Review

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HCV and the hepatic lipid pathway as a potential treatment target

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Atherosclerosis has been described as a liver disease of the heart [1]. The liver is the central regulatory organ of lipid pathways but since dyslipidaemias are major contributors to cardiovascular disease and type 2 diabetes rather than liver disease, research in this area has not been a major focus for hepatologists. Virus–host interaction is a continuous co-evolutionary process [2] involving the host immune system and viral escape mechanisms [3]. One of the strategies HCV has adopted to escape immune clearance and establish persistent infection is to make use of hepatic lipid pathways. This review aims to:

- update the hepatologist on lipid metabolism
- review the evidence that HCV exploits hepatic lipid pathways to its advantage
- discuss approaches to targeting host lipid pathways as adjunctive therapy.

The hepatic lipid pathway

Lipids act as energy sources for cells. Mammals have evolved a sophisticated mechanism to enable hydrophobic fat to be made soluble in the form of lipoproteins for delivery to peripheral tissues. The role of hepatically-derived very-low-density lipoprotein (VLDL) is thus to deliver energy, in the form of triglyceride (TG) to cells requiring energy (skeletal muscle) or to cells that store energy (adipose tissue). Lipoproteins can be characterised by their densities related to their core lipid (TG and cholesteryl ester) composition; the five main classes according to their density are chylomicrons (CM), VLDL, intermediate-density lipoproteins (IDL), low-density lipoprotein (LDL), and high-density lipoproteins (HDL). Lipoproteins can also be characterised by the major apolipoproteins present on their surfaces, some of which are structural (apolipoproteins B and AI), whilst others exchange between different lipoprotein particles (apolipoproteins AII, C, and E).

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Apolipoprotein B (apoB)

Apolipoprotein B (apoB) exists as two distinct forms, apoB100 and apoB48 (the amino-terminal 48% of apoB100).

ApoB100 is the full length protein produced by the liver and contains the low-density lipoprotein receptor (LDL-R)-binding domain. ApoB100 is essential for VLDL assembly in hepatocytes and is the structural lipoprotein present in VLDLs, IDL (also called VLDL remnants), and LDL. The number of secreted VLDL is regulated primarily by multiple degradative pathways for apoB within the cell which are, in turn, regulated by metabolic factors and pathways [4].

ApoB48, which lacks the LDL receptor-binding domain, is primarily synthesized by the postnatal small intestine and is essential for chylomicron assembly in enterocytes. Hence apoB48 is the intestinally-derived isoform of apoB100 on CM which transport dietary lipids [5].

Apolipoprotein E (apoE)

Apolipoprotein E (apoE) is a multifunctional protein that is synthesized by the liver and several peripheral tissues and cell types, including macrophages [6]. Insight into its multiple roles in lipid and energy metabolism has been provided by the transgenic *ApoE* (−/−) mouse model [7]. ApoE is a critical ligand for the receptor mediated removal of TG-rich lipoprotein (TRL) remnants (VLDL and CM remnants) by the liver. ApoE not only acts as a high affinity ligand for multiple members of the LDL receptor family including the LDL-R, the LDL receptor-related protein (LRP), and the VLDL receptor but also binds to heparan sulphate proteoglycans (HSPG), which act as hepatic receptors for TRL remnant clearance. In addition, apoE participates in the biogenesis of discoidal HDL particles which are then converted to the spherical form, by the action of lecithin:cholesterol acyltransferase (LCAT) allowing the recognition of HDL by the scavenger receptor-B1 (SR-B1) [8]. ApoE also has an immune function and mediates the presentation of serum-borne lipid antigens [9].

Human apoE has three common isoforms (apoE2, apoE3, and apoE4) which differ only by a single amino acid at two residues. These differences affect structural and biophysical properties of apoE resulting in different effects on lipid homeostasis. For example apoE2 binds LDL-R with reduced affinity [10] whilst apoE4 preferentially associates with VLDL.

Table 1. Distribution of major apolipoproteins among the lipoprotein particle family.

Apolipo-protein	Molecular Weight	Chylomicron (CM)	VLDL	IDL/CM remnants	LDL	HDL
AI	28,016	Ex	Ex			St
AII	17,414	Ex	Ex			Ex
B100	515,000		St	St	St	
B48	241,000	St*		St*		
CI	6600	Ex	Ex			Ex
CII	8800	Ex	Ex			
CIII	8750	Ex	Ex	Ex		Ex
E	34,100	Ex	Ex	Ex		Ex

*B48 is exclusive to chylomicrons and chylomicrons remnants. St, structural apolipoprotein; Ex, exchangeable apolipoprotein. Other apolipoproteins (AIV, AV, D, F, G, H, J, (a)) are beyond the scope of this review.

Apolipoprotein Cs (apoCs)

The apoCs are a family of small exchangeable lipoproteins (Table 1) which are important for the regulation of lipolysis. ApoCI is a basic apolipoprotein that is mainly secreted by the liver as a component of VLDLs. It can dissociate from the VLDL surface to rapidly associate with HDLs and promote discoidal particle morphology [11]. ApoC-I has multiple regulatory actions in plasma TRL metabolism including inhibiting the binding and/or uptake of VLDL by LDL-R and LRP. This is believed to be due to the ability of apoC-I to displace significant amounts of apoE from TRL, or alternatively to mask or alter the conformation of apoE on these particles [12]. ApoCI also functions as an activator of LCAT and an inhibitor of plasma cholesteryl ester transfer protein (CETP) [13].

Apolipoprotein CII (apoCII) is a necessary activator for lipoprotein lipase [14].

ApoCIII is synthesised by the liver and, in the fasting state, is mainly associated with high-density lipoprotein (HDL) whereas in the fed state, or in hyperlipidaemic individuals, it preferentially redistributes to VLDL and CM (Fig. 1). ApoCIII is an important regulator of lipoprotein metabolism [15]; it impairs the lipolysis of TRLs by inhibiting lipoprotein lipase and the hepatic uptake of TRLs by remnant receptors. VLDL particles rich in apoCIII have a reduced clearance rate whereas those rich in apoE rapidly clear from the circulation [16,17]. It has also been shown that apoCIII stimulates VLDL synthesis [18]. Hence total plasma apoCIII concentrations causally correlate with plasma TG concentrations.

Apolipoprotein As (ApoAs)

ApoAI is the principal apolipoprotein in HDL and has the ability to interact with the SR-B1 hepatic receptor as well as activate LCAT [19]. ApoAII is also associated with HDL where it is the second most abundant protein (Table 1). ApoAII exchanges from HDL to VLDL resulting in VLDL that is a poorer substrate for lipolytic activity (Fig. 1). It is thus a regulator of VLDL metabolism and apoAII levels in humans are associated with plasma concentrations of TGs [20].

