Review

Liver-derived lipoproteins and inflammation: from pathophysiology to pharmacological targets in metabolic liver disease

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Abstract

Low density lipoproteins (LDL) reduction remains the key goal for reducing the risk of atherosclerotic cardiovascular diseases (CVD) in people with high residual risk and metabolic complications including liver disease. Notwithstanding, epidemiological projections support a key role of liver-derived apolipoprotein B (ApoB) containing lipoproteins, namely very low density lipoproteins (VLDL) and their "remnants" (TG), undergoing the activity of lipases, in eliciting atherosclerotic inflammatory sequelae of a comparable order of magnitude to that of LDL. Disparate experimental evidence supports that triglycerides (TG), residual cholesterol content, or the large apolipoprotein set on the surface of these lipoproteins can elicit a number of plausible immune-inflammatory mechanisms that foster the vascular atherosclerotic process. Therapeutic options that convincingly lowered the plasma levels of liver-derived ApoB containing lipoproteins, either by reducing the hepatic synthesis or by improving the peripheral lipolysis of the lipid content, did not exert robust CVD risk reduction, and the effect on inflammation was questionable. Understanding the mechanisms linking liver-derived lipoproteins with chronic inflammation will provide pathophysiological insights for the identification of new therapeutic targets for people at high CVD risk and with metabolic complications. In this perspective, this topic is of immediate interest for the prevention of CVD in patients affected by non-alcoholic fatty liver disease (NAFLD) and, even more, for NAFLD patients with diabetes, insulin resistance, or other comorbidities (metabolic-associated fatty liver disease). This review resumes the principal physio-pathological insights regarding the metabolism of liver-derived lipoproteins and provides an update on the current pharmacological options that can be considered for improving CVD

prevention in metabolic liver diseases.

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a prevalent disease that increases the risk of cardiovascular disease (CVD) as compared to other liver diseases (e.g., infective liver disease)[1-3]. In the presence of systemic metabolic complications [i.e., metabolic-associated fatty liver disease (MAFLD)[4]], the CVD risk is even higher. MAFLD has predominate causes from nutrition overload to altered systemic metabolism and inflammation[3,4], together with persistent liver damage that eventually lead to the development of liver fibrosis and cirrhosis.

Mechanistically, the development of MAFLD encompasses complex molecular aspects which intertwine in pathological processes starting from alteration in lipid metabolism and pro-inflammatory activation[5]. All together, these mechanisms foster oxidative stress, cell apoptosis, and extracellular matrix formation up to the fibrogenesis process.

Alterations in lipid metabolism, defined as over-production of triglyceride-rich liver-derived lipoproteins [very low density lipoproteins (VLDL)], physiologically occurs in humans following consumption of a high-fat based meal (postprandial lipemia), but, in people with metabolic complications (including MAFLD), this is dramatically exacerbated[6-8]. This iterative process over time promotes a constantly elevated amount of VLDL, which over-engages the activity of peripheral lipases [lipoprotein lipase (LPL) and hepatic lipase (HL)], which are in charge of the lipolysis of the triglyceride (TG) content of VLDL[8].

Hence, the accumulation of VLDL will turn into a relative increase in the amount of remnant cholesterol in the downstream products of the VLDL, which are low density lipoproteins (LDL).

Mechanistically, both VLDL and LDL separately, which share apolipoprotein B (ApoB) on their surface, exert inflammatory and potent pro-atherogenic processes, which are discussed in this review. In response to the wealth of evidence from pre-clinical studies, the reduction of LDL is the first goal effectively outreached for reducing the risk of atherosclerotic CVD in people with high residual risk. By contrast, the pharmacological strategies thus far available are unable to provide a comparable magnitude of VLDL reduction[9,10].

Hence, the understanding of the alterations in lipid metabolism as a primum movens for these pathological sequelae is of immediate interest for NAFLD/MAFLD, by contrast to other types of liver disease. In fact, an in-depth study of these aspects might help to pave the road towards the development of future strategies controlling the over-production of VLDL and to more effectively reduce the entire set of atherogenic ApoB-containing liver-derived lipoproteins.

