucleus to the cytoplasm and decreased expression of MTP and VLDL secretion. In insulin resistance, this pathway is blunted, leading to an increase in MTP expression and hepatic overproduction of VLDL. However, recent data suggest that acute insulin treatment does not decrease MTP transcription in apoB^{-/-} mice, which lack the ability to edit apoB100 mRNA [86]. In addition, insulin decreases apoB secretion to a similar extent in hMTP overexpressed vs. wild type mice, suggesting that insulin may also decrease apoB secretion by MTP-independent mechanisms [86]. Insulin inactivation of FoxO1 also decreases apoCIII expression, which promotes VLDL production and provides yet another potential mechanism for insulin-mediated attenuation of VLDL assembly and secretion [87].

As discussed previously, hepatic autophagy mediates degradation of apoB [36,37]. Poor lipidation of hepatic VLDL apoB when insulin is high may shunt apoB from proteasomal to autophagosomal degradation [88]. However, insulin activates mTOR via PI3 kinase, inhibiting expression of vps34, atg12 and gabarapl1, all of which are involved in autophagosome formation [89,90]. Thus, additional support for insulin stimulation of apoB autophagosome degradation is needed to reconcile the evidence that insulin inhibits autophagy.

VLDL secretion via insulin signaling is modulated by other signaling molecules along the PI3 kinase-Akt pathway. Protein tyrosine phosphatase 1B (PTP1B) dampens insulin signaling by dephosphorylating phosphotyrosine residues on activated IRS to induce liver overproduction of VLDL in insulin-resistant states. Loss of liver PTP1B protects against fructose diet-induced overproduction of VLDL [91]. Phosphatase and tensin homolog (PTEN), a key negative regulator of the PI3 kinase pathway, also regulates VLDL secretion. Liver-specific PTEN knockout mice display reduced apoB production and MTP protein expression and activity [92].

3.4. Summary of regulation of VLDL secretion

Hepatic VLDL assembly and secretion is a complex process, involving numerous lipid and protein factors that integrate nutritional and hormonal cues to control hepatic TG output. Undoubtedly, the regulation of VLDL assembly and secretion is even more complex, with additional control points in cytosolic lipid mobilization and transport of lipid substrate to the secretory pathway. Other modulators of VLDL TG secretion have recently been described (see below) and more are likely to be discovered. A future challenge will be to integrate these findings and pathways into a comprehensive and rational model for understanding the pathophysiology of liver overproduction of VLDL during insulin resistance, so that better therapeutic strategies may be developed.

4. Hepatic ABCA1: another link between HDL and VLDL metabolism

There is a well-established inverse association between plasma HDL and TG concentrations [93]. One potential mechanism to explain this association involves cholesterol ester transfer protein (CETP)- mediated exchange of VLDL TG for HDL CE, leading to relative TG enrichment of HDL. Subsequent hydrolysis of HDL TG by hepatic lipase (HL) leads to smaller HDL particles that are rapidly removed from the circulation, resulting in lower plasma HDL concentrations [94]. This intravascular process likely accounts for the inverse association between HL levels and plasma HDL concentrations [95]. We now summarize recent evidence that links deficient hepatic ABCA1 expression to decreased HDL formation and increased VLDL TG production. This new pathway may explain, in part, the inverse association between VLDL TG and HDL concentrations, particularly in individuals with compromised ABCA1 expression due to single nucleotide polymorphisms in the ABCA1 gene [96].

4.1. Hepatic ABCA1 and HDL

Tangier disease (TD) is a rare inherited disorder characterized by severe HDL deficiency, rapid apoA-I clearance from plasma, massive sterol deposition in macrophage-enriched tissues, and premature coronary heart disease in some kindreds [9]. Despite discovery of the disease in the early 1960's, relatively little was known about the molecular pathogenesis of Tangier disease until the mid-1990's. The ABCA1 gene was cloned in 1995, but its function was unknown [97]. In 1995, Francis et al [98] demonstrated that fibroblasts from Tangier disease patients were defective in apoA-I-mediated FC and PL efflux compared to fibroblasts from unaffected individuals. In 1999, multiple groups reported mutations in ABCA1 as the molecular defect in Tangier disease [99–101].