High-density lipoproteins (HDLs)

HDLs are small, dense, particles [density range of 1.063–1.210 g/ml], some of which carry only ApoAI, whereas others contain both ApoAI and ApoAII. Other apolipoprotein species found in HDL particles include ApoAIV, Apo C (CI, CII, and CIII), and Apo E. The physical heterogeneity of HDLs is associated with multiple functions that involve both the protein and the lipid components of these particles [19]. A major role of HDL is in the reverse cholesterol transport process where cholesterol in peripheral tissues is transported to the liver for reuse or bile acid synthesis. In addition, recent data indicate that HDL participates in a mechanism of intercellular communication involving the transport and delivery of microRNAs [21].

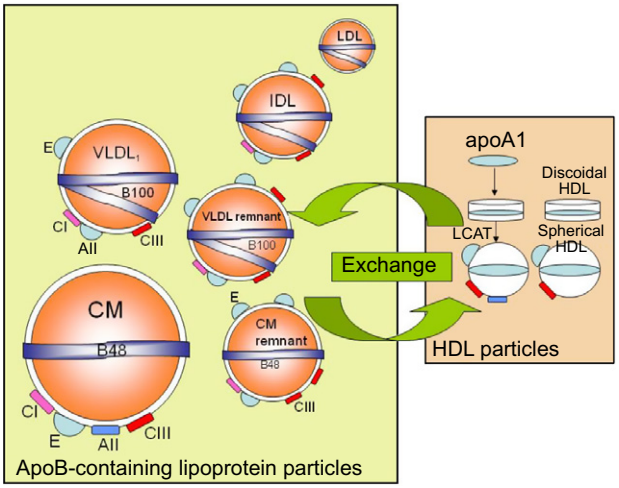

Fig. 1. Pictorial representation of the transfer of exchangeable apolipoproteins (ApoCs, apoAII, § and apoE) between apoB-containing lipoprotein particles (very-low density lipoprotein [VLDL], intermediate density lipoprotein [IDL], low density lipoprotein [LDL], and chlymicrons [CM]) and apoAI-containing high-density lipoprotein [HDL] particles in the circulation. For example in the fed state apoCIII preferentially redistributes to VLDL and CM. Similarly HDL serves as a plasma reservoir of apoAII that transfers to TRLs in much the same way as apoCs [20]. Discoidal HDL particles are converted to the spherical form by the action of lecithin:cholesterol acyltransferase (LCAT) [8]. [Adapted from Ooi *et al.* [18]].

VLDL assembly and secretion

VLDL particles secreted from the liver vary in size and composition and can be classified not only by their density (0.94–1.06 g/ml), but also by their diameter (20–75 nm), and flotation rate [Svedberg flotation rate (Sf) 20–400]. VLDL can be separated into two main classes: large, buoyant $VLDL_1$ particles (Sf 60–400) which contain more TG and smaller denser $VLDL_2$ particles (Sf 20–60).

Each VLDL contains a single non-exchangeable apoB100 molecule, which serves as its scaffolding, and exchangeable apolipoproteins including apoE and apoCs. The processes involved in the assembly and secretion of VLDL have been studied extensively *in vitro* and *in vivo* for the past two to three decades [5,22]. VLDL assembly occurs via a two-step mechanism (Fig. 2), involving the formation of apoB-containing VLDL precursor particles in the lumen of the endoplasmic reticulum (ER), a step which requires microsomal triglyceride-transfer protein (MTP) (reviewed in [23]). This initial lipidation of apoB prevents proteosome-mediated degradation. The VLDL precursor particles are then loaded with neutral lipids by lipid droplets (LDs) [24,25] to form TG rich $VLDL_1$. It is thought that interplay between the length of

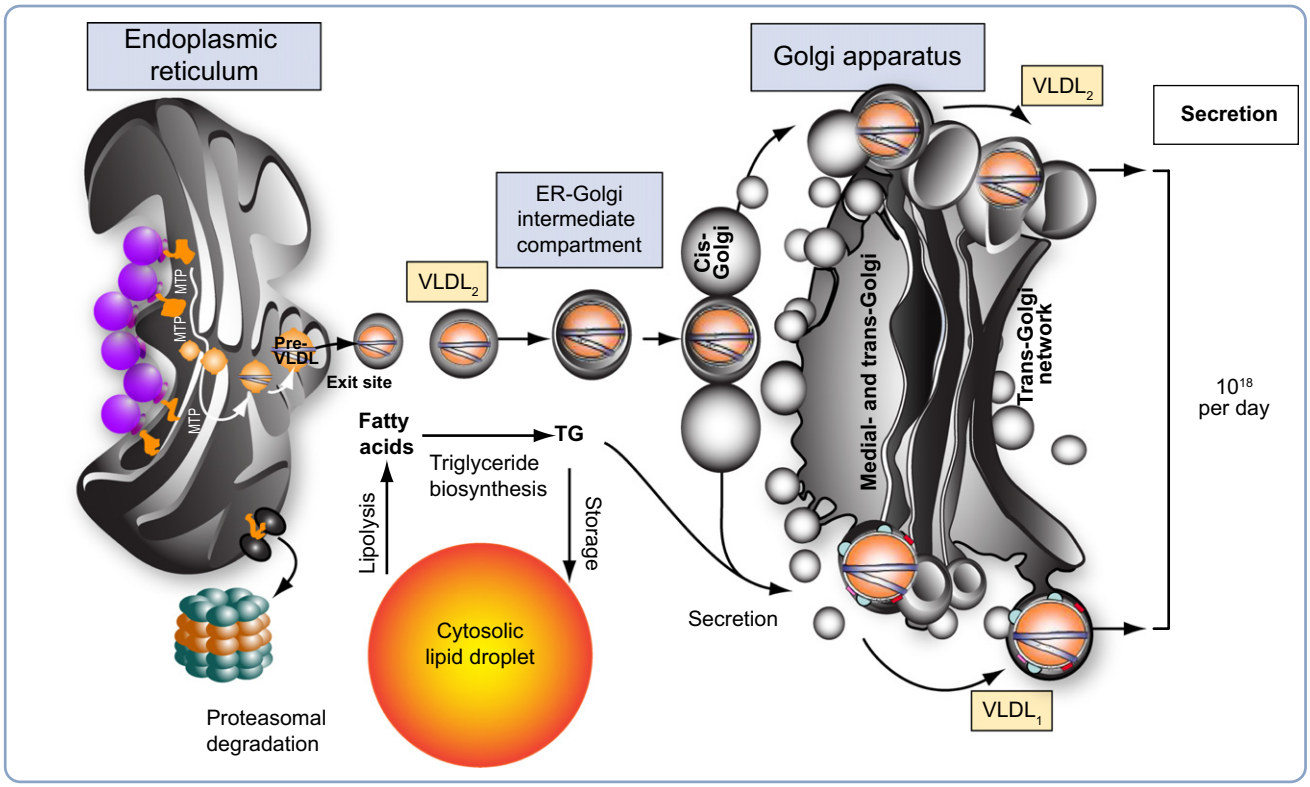

Fig. 2. Schematic representation of the two-stage VLDL assembly in hepatocytes. ApoB is translocated to the lumen of the ER and lipidated by microsomal triglyceride transfer protein [MTP] to form pre-VLDL. Pre-VLDL is further lipidated to form $VLDL_2$ or misfolded apoB can be degraded in the cell. $VLDL_2$ is transferred to the Golgi and is either secreted or further lipidated with a major load of triglyceride (TG) from the lipid droplet to form $VLDL_1$. [Adapted from Olofsson *et al.* [166]].

apoB polypeptide and palmitoylation of apoB regulates TG-rich lipoprotein assembly and secretion. In addition, apoE appears to play a role in the formation of fully lipidated VLDL, whilst apoCIII plays an intracellular role in stimulating VLDL assembly and secretion.