In this review, the concept of liver-derived lipoproteins and its relevance in inflammation and CVD are described and critically analyzed. Subsequently, this review summarizes in detail the pharmacological strategies and pipeline that are being tested and currently under development for future consideration in the prevention of CVD in metabolic liver disease.

THE METABOLISM OF LIPOPROTEINS DURING THE POSTPRANDIAL ENERGETIC CHARGE

Evolution developed the mechanism by which our body stores energy following consumption of a high fat meal and ensures a proper disposal in the case of long-term starvation. TGs are the principal forms of lipids that ensure an elevated potential energy per molecule as, when they are mobilized by LPL from visceral adipose tissue, they are fuel for high-energy-producing oxidative metabolism in high energy-demanding metabolic tissues, including the heart and skeletal muscle. Lipoproteins are key drivers of TG mobilization as, by virtue of their lipophilic nature, they cannot freely circulate in blood. Chylomicrons are the intestinally derived lipoproteins that increase in quantity immediately following the ingestion and absorption through the duodenal villi of the lipid content of a meal (no longer than 1 h[11]). Following arrival to the liver, the lipid material is immediately distributed to hepatocytes, which subsequently prepares this lipid material into another lipoprotein structure, i.e., VLDL. VLDLs are produced in a higher order of magnitude as compared to chylomicrons and, per particle, carry a TG as well as a proportion of cholesterol, which derives from the hepatic production pool. Both chylomicrons and VLDL undergo the activity of endothelium-bound lipases (both LPL and HL) to hydrolyze their triglyceride (TG) content, with VLDL being a preferential substrate of LPL and HL. A fine tuning of this enzymatic flow is essential to ensure the distribution of lipid energy sources to tissues. In fact, the activity of LPL and HL residing in visceral adipose tissue and the liver is enhanced following ingestion of a high fat meal, while it decreases in other oxidative tissues (e.g., skeletal muscle); conversely, in the fasting state, this ratio is the opposite, favoring the oxidative utilization and energy expenditure. These energetic flows among these metabolic sites are iterative over time, since the elevation of VLDL occurs in the so-called postprandial lipemia (PPL[12]), a physiological situation in which people from western societies spend the majority of their daily life, according to epidemiological projections. In fact, PPL has been recently described to last 6-8 h[13] following the consumption of a high fat meal (20-40 g of fats/meal) in affluent societies[14,15].

VLDL, although with a lower relative amount of TG as compared to chylomicrons, represents the predominant mediator of the energetic exchanges during PPL and over time, being higher in number as compared to chylomicrons and the preferential substrate of LPL. In addition, the iterative postprandial situation increases VLDL (which stays in circulation for 4-13 h on average)[16] [Figure 1]. As soon as VLDL undergoes the activity of lipases, it becomes smaller in size (from 30-70 to 20-25 nm in diameter) and with a higher residual proportion of remnant cholesterol (from 15% to 65% per particle)[16] [Figure 1]. This passage promotes the increase of lipoprotein density and makes the original VLDL remnant intermediate lipoproteins, and then low density lipoproteins (LDL). In the long term, the much higher half-life of LDL than that of VLDL (on average, 2.5-3.5 days for LDL [Figure 1]) explains the greater abundance of LDL particles, estimated at around 3-10 LDL per each VLDL in most individuals[16]. The quantity of cholesterol carried by these lipoproteins is much higher compared to that coming from the intestine. In fact, out of 20-40 g of fats/meal, the actual content of cholesterol in the majority of foods is 4-700 mg per quantity of food consumed[17,18]. It is therefore evident that the dietary source of cholesterol is minor as compared to the quantity of cholesterol that is continuously re-cycled through the complex enterohepatic circulation, under the regulation of farnesoid-X receptor (FXR)[19].