ABCA1 is a membrane protein required for nascent HDL formation [102]. It catalyzes the formation of heterogeneous-sized nascent HDL particles by facilitating the efflux of PL and FC across the cell membrane to apoA-I. Depending on the cell system used, the resulting nascent HDLs have pre- β or α mobility on agarose gels and vary in particle diameter from 7.1 to 15.7 nm [103–109]. Similar sized pre β particles are formed in most cell types including fibroblasts [103,107], macrophages [110], hepatoma cells [6,110], adipose tissue explants [111], and ABCA1-expressing human embryonic kidney (HEK) 293 cells [104], suggesting that the particle size heterogeneity is a fundamental property of ABCA1 activity and not due to cellular origin of the nascent HDL particles.

After the discoveries that ABCA1 is essential for nascent HDL particle formation in vitro and genetic deficiency of ABCA1 leads to near-absence of plasma HDL, one of the next challenges was to understand the role of ABCA1 in HDL formation in vivo. This was an intriguing question, in part, because ABCA1 is expressed at varying levels in nearly all tissues [112], yet only the liver and intestine secrete the major apolipoprotein of HDL, apoA-I. Using cell-specific deletion of ABCA1, we demonstrated that hepatocytes are the

predominant cell type responsible for HDL formation in vivo, contributing 70–80% of the plasma HDL pool [113], whereas the intestine contributes 20–30% [114]. Isolated hepatocytes from hepatocyte-specific ABCA1 knockout (HSKO) mice failed to efflux FC and PL to apoA-I and failed to generate heterogeneous-sized nascent HDL particles (Figure 1A) compared to wild type (WT) hepatocytes. The size range of nascent HDL generated by isolated hepatocytes is similar to that of HDL particles generated by a non-hepatic cell line stably expressing ABCA1 (Figure 1B), suggesting that hepatic and non-hepatic cells generate similarly sized nascent HDL particles with sufficient ABCA1 expression.

4.2. Hepatic ABCA1 and TG metabolism

In addition to very low plasma HDL concentrations, Tangier disease patients have elevated plasma TG levels and a 50% reduction in plasma LDL concentrations. Most Tangier disease subjects display elevated fasting or postprandial TG levels, but results vary [9]. Furthermore, there is an inverse association between dysfunctional ABCA1 alleles and plasma TG concentrations [115]. Several studies have suggested that increased apoC-III and apoA-II content of Tangier VLDL inhibits lipolysis of TG by LPL, resulting in increased plasma TG concentrations [9,116,117]. Another study has documented hypercatabolism of ¹²⁵I-human LDL tracer in Tangier disease subjects [118], suggesting a potential mechanism for reduced plasma LDL concentrations in these subjects. However, a detailed molecular explanation for increased plasma VLDL and decreased plasma LDL in Tangier disease remains to be elucidated.

Chow-fed HSKO mice demonstrate a plasma lipid phenotype similar to Tangier disease subjects, with low plasma HDL concentrations (<20% of WT), a two-fold increase in plasma TGs, a 50% lower plasma LDL level, and hypercatabolism of HDL and apoA-I [113]. Since the liver plays a major role in the production and catabolism of VLDL, LDL and HDL, we hypothesized that hepatic ABCA1 plays a pivotal role in the metabolism of all three major plasma lipoprotein species. To test this hypothesis, we used both in vitro and in vivo studies.

Initial studies were performed using McA cells because they respond to oleic acid treatment with a robust stimulation of VLDL TG secretion and express ABCA1, thus generating heterogeneous-sized nascent HDL particles when apoA-I is added to the medium [6]. Using siRNA, we successfully silenced ABCA1 protein expression to <30% of control siRNA-treated cells and studied VLDL secretion and nascent HDL particle formation. Silencing ABCA1 in oleate-stimulated McA cells resulted in a two-fold increase in TG secretion, accompanied by secretion of larger VLDL particles (1.6-fold increase in diameter, from 38 nm in control cells to 63 nm in ABCA1-silenced cells). There was only a marginal increase (10–15%) in apoB secretion in ABCA1-silenced cells; however, density gradient ultracentrifugation revealed an increase in VLDL1 (S_f 100–400) particles in the medium. These results suggested that ABCA1 expression affects the second step of VLDL particle maturation, which is affected by insulin and PI3 kinase signaling [82,84]. In ABCA1-silenced, oleate-stimulated McA cells, we observed a marked attenuation of PI3 kinase activation relative to control siRNA-treated cells. Treatment of these cells with PI3 kinase inhibitors (Ly 294002 or wortmannin) resulted in a two-fold increase in TG secretion in controls, but no change in ABCA1-silenced McA cells, suggesting a link between diminished PI3 kinase activation and increased TG secretion in ABCA1-silenced McA cells. In other studies, we determined that the increase in TG secretion with ABCA1 silencing was unrelated to liver X receptor activation, MTP protein expression, or mTOR and MEK/ERK activation [6]. Taken together, these results suggested a novel role for ABCA1 in regulating PI3 kinase activation and TG secretion in McA cells.