Both the hepatic production rate and the circulating number of $VLDL_1$ particles are significantly and strongly related to HOMA-estimated insulin resistance (IR) in normoglycaemic adults [26,27]. Disease phenotypes (e.g., type II diabetes, obesity) also dramatically alter the total numbers and size of LDs, the dynamic cellular structures involved in lipid homeostasis [25]. In contrast, the production rate and number of $VLDL_2$ are not significantly related to insulin resistance.

Chylomicrons

Dietary fat is absorbed by the enterocyte, where a unique assembly process similar to that of VLDL produces a "package" of lipid and cholesterol with phospholipids and apoB-48 to form a chylomicron that is stable in the aqueous environment of the bloodstream. Chylomicrons are the largest lipoproteins; CM access the circulation via the thoracic duct where they are converted to remnants by the TG hydrolysing action of lipoprotein lipase, with apoCII acting as a co-factor and activator. The resultant relatively TG-depleted, cholesterol-enriched CM remnant particle is then cleared by the liver via an apoE dependent receptor-mediated process. Thus, chylomicrons facilitate delivery of dietary lipid and cholesterol to the liver [28].

Intravascular re-modelling of lipoproteins and removal of lipid

Intravascular re-modelling of lipoproteins

Re-modelling entails the exchange of core and surface lipids and apolipoproteins, mediated largely by the action of plasma lipid transfer enzymes [lipoprotein lipase, hepatic lipase (HL), lipid transfer proteins (CETP and phospholipid transfer protein (PLTP)) and LCAT].

Lipoprotein lipase (LPL) is the enzyme responsible for the hydrolysis of core TG in VLDL and CM, producing IDL and chylomicron remnants, respectively. LPL is made in tissue parenchymal cells and then translocated to functional binding sites at the luminal surface of endothelial cells. LPL is anchored by ion interaction with heparin sulphate proteoglycans (HSPG) and/or glycosyl phosphatidylinositol. Once TRLs are bound to this platform, LPL mediates TG hydrolysis, causing the release of fatty acids that are then taken up by receptors located on the plasma membrane of cells. Within these cells, the fatty acids are re-esterified and used for storage in adipocytes or for energy production in muscle. LPL requires a specific co-factor, apoCII, to be fully active [14,29]. The enzymatic activity of LPL is regulated in a complex manner in response to energy requirements and hormonal changes. For example insulin not only increases the level of LPL mRNA in mature adipocytes but also regulates LPL activity through post-transcriptional and post-translational mechanisms. Conversely, interferon (IFN) decreases LPL activity

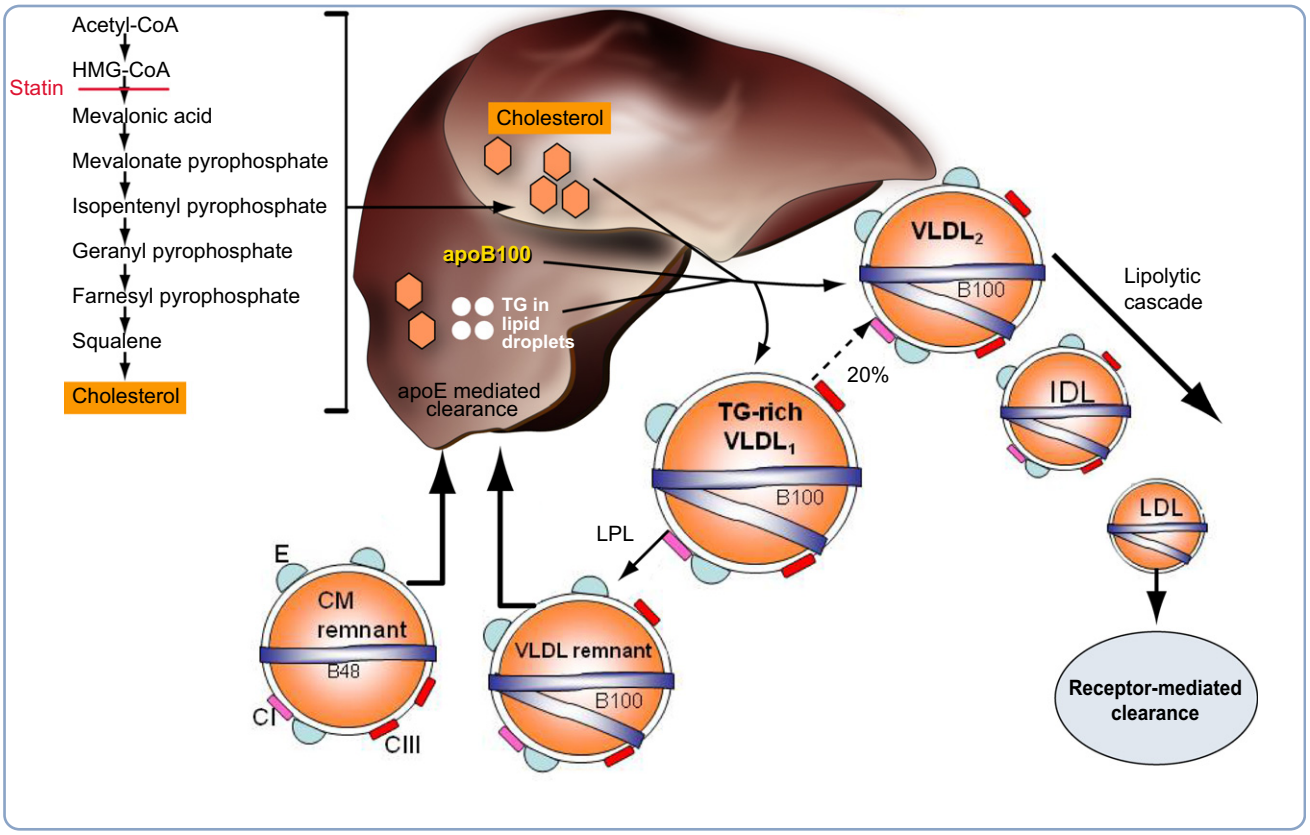

Fig. 3. Schematic representation of the life cycle of apoB-containing lipoprotein particles. Triglyceride-rich lipoproteins ($VLDL_1$ and chylomicron (CM)) remnants undergo rapid apoE-mediated clearance from the plasma (see Fig. 4). The lipolytic cascade produces low-density lipoprotein (LDL) particles largely from $VLDL_2$ catabolism. Statins have the largest LDL-cholesterol lowering effect among current licensed drugs by inhibiting the rate-limiting step in hepatic cholesterol synthesis, 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA).

resulting in an increase in plasma TG [30–33]. Hepatic lipase plays a major role in lipoprotein metabolism as a lipolytic enzyme that hydrolyses TG and phospholipids in $VLDL_2$, IDL, CM remnants, and HDL [34].