LIVER-DERIVED LIPOPROTEINS AS POTENT CARDIOVASCULAR RISK PREDICTORS IN CLINIC

The clinical evidence, as does the epidemiological projection, supports that it is this exchange of cholesterol among lipoproteins that matters in the development of the atherosclerotic process. In fact, hyperchylomicronemia (the condition of elevated triglyceride rich, intestinally-derived chylomicrons in fasting and during PPL), as with severe hypertrigliceridemia (TG over 880 mg/dL), results in pancreatitis,
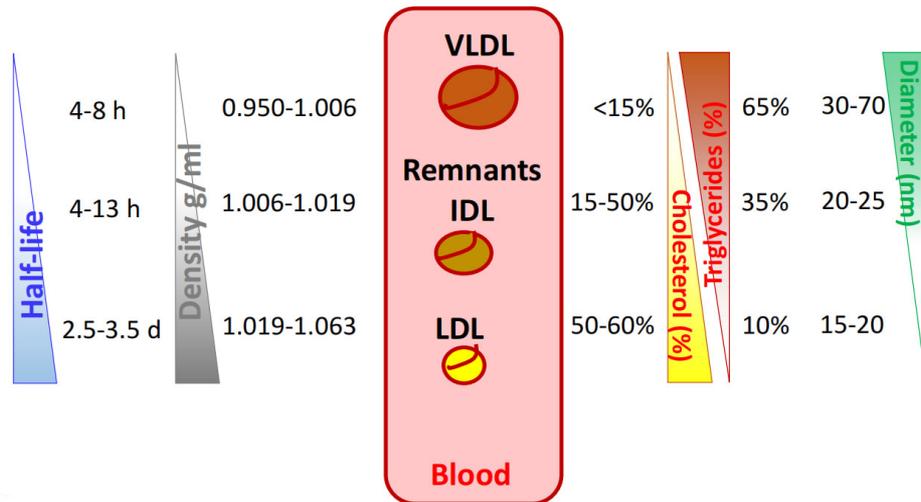
Figure 1. Half-life, density, cholesterol, triglyceride relative content, and diameter of liver-derived lipoproteins. This figure summarizes the ranges (average) of (from left to right) half-life, density, cholesterol, triglycerides relative content (percentage), and diameter of VLDL, Remnant/IDL, and LDL. ApoB: Apolipoprotein B; VLDL: very low density lipoproteins; IDL: intermediate density lipoproteins; LDL: low density lipoproteins.

metabolic complications, and liver alterations, but it does not increase CVD risk[20-22]. Mechanistically, in fact, large diameter chylomicrons (> 80 nm) and VLDL of immediate liver secretion (60-80 nm) are not able to transmigrate over the vascular endothelial layer. Conversely, CVD risk triples as TG increases from 250 to 450 mg/dL, indicating that only VLDL remnants and cholesterol-enriched LDL, the entire set of apolipoprotein B (ApoB)-containing lipoproteins with less TG content and with diameter less than 60 nm[23,24], can penetrate in the endothelial layer.

ApoB, by reflecting both the acute raise of triglyceride-rich ApoB-containing lipoproteins during PPL and the chronic accumulation of cholesterol, represents the key target of clinical situations associated with high CVD risk and characterized by elevated production of liver-derived lipoproteins.

ApoB is the protein structure present in equimolar ratio per each lipoprotein in VLDL, LDL, and all remnants, and it is in charge of interacting with receptors and the internalization of lipoproteins in peripheral tissues[25-27]. Statins, which inhibit the synthesis of cholesterol in the liver, lower LDL-C more than non-HDL-C and relatively more than the molar quantity of ApoB[28], indicating that reduction of cholesterol is not sufficient to control the hepatic secretion of all the ApoB-containing lipoproteins. Elegant Mendelian randomization studies that mimic the effects of CETP inhibitors and statins, by combining variants in the cholesterol ester transfer protein (*CETP*, in charge of exchanging cholesterol between VLDL and high density lipoproteins) and the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR, the rate limiting step enzyme for cholesterol synthesis) genes (to create genetic scores) robustly affirmed the relevance in targeting ApoB, as a unique direct marker of both LDL and liver-derived VLDL and remnants[29]. In fact, a CETP score at or above the median was associated with lower levels of LDL-C, lower ApoB, and consistently lower CVD risk. This effect was similarly exerted by an HMGCR score at or above the median, which was associated with lower levels of LDL-C, ApoB, and CVD risk as well. However, the effect of both scores together was additive for LDL-C but not for ApoB or CVD risk. Indeed, the reduction of LDL-C in people harboring both scores equated to the sum of each independent score, although the extent of the reduction in ApoB and CVD risk were attenuated in people harboring both scores *vs.* those achieved by a single score[29]. Thus, Mendelian randomization indicates that the primary mechanism of benefit from

lowering LDL-C relates to the lowering of the number of LDL particles, i.e., to the lowering of ApoB. Hence, ApoB unifies, amplifies, and simplifies the information from the conventional LDL-C lipid marker as to the atherogenic risk attributable to the liver-derived lipoproteins.