Since ABCA1 has no known direct role in intracellular TG transport, we hypothesized that a product of ABCA1, specifically nascent HDL, affects TG secretion indirectly. To test this

hypothesis, we monitored nascent HDL formation in oleate-stimulated, ABCA1-silenced McA cells in the presence of ^{125}I -apoA-I in serum-free medium [6]. Compared to control siRNA-treated cells, ABCA1 silenced cells generated fewer of the larger (>10 nm diameter, pre β 3 and pre β 4 HDL) nascent HDL particles and relatively more of the smaller pre β 2 particles. To determine whether the decrease in larger nascent HDL particles was associated with an increase in TG secretion, isolated nascent HDL particles from McA cells or HEK-293 cells expressing ABCA1 were added back to control and ABCA1-silenced McA cells and TG secretion and PI3 kinase activation were measured. Larger (>10 nm), but not smaller (<10 nm), nascent HDL particles attenuated TG secretion and restored PI3 kinase activation in ABCA1-silenced cells to levels near those in control siRNA-treated cells. The attenuation of TG secretion and restoration of PI3 kinase activation in ABCA1-silenced cells was specific for nascent HDL and did not occur with apoA-I or human plasma HDL [6].

Based on the results of our McA cell studies, we proposed a hypothetical model (Figure 2) in which large nascent HDL particles assembled by ABCA1 at the hepatocyte plasma membrane bind to a putative membrane receptor, target of pre β (Top β), resulting in activation of PI3 kinase, decreased lipid mobilization into VLDL particles during second-step assembly, and secretion of normal-sized VLDL particles (i.e., VLDL2) (Figure 2, left panel). When ABCA1 function is diminished or absent, large nascent HDL assembly decreases, leading to less binding to Top β , decreased PI3 kinase activation, increased lipid mobilization into maturing VLDL particles, and secretion of larger, TG-enriched VLDL particles (VLDL1, Figure 2, right panel). Teleologically, this hypothetical pathway may compensate for loss of ABCA1-mediated FC and PL efflux from hepatocytes to extrahepatic tissues by increasing hepatic lipid efflux through increased VLDL secretion. In support of this idea, several studies have suggested that decreased hepatic FC biosynthesis [119,120] or increased ABCA1-mediated FC efflux [121], decreases hepatic VLDL TG secretion. Although we saw no evidence for increased cellular cholesterol in our study, it is possible that small changes in a regulatory pool of cholesterol might stimulate VLDL secretion when ABCA1 expression is diminished.

Further support for our in vitro observations came from studies in HSKO mice. Compared to WT controls, chow-fed HSKO mice had increased plasma TG concentrations, a two-fold increase in hepatic TG production in vivo and hepatocyte TG secretion in vitro, and larger plasma VLDL1 particles, with no significant alteration in hepatic lipid content or MTP protein expression [7]. Furthermore, hepatic PI3 kinase activation was attenuated in HSKO mice compared to WT mice after acute insulin injection or in response to fasting and refeeding. In vivo acute pharmacological inhibition of PI3 kinase with wortmannin resulted in increased TG secretion in WT mice, similar to that observed in HSKO mice without PI3 kinase inhibition. These data are remarkably similar to those obtained with silencing of ABCA1 in McA cells and support an essential role for hepatic ABCA1 in regulation of VLDL TG secretion, in vivo.