CETP primarily transfers TG from VLDL in exchange for cholesteryl esters from other lipoproteins, especially HDL [35].

Low-density lipoprotein is thus not directly secreted from hepatocytes but is produced by VLDL catabolism. The lipolytic cascade produces LDL particles which are depleted of TG and enriched with cholesterol. During lipolysis of TRLs, apolipoproteins in their surface phospholipid coats are released and recycled into HDL in serum. HDL particles are also secreted *de novo* from hepatocytes.

Clearance of TRLs

The size of VLDL is a critical determinant in deciding the fate of the particles in the circulation [36]. Thus, most of the large TG-rich $VLDL_1$ are cleared directly from the plasma [37] and less than 20% of $VLDL_1$ undergoes lipolysis all the way to LDL [38] (Fig. 3).

Once TG-rich lipoproteins ($VLDL_1$ and CM) are hydrolysed by LPL, the resultant apoE-enriched TG-rich lipoprotein remnant particles are removed in the liver by the concerted action of a number of receptors [39,40]. Hepatic lipase on the basolateral surface of hepatocytes not only functions as a lipase but also serves as a bridge/ligand that facilitates lipoprotein uptake. The lipase-lipoprotein complex can then undergo internalisation, a process that is independent of lipolysis and can be mediated by HSPG, LDL-R, and LRP (Fig. 4), as well as SR-B1 (reviewed in [40,41]). Syndecan1 is a HSPG that is essential for the binding and clearance of TG-rich remnant lipoproteins [42,43]. SR-B1 might function as an initial recognition site for CM remnants with subsequent internalisation by additional receptors such as LDL-R [44]. Thus SRB1 not only promotes cellular uptake of cholesteryl esters from HDL but also binds and facilitates the catabolism of VLDL and CM [40,45]. Recent data confirm complexity in the interactions of lipoproteins with SR-B1 [46].

The ligand for these receptors is likely to be apoE since apoB appears not to be in a receptor competent conformation on large triglyceride-rich $VLDL_1$. ApoCIII strongly inhibits hepatic uptake of VLDL and IDL overriding the opposite influence of apoE when both are present [17].

HCV co-opts the hepatic lipid pathway

HCV infection is a highly dynamic process with a viral half life in plasma of less than 3 h and production/clearance of an estimated 10^{12} virions per day in an infected individual [47]. Currently, it is

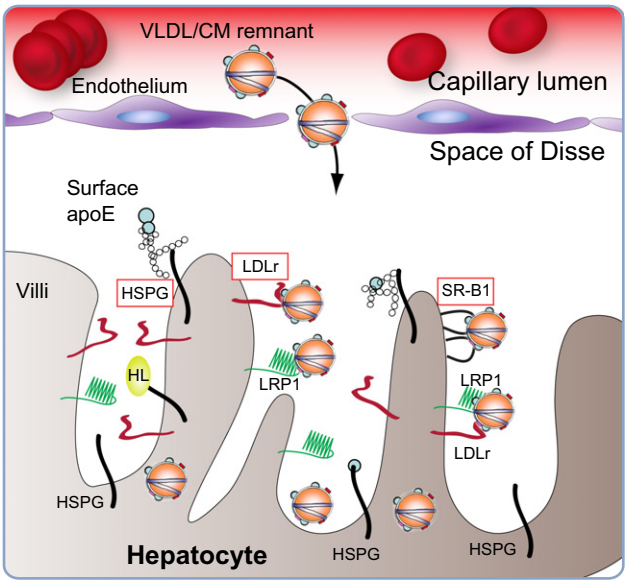

Fig. 4. Hepatic TRL remnant clearance involves sequestration of the particles through the fenestrae in the endothelium into the space of Disse. Two endocytic receptors, the syndecan-1 heparan sulfate proteoglycan (HSPG) and the LDL receptor, plus one docking receptor, SR-BI, significantly contribute to normal hepatic remnant catabolism [40], all of which have been implicated as cellular receptors for HCV (red box). Hepatic lipase (HL) and apoE, secreted by the hepatocytes (H), appear to bind to the HSPG and be available to enrich the remnant lipoproteins and facilitate their uptake. [Adapted from Van Eck *et al.* [167]].

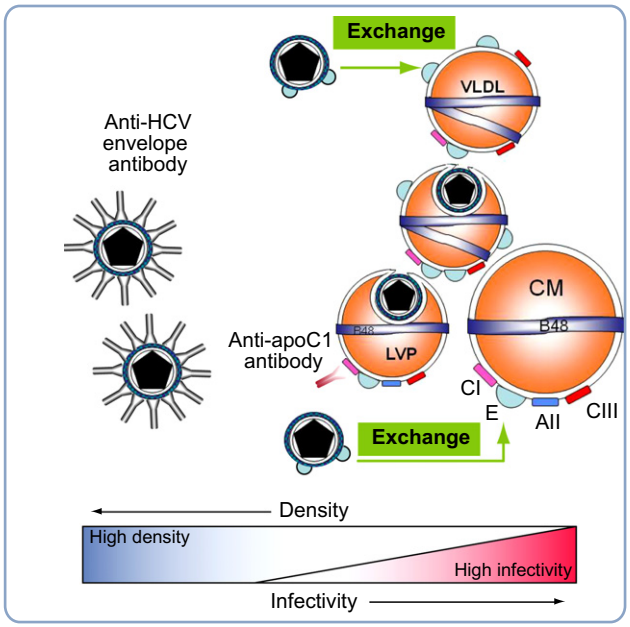

Fig. 5. The buoyant density of HCV particles in human plasma is heterogeneous. High density HCV is associated with immunoglobulins, whilst low density infectious HCV-lipo-viral particles (LVP) contain at least HCV RNA, HCV core protein, TG and VLDL components apoB and apoE. HCV is able to transfer onto TRLs in a manner similar to exchangeable lipoproteins [77]. Antibodies to apoCI are able to neutralise >75% of infectious particles *in vitro* [87]. It is not known whether immunoglobulin-loaded HCV can be exchanged.

believed that HCV co-opts the VLDL assembly, maturation, degradation, and secretory machinery of the cell [48,49] and that the production of infectious HCV virions coincides with the pathway for producing VLDL/TRLs (reviewed in [50]. This virus–host interaction impacts on host lipid metabolism in ways which may be HCV genotype (G) specific, such as induction of hepatic steatosis and hypobetalipoproteinaemia, both of which are more frequent and severe in HCV G3 infection [51,52].