Familial combined hyperlipidemia (FCHL), a polygenic situation of elevated liver-derived ApoB particle production, reduced TG hydrolysis, resulting in liver steatosis, metabolic complications, and elevated CVD risk. Conversely, familial combined hypolipidemia (FHBL2), a rare genetic condition (OMIM#605019) driven by deficiency of angiopoietin-like 3 (Angptl3), a natural inhibitor of peripheral lipases, results in an improved postprandial response to fatty meal, minimal liver-derived lipoprotein production, and neutralized CVD risk[30,31]. Similarly, non-alcoholic fatty liver disease (NAFLD), where PPL increases because of elevated quantity of liver-derived VLDL, results in elevated susceptibility to CVD[1,2], which ranks as the first comorbidity, even before that of extrahepatic malignancies and liver-related complications[32,33]. Furthermore, when systemic metabolic complications are also present (i.e., MAFLD)[34], the peripheral hydrolysis of TG in VLDL by lipases is also reduced[6-8], further increasing CVD risk. The hardwired pathophysiological connections in MAFLD, however, complicate the understanding of the metabolism of lipoproteins as the primum movens. Indeed, the excess caloric intake is the first mover in promoting the systemic inflammation associated with metabolic complications and insulin resistance. In this scenario, liver-derived lipoproteins might act as both mediators of the link caloric excess-inflammation and simply an epiphenomenon of altered handling of the lipid energetic sources in liver and adipose tissue.

THE INFLAMMATORY POTENTIAL OF LIVER-DERIVED LIPOPROTEINS

Around 75 years ago, Mereton wrote that "*the lipid particles must be assumed to be retained and deposited from the plasma-derived nutrient lymph stream which normally passes from the lumen through the intramural structures towards the adventitial venules and lymphatics. It may be theorised that the increased particle size of the lipids in sustained or alimentary hyperlipemia is the stimulus to the phagocytosis in the intima by macrophages and the formation of the typical foam cells*"[35,36].

This pioneering concept anticipated the subsequent data indicating that ApoB containing VLDL is surveilled by immune-inflammatory checkpoints, and, by entering the sub-endothelial layer of vasculature, they directly contribute to the inflammatory mechanisms, including cholesterol deposition and pro-thrombotic effects[37-42], involved in the progression of atherosclerosis. Within these mechanisms, however, the experimental evidence produced thus far still questions whether it is the ApoB lipoprotein per se, or it is more likely their content of the aryl carbon chains of TG (fatty acids) or that of cholesterol[43].

Mechanisms elicited by TG in liver-derived lipoproteins

Fatty acids in lipoproteins can be medium chain (6-12 carbons) and long chain (up to 22 carbons) and can be saturated (SFA), monounsaturated (MUFA), or polyunsaturated (PUFA). Among PUFAs, those with a first double bond on the third carbon are referred to as n-3, whereas those with a first double bond on the sixth carbon are called n-6.

SFAs stimulate the inflammatory activation of macrophages by a process that involves toll-like receptor 4 (TLR4), a pattern recognition receptor that plays a key role in the innate patrolling of bacterial pathogens, including lipopolysaccharide (LPS). In fact, the activation of TLR4 by SFA induces an over-activation of IL-6 and TNF-α inflammatory genes through a nuclear factor-κB (NFκB)-dependent mechanism[44,45]. The TLR4-mediated engagement of NFkB is tightly linked with the activation of NLRP3 in macrophages[46]. Similarly, *in vitro*, DHA and eicosapentaenoic acid (EPA, an n-3 PUFA with 20 carbons) inhibit the LPS-induced gene expression of cyclooxygenase-2 (COX-2), which is instead increased by treatment with

lauric acid[47]. Furthermore, *in vitro* data suggest that inflammatory pathways are triggered by different fatty acids[48-54] as a direct function of their degree of saturation, while others are in contrast[55].