An additional mechanism for the elevated plasma TG concentrations in HSKO mice was a decrease in post-heparin HL and LPL activity compared with WT mice [7]. This evidence was further supported by delayed in vivo clearance of postprandial TG after an oral fat load. The molecular explanation for this decrease is unknown at this time. However, our observations in HSKO mice agree with similar data demonstrating decreased LPL activity [122] and delayed clearance of postprandial lipid in Tangier disease subjects [123].

Low plasma LDL concentrations in Tangier disease subjects may be due to increased plasma clearance of LDL particles [118]. We tested this possibility in HSKO mice using ^{125}I -LDL tracer and observed a two-fold increase in fractional catabolic rate for the LDL tracer relative to WT mice [7]. The increased clearance of LDL tracer in HSKO mice appeared due

to a two-fold increase in hepatic LDL mRNA and protein expression. In vivo verification of this result came by crossing the HSKO mice with LDL receptor-deficient (LDLrKO) mice. HSKO mice lacking LDL receptor expression had plasma LDL concentrations similar to those of LDLrKO mice, but higher plasma TG concentrations [7]. Taken together, these results suggest that two distinct mechanisms are responsible for the elevated plasma TG concentrations and reduced LDL levels in ABCA1-deficient states, hepatic overproduction of TG, and reduced TG lipolysis for the former and increased hepatic LDL receptor expression for the latter.

4.3. Hepatic ABCA1 deficiency and hepatic insulin resistance

Chow-fed HSKO mice had several phenotypic changes indicative of early insulin resistance, including increased hepatic secretion of VLDL1 particles and defective activation of PI3 kinase with acute insulin stimulation or fasting-refeeding [7]. Insulin resistance has not been documented in Tangier subjects, perhaps because the disease is so rare and too few subjects have been studied [124]. However, there is mounting evidence that decreased ABCA1 expression is associated with altered glucose metabolism and insulin resistance. A polymorphism in the 5' flanking region of the ABCA1 gene is more frequent in Japanese type 2 diabetics than controls and is independent of plasma HDL concentrations [125]. ABCA1 polymorphisms have also been associated with obesity in Mexican and French populations [126,127] and with early onset T2D in a Mexican population [128]. Another study has reported an inverse correlation between leukocyte ABCA1 expression and fasting plasma glucose levels in normoglycemic men [129]. Studies in mice have also found an association between ABCA1 expression and abnormal glucose metabolism. Chow-fed ABCA1 total knockout mice develop hyperglycemia by four months of age and pancreatic β cell-specific deletion of ABCA1 resulted in accumulation of FC in islet cells and defective insulin release, resulting in hyperglycemia [130]. While these studies suggest an association between ABCA1 expression and glucose metabolism, the role of cell-specific expression of ABCA1 in glucose metabolism, except for that of the pancreatic β cell, remains unknown. Finally, type 2 diabetics given intravenous reconstituted HDL had increased plasma insulin and reduced glucose compared to placebo treatment, suggesting that HDL-raising therapies may be a beneficial treatment for type 2 diabetes [131].

5. Summary and conclusions

Human genetic, cell culture, and animal studies all suggest a significant role for ABCA1 in modulating lipoprotein metabolism; several key points can be emphasized from the published literature. First, deletion of hepatic ABCA1 in the mouse nearly replicates the Tangier disease lipid phenotype of increased plasma TG and decreased LDL and HDL concentrations, suggesting a central role for hepatic ABCA1 expression in manifestation of the altered plasma lipid phenotype in these individuals. The slightly higher HDL concentrations in HSKO mice (20% of normal) compared to Tangier disease subjects (<5% of normal) likely reflects HDL production from extrahepatic tissues, such as the intestine [114]. Second, new insights into the molecular pathogenesis of Tangier disease have emerged from cell and mouse studies, suggesting that multiple mechanisms are involved in the abnormal lipid phenotype including VLDL overproduction, decreased postheparin lipolytic activity, and increased LDL clearance. Third, an important and novel role for hepatic ABCA1 expression in the regulation of production and catabolism of plasma apoB lipoproteins has been elucidated. Finally, an association between ABCA1 and insulin resistance is emerging. HSKO mice should provide a valuable animal model for exploring the relationships among hepatic ABCA1 expression, PI3 kinase activation, and VLDL particle maturation.