The *in vitro* study of HCV replication has benefitted from the use of the replicon system and, more recently from the infection competent HCV cell culture (HCVcc) system (reviewed in [53]). However, the hepatoma cell lines used secrete relatively dense lipid-poor apoB-containing particles, unlike the buoyant VLDL particles secreted *in vivo* by the human liver [54]. The density profile of HCVcc particles shows an HCV RNA distribution from 1.0 to 1.18 g/ml with no infectivity at densities >1.12 g/ml [55]. It appears that HCV has evolved a mechanism of replication in which lipid droplets, the intracellular storage sites for TG and cholesteryl esters, are used to produce infectious virus. Association of replication complexes with LDs occurs in a core and NS5A-dependent manner [56–58], (reviewed in [59]). Recently, it has been shown that the triglyceride-synthesizing enzyme diacylglycerol acyltransferase-1 (DGAT1), an enzyme involved in lipid droplet biogenesis, is another key host factor for HCV infectious particle production [60].

Although apoB is essential for production of infectious virus [61], HCV infectivity is closely related to the level of apoE present in HCVcc particles [62] and biochemical analysis shows that these particles have 290 ± 41 apoE molecules per viral RNA [63]. Knockdown of apoE by a specific siRNA results in reductions in infectious HCV [64] and an interaction between apoE and NS5A is required for assembly of infectious HCV particles [65,66]. Virion associated cholesterol contributes to the interaction between HCV particles and apoE [67]. Production of HCVcc from serum free culture has shown that these viral particles have a lower level of associated apoE and that lipids conjugated with HCV affect infection and neutralization [68]. Recently, it has also been shown that specific siRNA-mediated downregulation of apoAI leads to a reduction of HCV RNA and viral particles *in vitro* [69].

Infectious HCV particles can also be produced in primary hepatocytes (HCVpc) [70]. Compared with HCVcc, HCVpc had lower average buoyant density and higher specific infectivity, similar to the characteristics of virus particles associated with VLDL that are produced during *in vivo* infection.

The infectivity of hepatitis C viral particles is thus inversely related to their density and low density particles have been termed lipo-viral particles (LVP). LVPs *in vivo* are TG-rich and contain at least viral RNA, HCV core protein, and the VLDL components apoB and apoE [71–73]. It has long been recognised that HCV in serum has a wide range of buoyant densities due to this association with lipoproteins and immunoglobulins [74,75]. The contribution of LVP to total HCV viral load varies widely in a cohort of fasting chronic HCV G1 patients and correlates not only with TG:HDL ratio and HOMA-IR but also with non-response to anti-viral therapy [76]. The buoyant density of HCV particles in human plasma is not only heterogeneous, but also dynamic and dependent on TRL in circulation [77]. Very-low-density HCV (density <1.025 g/ml) associated with TRLs ($VLDL_1$, IDL, CM, and CM remnants) rise 26-fold after a fatty meal and are rapidly cleared from the circulation, rather than entering the lipolytic cascade, suggesting removal via the hepatic TRL receptors [77]. These post-prandial LVP are associated with both apoB100 and apoB48 TRLs [77], confirming a previous study [72]. The marked

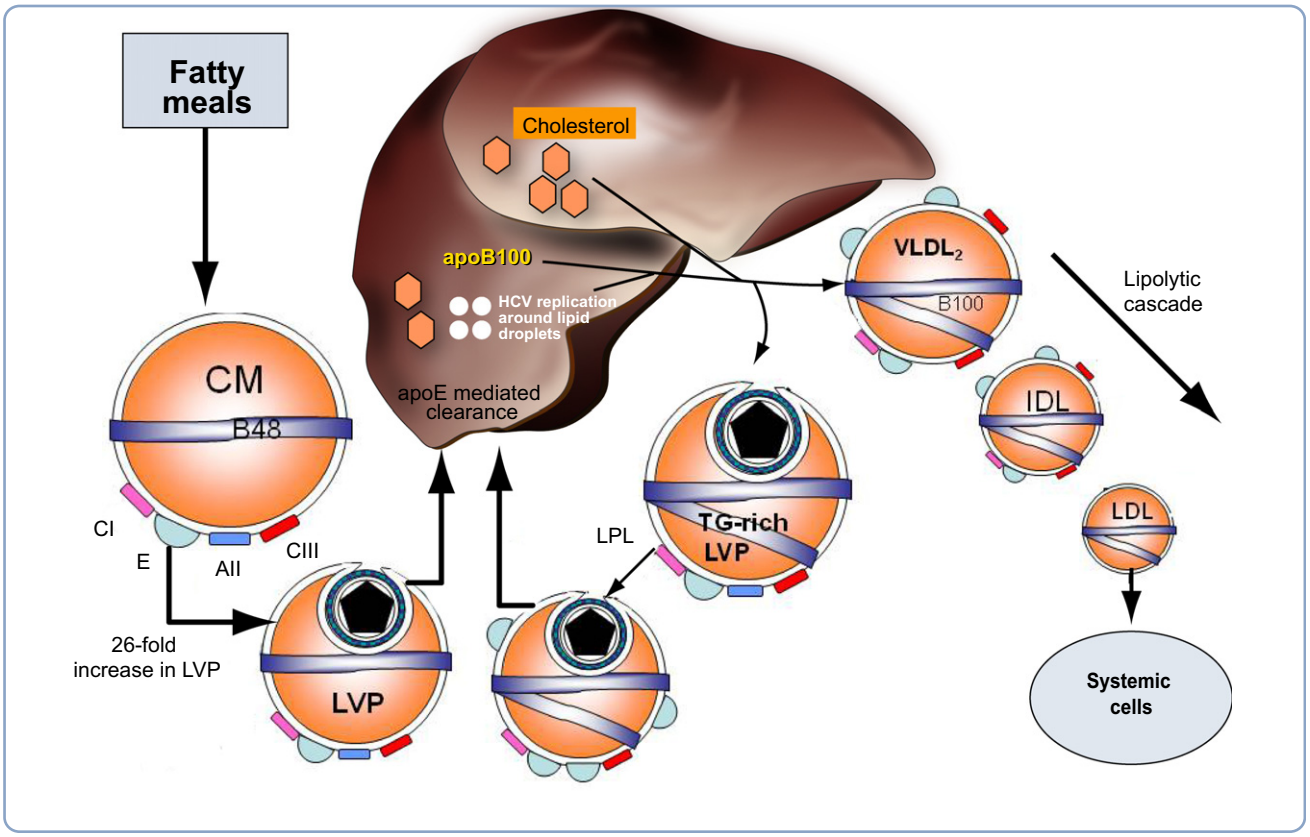

Fig. 6. HCV co-opts the hepatic TRL remnant pathway. After a fatty meal, there is a 26-fold increase in very-low density lipo-viral particles (LVP) which are rapidly cleared from the plasma ($t_{1/2}$ of 95 min) [77].

increase in these putative infectious LVP after a fatty meal appears to be due not only to *de novo* production from infected hepatocytes but also to intravascular transfer onto TRLs in a manner similar to exchangeable lipoproteins (Fig. 5). These studies in patients with chronic HCV suggest that HCV has not only co-opted the VLDL assembly, maturation, degradation, and secretory machinery of the hepatocyte but also utilises the intravascular remodelling of lipoproteins and the rapid removal of TRL remnants by the liver to its advantage (Fig. 6).