Mechanisms elicited by cholesterol in liver-derived lipoproteins

Cholesterol can be oxidized into different types of oxysterols by a number of cardiovascular risk determinants as well as by factors[56,57]. Oxysterols contribute to the formation of modified LDLs [namely, oxidized LDL (oxLDL)], which are taken up by macrophages in the atheroma. Within cells, the crystallization of excess cholesterol occurs, further increasing its atherogenic potential and the ability to evoke the inflammatory activation of effector inflammatory lymphocytes[58] and the induction of the inflammasome (NOD-like receptor protein 3) complex[59,60]. Acute exposure of macrophages to oxLDL prolongs these mechanisms by inducing epigenetic priming of a complex set of inflammatory players[61]. In addition, NLRP3 undergoes this epigenetic long-lasting activation, a process that has been described to favor an inflammatory phenotype of the myeloid hematopoietic immune compartment[62].

The apolipoprotein content of liver-derived lipoproteins

By *in vivo*[18] fluoro-deoxyglucose position emission tomography (PET) blood labeling to track metabolic circulating leukocytes during vascular inflammation, it was demonstrated that elevated hepatic VLDL and their remnants (such as in the case of FCHL), despite lower levels of LDL-C, elicit arterial inflammation compared with subjects with familial hypercholesterolemia (a genetic condition of unique elevated LDL-C) [63,64]. In addition, studies suggest that elevated TG and remnant cholesterol levels are causally related to whole body low-grade inflammation, in contrast to LDL-C[22]; more recently, a significant association of vascular inflammation with TG levels ≥ 150 mg/dL has been reported versus lower TG, independently of LDL-C alone[65].

These clinical observations recapitulate findings ex vivo as, per particle basis, cholesterol-rich liver-derived lipoproteins are more potent inducers of macrophage inflammatory foam cells than LDL alone and do not need structural modification to trigger uptake[66-68].

Human endothelial cells stimulated *in vitro* with fasting VLDL (concentration of 50 μg/mL in ApoB) isolated from patients with hypertriglyceridemia showed an exaggerated expression pattern of multiple inflammatory and adhesion molecule (VCAM-1 and PECAM-1). These mechanisms are even further induced by stimulation of these cells with VLDL for 4 h during PPL[69]. At the same time point, VLDL induced *in vivo* an increased number of circulating leukocytes[69], intracellular lipid accumulation[70], and cell activation, leading to adhesion to endothelium, thus suggesting that the endothelial activation during PPL is associated with immune response switching[70,71].

Furthermore, the inflammatory potential of liver-derived lipoproteins is supported by elegant pre-clinical studies showing that improving their catabolism (by improving the expression of their hepatic receptors via gene therapy approaches) would result in a significant regulation of the systemic inflammation, thus an improvement of metabolic fitness and cardioprotection[72].

In addition to ApoB, a large set of apolipoproteins characterize the membrane of liver-derived lipoproteins. In fact, the proteome of liver-derived lipoproteins includes other apolipoproteins (smaller-sized as compared to ApoB), including ApoCIII, ApoCII, ApoCI, ApoAIV, ApoAV, and ApoE, as well as regulators of the LPL activity, including ANGPTL-3, -4, and -8.

Of these, ApoE is a physiological mediator of the uptake of liver-derived lipoproteins by peripheral tissues and macrophages[73]. In humans, ApoE is highly polymorphic, and a relatively frequent isoform (ApoE-4 isoform[74]) causes defective uptake of liver-derived lipoproteins and plasma cholesterol increase; is associated with inflammation; increases phagocytosis and foam cell formation; alters efferocytotic activity; increases antigen presentation potential[75]; and favors fibrous cap thinning due to activation of metalloprotease expression[76]. In addition, ApoE deficiency in mice translates into an increased distribution of GM-CSF receptors by hematopoietic stem cells in the bone marrow, leading to increased and inflammatory myelopoiesis[77,78].