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Highlights

- Hepatic VLDL overproduction is a prominent cause of elevated plasma TGs
- Loss of hepatic ABCA1 expression leads to VLDL1 overproduction
- Loss of hepatic ABCA1 results in plasma LDL hypercatabolism
- Hepatic ABCA1 affects metabolism of all major plasma lipoprotein classes

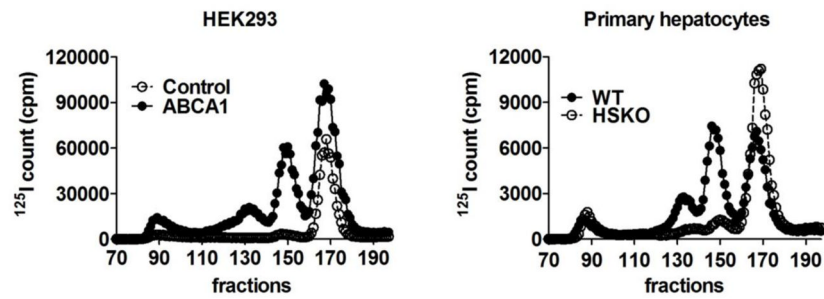


Figure 1. Nascent HDL particle formation by primary hepatocytes from wild type (WT) or hepatocyte-specific ABCA1 knockout (HSKO) mice (panel A) and control or ABCA1-expressing HEK293 cells (panel B). Cells were incubated for 24h with ^{125}I -human apoA-I and media were harvested and concentrated for fractionation by high resolution fast protein liquid chromatography. Fractions were quantified for radiolabel and values (cpm) plotted.

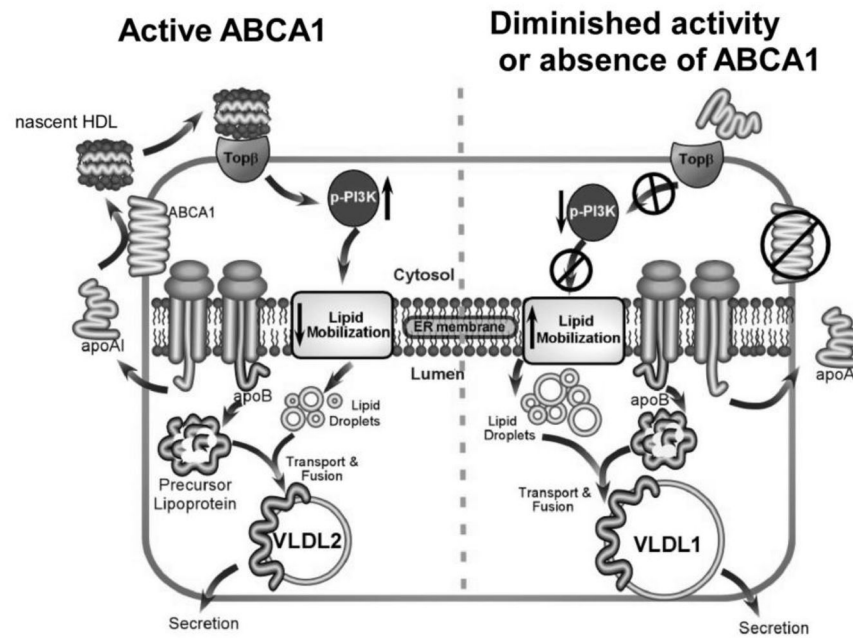


Figure 2. In the presence of active ABCA1 (left), lipid free apoA-I is secreted by hepatocytes and lipidated by hepatocyte ABCA1, forming nascent HDL particles. Large nascent HDLs (>10 nm diameter) bind to a putative receptor (Topβ, target of pre-β), which in turn stimulates PI3 kinase activation resulting in reduced lipid mobilization and secretion of normal-sized VLDL particles (i.e., VLDL2). In the absence of hepatic ABCA1 or diminished ABCA1 activity (right), newly secreted lipid-free apoA-I fails to form large nascent HDL particles. Reduced nascent HDL particle formation leads to diminished signaling through Topβ, resulting in reduced PI3 kinase activation, increased lipid mobilization, and increased secretion of larger TG-enriched VLDL particles (VLDL1). Reprinted with permission (J Lipid Res 2010;51:729–742).