HCV entry

The current model for HCV entry involves the initial docking of the virus onto the cell surface through interactions of LVP with cell surface HSPG and lipoprotein receptors (e.g. LDL-R, SR-B1) which lead to conformational change(s) in the viral particle allowing the engagement of other hepatocyte membrane co-receptors (CD81, Claudin 1 (CLDN1) and Occludin (OCLN) (reviewed in [78–80]). HCVcc infectivity is increased 18-fold in Huh-7 cells over-expressing SR-B1 [81] and SR-BI appears to be an essential HCV entry factor [82]. Thus, cellular receptors involved in the uptake of VLDL and CM remnants *in vivo* (HSPG, SR-B1 and LDL-R; Fig. 4) are also implicated as receptors for infectious HCVcc particles. Blocking experiments show that VLDL itself or anti-apoE antibody can block HCV LVP entry [83]. ApoE isoforms have been found to influence infectivity with the suggestion that the apoE2 isoform, which has low affinity for LDL-R, being associated with poor infectivity both *in vitro* [84] and *in vivo* [85]. It is likely that the balance of apoE and apoCs on LVPs is involved in cell entry. ApoCI appears to be involved in infectivity; it is recruited by HCV glycoproteins on the viral surface and promotes fusion of HCV particles with membranes [86]. ApoCI also enhances HCVpp infectivity [87].

The lipid pathway as a therapeutic target in chronic HCV

The current standard of care (SoC) for the treatment of hepatitis C virus (HCV) infection is a combination of pegylated IFN and ribavirin (Peg-IFN/RBV) [88], but this cures around 50% (sustained virological response (SVR) = undetectable HCV RNA for greater than 24 weeks after cessation of therapy). There is hence an urgent need for better treatment and a huge array of potential targets for the development of new classes of HCV therapies [89], which has focused initially on "direct-acting anti-virals" (DAAs) [90]. These represent a major step forward but the emergence of drug-resistant virus in patients with viral breakthrough on treatment needs to be addressed [91,92].

HCV–lipid interactions represent attractive targets for indirect-acting antiviral (IDAA) development because it is more difficult for the virus to develop escape mutations against therapeutics that target host cell factors. In addition, as dyslipidaemia in the metabolic syndrome is central to cardiovascular disease, a number of therapeutic interventions are already in use or under development for this indication [93]. An understanding of how HCV genotypes interact with lipid pathways is

thus central to the use of these treatments "off-label" for a condition not approved in the marketing authorization [94].

Diet and lifestyle modifications

Obesity may be associated with decreased efficacy of current SOC [95]. Modest weight loss optimally decreases plasma TG and LDL-cholesterol via reduction in hepatic apoB secretion [96]. One small unconfirmed study in patients with chronic hepatitis C (CHC) has reported an association of a mean weight loss of only 5.9 kg with a reduction in steatosis and abnormal liver enzymes and an improvement in fibrosis, despite the persistence of HCV [97]. Conversely, high visceral adiposity is associated with both steatosis and high viral load in G1-CHC [98]. Hence, management of dyslipidaemia with diet and exercise may provide an adjunct treatment strategy for obese patients with CHC [99].

Omega-3-fatty acids

Fish oils are a rich source of omega-3-fatty acids, eicosapentaenoic acid and docosahexanoic acid. Fish oils diminish hepatic secretion of VLDL-apoB [96]. Several polyunsaturated fatty acids (PUFA) including eicosapentaenoic acid and docosahexanoic acid dramatically inhibit HCV replication *in vitro* using the HCV RNA replicon system [100]. When combined with IFN-alpha, PUFAs have a strong synergistic anti-HCV effect *in vitro* [101], but no studies have evaluated whether omega-3-fatty acids can provide synergistic antiviral effects when given as food supplements during IFN-based anti-HCV therapy in patients.

Pharmacological interventions

Targeting LVP assembly, maturation and secretion

Lipid lowering drugs differ strongly with respect to the types of lipids or lipoproteins that they predominantly affect. The major classes of drugs that lower cholesterol are statins and cholesterol absorption inhibitors, whilst those that lower triglycerides are fibrates and omega-3 fatty acids. Nicotinic acid has a hybrid position in that it decreases both TG and cholesterol. As a rule, TG lowering drugs increase HDL-cholesterol and nicotinic acid is the strongest HDL raising drug in current clinical use.

Statins

Statins inhibit the *de novo* synthesis of cholesterol in the liver by blocking mevalonate production via inhibition of 3-hydroxy-3-methylglutaryl CoA reductase (HMG CoA reductase) (Fig. 3) and have other pleiotropic effects. Initial *in vitro* studies in genomic and sub-genomic HCV RNA replicons showed that lovastatin markedly reduced HCV RNA levels [102]. This effect was shown to be mediated by inhibiting geranylgeranylation of a host protein FBL2 required for HCV replication [100]. *In vitro,* different statins show a hierarchy of inhibition of HCV replication, with pravastatin being devoid of an anti-viral effect [103,104]. The sensitivity of HCV to an individual statin may also vary [105].

In combination with IFN-α, both fluvastatin and pitavastatin show strong synergistic activity and enhance the anti-HCV effect of IFN-α [103,106]. Statins may be a useful adjunctive therapy with HCV polymerase or protease inhibitors as an additive antiviral effect has been reported with mevastatin or simvastatin *in vitro* in short-term (3 days) antiviral assays [104]. However, the potential for clinically relevant drug–drug interactions between statins and DAAs via CYP450-dependent metabolism [107] could present a problem, similar to that encountered in the treatment of HIV infection with "ritonovir-boosted" protease inhibitors [108].

The clinical data with statins are limited, but indicate that statin monotherapy has little anti-viral effect [109–113], although combination therapy is more promising and adequately powered randomised controlled trials are needed.

The disappointing and sometimes contradictory results using statin monotherapy *in vivo* may be partially explained by dose effects. With conventional doses, the serum concentration of the statin may be 10-fold lower than that found be effective in the HCV replicon systems. Also, besides their effect on HCV replication, statins cause up-regulation of LDL-R and hence may enhance HCV infectivity of hepatocytes, abrogating or even reversing any beneficial effects of monotherapy *in vivo*.

Combining a statin with Peg-IFN/RBV appears more promising [114] (Table 2). In HCV/HIVco-infection, combination of fluvastatin with Peg-IFN/RBV significantly improved the rapid virological response (RVR) rate [115]. In patients with HCV-G1 infection treated in the IDEAL study [116], multivariate logistic regression analysis showed that pre-emptive statin use was independently associated with SVR. This has been confirmed by a large retrospective study utilising the USA Veterans Affairs administrative database where statin use was associated with an improved SVR among both diabetic patients and nondiabetic patients receiving combination antiviral therapy [117].