Angptl3 is another protein component of liver-derived lipoproteins which recently appears to be involved in inflammatory mechanisms. Angptl3 physiologically inhibits LPL and EL and, when over-expressed/activated, significantly reduces the hydrolysis of TGs, limiting the distribution of FAs to peripheral cells, including endothelial cells. By contrast to other angiopoietins on liver-derived lipoproteins (Angptl-4 and -8), Angptl3 is active during PPL, sensing the postprandial increase of liver-derived lipoprotein production under the regulation of liver X receptor (LXR)[79].

Reduced medullary hematopoietic homing was found in Angptl3-null mice[80], a finding not confirmed by Angptl3 gene editing on hematopoietic stem cells in hypercholesteremic mice (on a hypercholesterolemia background due to LDL receptor deficiency[81]). By contrast, hematopoietic stem cells transplanted in Angptl3-null recipients exhibited impaired repopulation[80,82]. Hence, although a tropism for the development of EPCs has peculiarly not been elucidated, it is plausible that Angptl3 acts as regulator of the hematopoietic stemness, dependent on the quantity of liver-derived lipoproteins.

LOWERING LIVER-DERIVED LIPOPROTEINS TO REDUCE INFLAMMATION: WHICH EVIDENCE SO FAR?

Statins (inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A, a rate-limiting step of cholesterol synthesis) and fibrates [agonists of peroxisome proliferator-activated receptor alpha (PPAR-α) that reduce liver-derived lipoprotein assembly, enhances LPL activity, and increases fatty acids oxidation in the liver] currently dominate the pharmacological perspective to reduce liver-derived lipoproteins.

Statins consistently reduce by up to 15% the basal TG levels per each -50% in LDL-C content in every population and degree of residual CVD risk they have been tested (merely because of a mass effect in reducing the quantity of LDL which carries this TG quantity per particle[83]). Consequently, the efficacy of statins in metabolic liver disease is indisputable. In addition, statins exert the same beneficial role in secondary prevention of CVD, being an optimal start of treatment for patients with metabolic liver disease with still clinical manifestation of atherosclerosis. In addition, statins are safe for patients with different degrees of NAFLD and MAFLD, starting from those with mild baseline elevation in transaminases (< 3× upper limit of normal (ULN)) up to those with compensated cirrhosis[84-89]. Some evidence is available also regarding the potential benefit of statins in reducing the degree of liver fibrosis[90], although more data are needed.

Besides statins, fibrates effectively lower TG across the range of TG levels but only modestly reduce ApoB levels. In addition the cholesterol reduction of fibrates differs between moderate hypertriglyceridemia (TG 150-500 mg/dL, where LDL-C is within normal ranges according to guidelines) and severe hypertriglyceridemia (TG 500-880 mg/dL, where LDL-C is increased, albeit from a low baseline level[24,91,92]). Bezafibrate and fenofibrate demonstrated beneficial effects on both lipid metabolism and liver function in patients with advanced metabolic liver disease and NASH. By contrast to statins, however, the

atheroprotective effect of fibrates has been more questioned. More probable beneficial effects in liver histology have been indicated for fibrates. In fact, fenofibrate treatment in patients with advanced MAFLD and NASH decreased transaminases together with hepatocellular ballooning evaluated by biopsy; by contrast, short-term treatment with bezafibrate appeared to reduce microvesicular steatosis[93]. Conversely, no beneficial effects in reducing local tissue inflammation and fibrosis were reported following treatment with fibrates. In addition, the atheroprotective effect of fibrates has been generally questioned. Indeed, while experimental data show mice models of atherosclerosis treated with fibrates benefit from reduced monocyte/macrophage infiltration in the atheroma[94], less is clear regarding data in humans[95,96]. The ACCORD study, combining the use of fenofibrate with simvastatin, failed in providing evidence of an anti-atherosclerotic benefit as compared to the treatment with statin alone in patients with type 2 diabetes, metabolic complication, and atherogenic dyslipidemia[97,98]. These data are of particular interest for figuring out the possible efficacy of this combination in NAFLD/MAFLD patients where atherogenic dyslipidemia is generally combined with such comorbidities[99]. Pemafibrate, a recently developed selective peroxisome proliferator activated receptor α modulator (SPPARMα), was reported to exert beneficial effects on liver function among patients with atherogenic dyslipidemias[100,101], potentially regulating the ratio between circulating SFAs and PUFAs[100]. However, a large multi-center, phase 3 study investigating the effects of pemafibrate on the risk of occurring CV events in high-risk patients with type 2 diabetes, atherogenic dyslipidemia, still treated with maximally tolerated statins (PROMINENT), was stopped very recently following recommendations of futility[102].

Apart from these classical strategies and the pemafibrate experience, further options to reduce the burden of liver-derived lipoproteins are currently in the pipeline, harnessing forefront biotechnological techniques[9].

Inhibiting liver-derived lipoprotein production
Drugs such as mipomersen [an antisense oligonucleotide (ASO) inhibitor of ApoB translation] and lomitapide [an inhibitor of microsomal triglyceride transport protein (MTTP) activity] block either ApoB synthesis or the addition of lipid during chylomicron and VLDL assembly in the intestine and liver, respectively. However, the initial clinical use of these compounds found that they both promote hepatic TG accumulation and possible development of NAFLD. Despite representing a valuable alternative in rare severe statin resistant hypercholesterolemia, it is questionable whether their long-term use could be considered in FCHL or the presence of metabolic complications.

Hence, novel or combination therapies that inhibit the assembly of apoB lipoproteins and protect against excess intracellular lipid by promoting FA oxidation or decreasing TG synthesis are needed.

Reducing TG availability for VLDL assembly
High-dose omega-3 FA (3-4 g/day, usually the combination of DHA and EPA) reduces TG and apoB secretion by ~25%-30% and promotes to a very variable extent the peripheral catabolism[103,104]. However, some studies showed an increase in conversion of VLDL to their remnants and LDL; hence, omega-3 FA may have limited impact on remnant populations or the total number of atherogenic lipoproteins.

By contrast, a new formulation of high-dose EPA (icosapent ethyl) induced potent reduction of CVD risk in high-risk patients still on aggressive ongoing statin treatment[105]. Of note, in the same trial, icosapent ethyl provided up to -37% reduction at the end of a 5-year follow-up in high-sensitivity C-reactive protein (CRP), a liver-derived marker of systemic inflammation. In secondary analysis of the same trial, icosapent ethyl was effective in reducing the risk of CVD independently from the presence of diabetes and in the presence of atherogenic dyslipidemia, supporting its potential consideration in NAFLD/MAFLD. Besides, it is questioned whether the actual effect of this EPA formulation was more likely induced by the reduction in

liver production of ApoB lipoproteins rather than the peripheral catabolism of their remnants and LDL. Kinetic studies regarding the catabolism of these fractions are required.

Enhancing the peripheral clearance of liver-derived lipoproteins
This option is actually not addressed by fibrates, which, despite promoting the expression and activity of LPL, failed to provide benefit in reducing inflammation and CVD risk[97,98].

The inhibition of ApoCIII and Angplt3, two important down-regulators of LPL activity on the surface of liver-derived lipoproteins, have more recently been addressed by pharmacological research as promising alternatives. An ASO targeting *APOC3* gene markedly reduced plasma TG levels in severe hypertriglyceridemia, an effect equally evident in the absence of LPL activity[106,107].

More robust reductions on TG, cholesterol, and ApoB plasma levels have been reached via the inhibition of Angptl3, by both monoclonal antibodies[108] and ASO therapy reducing the synthesis of the protein in patients with metabolic complications and liver disease[109]. Of note, regarding the second option, the robust effect on TG levels in patients with hepatic steatosis was not associated with rebound accumulation of TG in the liver, in contrast to other biotechnological drugs directed to alternative targets (e.g., mipomersen and lomitapide). Long-term studies addressing the anti-atherosclerotic potential of Angplt3 inhibition will be seminal to provide evidence for the hypothesis of this therapeutic strategy in high-risk patients with metabolic complications, including NAFLD/MAFLD.