Peroxisome proliferator-activated receptor (PPAR) agonists

PPARs belong to the steroid/thyroid/retinoid receptor superfamily and are nuclear lipid-activable receptors that control a variety of genes in several pathways of lipid metabolism [118]. In addition to being activated by fatty acids, they respond to fibric acid derivatives and thiazolidinediones.

PPARα agonists

Fibrates decrease hepatic VLDL secretion and enhance clearance, hence reducing plasma TG by 30–50% [119]. They are particularly beneficial in overweight people with high plasma TG levels and low levels of HDL cholesterol (HDL-C). Lower TGs associate with higher rates of SVR in CHC-G1 [120], but there is conflicting data on the use of fibrates in HCV. One *in vitro* study showing binding of HCV core protein to apoAII reported that fenofibrate treatment resulted in a parallel increase in apoAII and core protein secretion [121]. Another found that use of a PPAR-α *antagonist* resulted in disruption of HCV replication complexes [122]. However, a recent study in a sub-genomic replicon model for HCV-induced steatosis shows that fenofibrate reduced ER stress [123] whilst a pilot study in patients has suggested that bezafibrate may be useful in combination with Peg-IFN/RBV [124].

PPAR-γ agonists

Both pioglitazone and rosiglitazone are effective in reducing liver fat content by 30–50% and sensitizing the liver to insulin but they have different effects on serum lipids [125]. Pioglitazone has a beneficial effect on fasting and post-prandial plasma triglycerides and reduces hepatic *de novo* lipogenesis

Table 2. Examples of studies evaluating lipid-modulating therapies *in vivo* and their effect on HCV.

Lipid-modulating drug	Study design	Effect on HCV	[Reference]
Fluvastatin 80 mg daily + SoC	Pilot: (n = 44 HIV/HCV genotype 1 co-infected patients)	Improved RVR (p = 0.02) and trend to improved SVR (p = 0.08)	Milazzo, L *et al.,* 2010 [115]
Use of any statin + SoC	Retrospective analysis of IDEAL study [116] (n = 66 on statin)	Improved SVR (OR = 2.0, p = 0.02)	Harrison, SA *et al.,* 2010 [156]
Use of any statin + SoC	Retrospective analysis of VA database (n = 1704 diabetic patients and 6589 nondiabetic patients)	Improved SVR among both diabetic patients (OR, 1.52; p = 0.0124) and nondiabetic patients	Rao, GA and Pandya, PK, 2011 [117]
Bezafibrate (400 mg/day) for 8 weeks	Pilot study (n = 15)	Fall in viral load	Fujita, N *et al.,* 2006 [124]
Pioglitazone	Randomised controlled (n = 97 HCV genotype 4 patients with HOMA >2)	Improved RVR (p = 0.006) and SVR (p = 0.04)	Khattab, M *et al.,* 2010 [128]
Niacin (and other dietary factors) + SoC	Prospective study of dietary habits (n = 1084)	Improved SVR	Loguercio, C *et al.,* 2008 [135]
Silibinin	Pilot study (n = 16 for 7 days IV and n = 20 for 14 days IV)	Dose dependent suppression of HCV viraemia	Ferenci, P *et al.,* 2008 [138]
Therapeutic silencing of microRNA-122 (SPC3649)	Study in primates	Long lasting suppression of HCV viraemia	Lanford, RE *et al.,* 2010 [149]
SR-BI antagonist, (ITX 5061)	Phase 1 pilot studies (n ≥280 subjects)	Inhibition of HCV entry	Syder, AJ *et al.,* 2011 *(in vitro* data) [160]
Double filtration plasmapheresis + SoC	Pilot study (genotype 2a: n = 2, genotype 1b: n = 10 NR patients)	Improved SVR	Ohara, T *et al.,* 2011 [162]

SoC, standard of care [168]: NR, non-responder; RVR, rapid virological response; SVR, sustained virological response.

[126,127]. Pioglitazone has been used in chronic HCV with the rationale that insulin resistance affects SVR. A study in treatment naïve HCV genotype 4 patients with HOMA-IR >2 has reported increased RVR, SVR and decreased IR in those receiving triple combination therapy (pioglitazone/Peg-IFN/RBV) (Table 2) [128]. The dose and schedule of use of pioglitazone may be important [129], with the suggestion that sequential rather than concomitant use is preferable [130]. In addition the response may be affected by genotype-dependent HCV–host interactions [131]. Further understanding of the effect of these compounds on HCV–lipid interaction in addition to simple insulin sensitisation is required to inform trial design. Measurement of LVP [76] in addition to total HCV viral load and HOMA-IR is likely to be informative.

Niacin (Nicotinic acid)

Niacin is a water-soluble B vitamin (B3) which decreases plasma triglycerides by 25%, increases HDL-C and reduces LDL cholesterol modestly [132]. Its use has been hampered because it can cause frequent flushing of the skin, but addition of a prostaglandin receptor blocker, laropiprant, improves this side effect [133,134]. In patients with CHC G1 there is a strong correlation between fasting LVP and TG:HDL ratio [76] and a post-prandial surge in TG-rich LVPs [77], suggesting that niacin may be beneficial as an adjunct to SoC. This is supported by a recent cross-sectional and longitudinal analysis of HCV RNA viral loads in chronic HCV patients with dyslipidaemia in which hypertriglycerideamia was found to correlate with HCV titres and niacin exposure was associated with significantly lower viral titres [113]. Another study has also reported significant differences in niacin intake between responders and non-responders to interferon therapy [135]. Niacin warrants further evaluation, either alone or in combination with a statin [136] as adjunctive therapy, particularly in patients with HCV genotype 1, body weight ⩾ 85 kg, and high baseline viral load who respond poorly to Peg-IFN/RBV [137].

Silymarin

Silymarin, a mixture of flavolignans extracted from the milk thistle plant *Silybum marianum*, is widely used as a traditional herbal remedy for self-treatment of chronic HCV. The main component of silymarin is silibinin which has been shown to inhibit HCV infection both *in vitro* and *in vivo* [138].The mechanism of action of silymarin is complex but it includes decreasing infectious virion production by blocking MTP-dependent apoB secretion [139]. The plant flavanoid taxifolin, also present in milk thistle, has likewise been shown to decrease hepatic lipid synthesis by decreasing apoB and increasing apoAI secretion [140]. As silymarin-derived compounds may influence HCV disease course in some patients [141], studies where standardized compounds are dosed to identify specific clinical endpoints are urgently needed. Initial case reports using silibinin to prevent graft reinfection after orthotopic liver transplantation are encouraging [142,143].