Further pharmacological options and drugs in the pipeline
Glucagon-like peptide-1 (GLP-1), an incretin hormone produced by intestinal L cells and the brain, is physiologically released during the postprandial phase to stimulate glucose-dependent insulin secretion by the pancreas. The agonism of its receptor (GLP-1R) has been considered in diabetology, with successful data regarding atheroprotection[110]. Additionally, given the less efficient activity of insulin in regulating the excessive secretion of intestinal and liver-derived lipoproteins in diabetes[111], there is a rationale in conceiving such strategy to reduce atherogenic dyslipidemia. Pre-clinical data in rodents demonstrate that GLP1-R agonism reduces VLDL production and hepatic steatosis in addition to an improvement of glycemic control[112]. This paved the road during the last years to consider this option for the treatment of atherogenic dyslipidemia in NAFLD/MAFLD as well. Recent metanalysis of different trials with up to 26 weeks of follow-up (testing multiple GLP1-R agonists, namely liraglutide, exenatide, dulaglutide, and semaglutide) concluded that increasing the signaling of GLP1 resulted in reductions in the absolute percentage of liver fat content (magnetic resonance-based techniques), lowered serum liver enzymes, and improved histology of NASH, without worsening the degree of fibrosis[113]. Further data are needed regarding the effect on lipid profile, as this would further strengthen the relevance of targeting the excessive production of liver-derived lipoproteins to control metabolic liver disease.

Metformin, by increasing AMP-activated protein kinase (AMPK), has been shown to inhibit the differentiation of monocyte to pro-inflammatory macrophages and blunt cytokines secretion by reducing signal transducer and activator of transcription 3 (STAT3) activity. As AMPK is essential for brown adipose tissue (BAT) development and homeostasis, metformin was demonstrated to increase liver-derived lipoproteins uptake and lipolysis by BAT[114].

LXR agonism, by virtue of its presumable effect on liver-derived lipoproteins uptake by the periphery, has been tested. Although LXR agonists exhibited anti-inflammatory effects in the pre-clinical setting, inducing monocytes egression from atherosclerotic plaque[115] and subsequent reduction of plaque size, this option

resulted in increased hepatic steatosis, both in healthy subjects and in statin-treated hypercholesterolemic patients[116], questioning the translation *in vivo*.

Obeticholic acid, an FXR agonist promoting the re-cycle of the cholesterol pool in the entero-hepatic circulation, provided encouraging data in a phase III trial[117] regarding the regulation of liver fibrosis with up to 38% of patients in the 25 mg arm displaying improvement of biopsy-proven fibrosis, even in the presence of metabolic complication. Despite the withdrawal of the compound being announced by the developing company[118], from a lipidological point of view, obeticholic acid induced questionable effects on TG (not consistently reduced as a function of FXR agonism) and LDL cholesterol content (which increased as compared to placebo).

CONCLUSION

The number of subjects who are at risk of liver disease is expected to grow over the coming years and will determine a serious escalation in the incidences of fatal and non-fatal atherosclerotic cardiovascular diseases[119]. This rate will be increased by the elevated pressure of additional comorbidities, with type 2 diabetes and metabolic syndrome *in primis*[120] constantly increasing in affluent societies with unhealthy lifestyles. Together, these well-described projections call for the worldwide guidelines to determine algorithms for coordinated methods of interventions. At the same time, all the pharmacological trials targeting liver-derived lipoproteins provide a wealth of evidence supporting that the lipid content of the liver is causal for the evolution of CVD risk. In addition, data from these trials are paralleled to those from forefront trials, posing inflammation per se as the real culprit. Under this perspective, for example, PCSK9 monoclonal antibody reduced LDL cholesterol (FOURIER trial[121]) and advanced EPA formulations (REDUCE-IT trial[105]) reduced VLDL quantity and cholesterol, together with convincing atherosclerotic CVD risk in high-risk patients and subjects with advanced metabolic complications including liver disease. A similar degree of benefit was not achieved in the same comorbid patients by neither anti-Interleukin Beta (IL-1b) monoclonal antibody (canakinumab[122]) nor colchicine[123], despite potently reducing a large set of inflammatory markers[124]. Lessons from the critical comparison of these trials[125] will improve the sensitization and understanding of the pathophysiological mechanisms involving dyslipidemias and elevated ApoB liver-derived lipoprotein levels. The pre-clinical evidence regarding their causal effects in the evolution of atherosclerosis and related inflammation will certainly help to foster the sensitization of physicians and pharmacological research and companies in the development of new and more effective therapeutic weapons.

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