ApoB antisense

An antisense inhibitor of apoB synthesis, mipomersen, has recently been shown to be effective and safe as an adjunctive agent to lower LDL cholesterol concentrations in patients with familial hypercholesterolaemia [144,145]. Mipomersen contains an endonuclease resistant modified sequence of nucleotides complementary to ApoB mRNA which binds to and inhibits the translation of the encoded sequence into the mature protein, reducing the synthesis and secretion of the apolipoprotein B lipoproteins VLDL and LDL [146]. As silencing *ApoB* mRNA in the HCVcc system causes a 70% reduction in the secretion of both ApoB100

and HCV [49,61,147], this novel drug may warrant evaluation in chronic HCV infection.

MicroRNA-122 inhibition

MicroRNAs (miRNAs) are small noncoding RNAs that regulate gene expression at post-transcriptional level. Some miRNAs have been associated with lipid metabolism; for example *miR-122* inhibition in normal mice results in reduced plasma cholesterol levels [148]. Recent work has shown that therapeutic silencing of *miRNA-122* in primates with CHC leads to long lasting suppression of HCV viraemia [149].

Novel targets

3β-Hydroxysterol Δ^{24}-reductase (DHCR24) inhibitors

DHCR24 is an enzyme in the cholesterol biosynthetic pathway, converting desmosterol to cholesterol [150]. This enzyme is induced by HCV infection in human hepatocytes *in vitro* and an inhibitor of DHCR24 has recently been shown to have an antiviral effect in chimeric mice with HCV infection in their humanised liver [151].

Diacylglycerol acyltransferase 1 (DGAT1) inhibitors

DGAT1 interacts with HCV core and is required for its trafficking to lipid droplets. Inhibition of DGAT1 activity or RNAi-mediated knockdown of DGAT1 severely impairs infectious virion production, implicating DGAT1 as a new target for antiviral therapy [60].

Long chain acyl-CoA synthetase 3 (ACSL3) inhibitors

ACSL3 is required for incorporation of fatty acids into phosphatidylcholine, a reaction that is essential for VLDL assembly. It has been shown that secretion of VLDL as well as HCV is inhibited when expression of ACSL3 is reduced by RNA interference [152]. This study identified ACSL3 as a new enzyme required for VLDL assembly, confirmed the link between VLDL assembly and HCV production and suggested that ACSL3 is a new enzymatic target for limiting both VLDL secretion and HCV infection.

Polyunsaturated liposomes (PERLS)

Liposomes capable of ER-targeted drug delivery have been developed [153] and polyunsaturated ER-targeting liposomes have been reported to decrease secretion and infectivity of HCVcc [154]. PERLS may target multiple points in the lipid pathway, not only lowering intracellular cholesterol and LD number but also competing with lipoproteins for cell entry.

Targeting intravascular re-modelling of LVP and clearance via TRL receptors

The demonstration that HCV particles behave in a manner similar to exchangeable lipoproteins and transfer onto TRLs [77] opens up further potential targets once the pathophysiology of this process is understood. Drugs that lower TG such as niacin or target LD biogenesis may be more effective *in vivo* than interventions aimed at lowering LDL via the cholesterol pathway such as statins. Indeed high LDL-cholesterol is associated with SVR in patients receiving Peg-IFN/RBV [155,156] whilst high TG is associated with both viral load and hepatic steatosis [120].

CETP inhibitors

Successful HCV infection of SCID/uPA mice transplanted with human hepatocytes correlates with expression of markers of human lipoprotein biosynthesis, human apoB and CETP, suggesting that CETP may be involved in the infectious cycle of HCV [157]. This raises the intriguing question of whether small-molecule CETP inhibitors (dalcetrapib, torcetrapib, and anacetrapib) which have been or are being tested in phase 3 clinical studies, may be of benefit in chronic HCV [158].

HCV entry inhibitors

HCV entry inhibitors would limit the expansion of the infected cell reservoir and complement other approaches to therapy. SR-BI appears to be an essential HCV entry factor [82]. SR-B1 residues involved in HCV recognition are not required for HDL binding or SR-BI-mediated cholesterol efflux, suggesting that the development of agents selectively inhibiting HCV infection, but with no or low impact on the reverse cholesterol pathway, is a feasible task [46,159]. Recently a small molecule SR-BI antagonist, ITX 5061, has been shown to have potent antiviral activity against HCVpp and HCVcc [160]. This orally active agent is now being evaluated in the clinic in chronic HCV patients and patients undergoing liver transplantation.

Double filtration plasmaphoresis (DFPP)

Heparin-induced extracorporeal low-density lipoprotein precipitation (HELP) apheresis is an effective tool to eliminate apoB containing lipoproteins from the circulation and has been found to reduce HCV–RNA by 77% [161]. DFPP is a similar technique which uses a plasma separator (first filter) to separate plasma and blood cells from blood and a plasma fractionator (second filter) to eliminate high-molecular-weight substances. DFPP can mechanically eliminate not only lipoproteins but also HCV from the blood. It was approved in Japan in 2008 for the retreatment of chronic HCV G1b patients with high viral loads but has similar effects in chronic HCV G2 infection [162]. DFPP may be useful in combination with IFN in some patients, especially those with cryoglobulinaemia [163,164] but there are safety issues as DFPP also decreases other plasma proteins including factor XIII [165].

In summary, HCV–host interaction is a continuous co-evolutionary process and phylogenetic analysis of 345 full-length HCV genomic sequences suggests different evolutionary ages of the major HCV genotypes [2]. It is now clear that the equilibrium established between HCV and the host in chronic infection involves not only the immune response but also lipid pathways, some of which may be genotype-specific. Better understanding of how HCV utilises these pathways in its life cycle will lead to more options for therapy. These include not only lipid-modulating drugs already in use or in development for other indications [e.g. statins, niacin, CETP inhibitors, apoB antisense, *miRNA-122* inhibitors] but also entry inhibitors [e.g. SR-BI antagonists] and novel targets [e.g. diacylglycerol acyltransferase 1 inhibitors, long chain acyl-CoA synthetase 3 inhibitors].

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Key Points

- One of the strategies HCV has used to establish persistent infection is to utilise lipid pathways.
- HCV has evolved a mechanism of replication in which lipid droplets, the intracellular storage sites for triglyceride (TG) and cholesteryl esters, are used to produce infectious virus.
- HCV particles produced in primary human hepatocytes have lower buoyant density and higher specific infectivity than HCV particles produced in Huh7 hepatoma cells, similar to the characteristics of virus particles associated with VLDL that are produced during *in vivo* infection. These infectious lipo-viral particles (LVP) are TG-rich and contain at least viral RNA, HCV core protein and the VLDL components apoB and apoE.
- Understanding the complex interaction of HCV with lipid metabolism, which may be genotype dependent, will lead to a number of possible new indirect-acting antiviral (IDAA) treatment options. These include not only lipid-modulating drugs already in use or in development for other indications but also LVP entry inhibitors and novel targets.
- IDAAs have the advantage that it is more difficult for HCV to develop escape mutations against therapeutics that target host factors. They may be useful as adjunctive therapy in chronic HCV, either with current standard of care or with the new classes of DAAs.

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