Intervention in hepatic lipid metabolism : implications for atherosclerosis progression and regression

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Chapter 1

General introduction

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1. LIPID METABOLISM

Hypercholesterolemia holds a key role in the development and progression of atherosclerosis and is a causative factor for coronary artery disease [1]. Hyperlipidemia is a metabolic disorder defined by either elevated levels of plasma concentrations of low-density lipoprotein (LDL) cholesterol and triglycerides, or decreased levels of the athero-protective lipid biomarker high-density lipoprotein (HDL) cholesterol[2]. Lowering of very-low-density lipoprotein- (VLDL-) and low-density lipoprotein- (LDL-) cholesterol levels leads to a reduction in cardiovascular morbidity and mortality[2]. In contrast, high levels of HDL cholesterol are associated with a decreased risk of cardiovascular disease[3].

1.1 Lipoproteins

Lipoproteins are spherical macromolecular complexes in which hydrophobic molecules, in particular triglyceride and cholesteryl ester, are enveloped within a monolayer of amphipathic molecules of phospholipids, free cholesterol, and apoproteins [4]. The major lipoprotein classes include intestinally derived chylomicrons that transport dietary fats and cholesterol, hepatic-derived VLDL, intermediate-density lipoprotein (IDL), and LDL that are considered to be pro-atherogenic, and hepatic- and intestinally derived HDL that are considered to be anti-atherogenic. Figure 1 illustrates the major lipoprotein classes and their compositions.

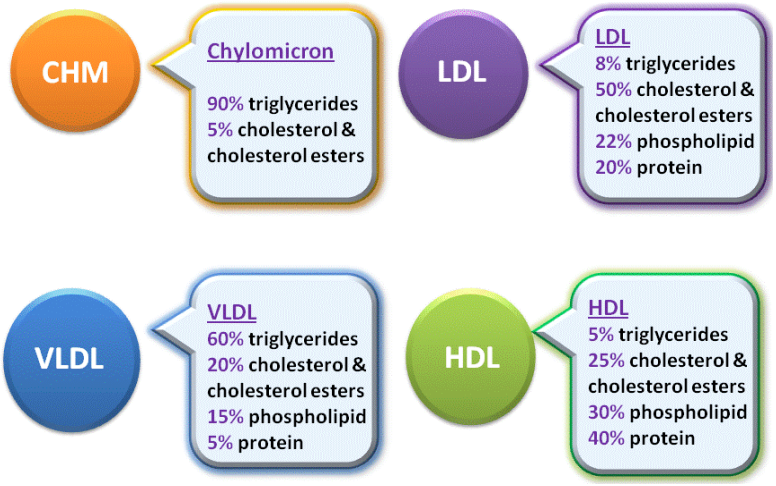

Figure 1. Illustration of the composition of four major classes of lipoproteins[5]. See text for explanation.

1.1.1 Chylomicron, VLDL, and LDL

Apolipoprotein B (apoB) is necessary for the assembly and secretion of chylomicrons by the intestine while it is also an essential component for VLDL, IDL, and LDL[6]. As shown in Figure 2, the liver secretes VLDL particles, which contain triglycerides and cholesterol esters. Capillaries in muscle and adipose tissue remove the triglycerides, and the lipid particle is modified into LDL, with its

cholesteryl ester core and apoB-100 coat. LDLs circulate in the plasma and the apoB-100 component binds to LDL receptors on the surface of hepatocytes. Through receptor-mediated endocytosis, receptor-bound LDLs enter hepatocytes and undergo degradation in lysosomes, and the cholesterol remnants enter a cellular cholesterol pool[7]. Plasma levels of apoB containing lipoproteins are regulated by both environmental effects on lipid metabolism and by genetic factors affecting the surface of the lipoproteins and enzymes in plasma[8].

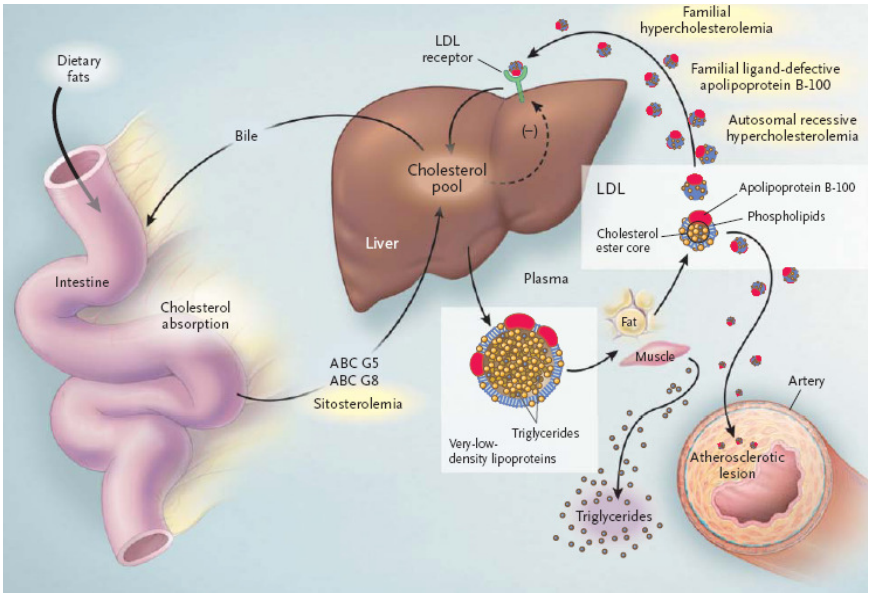

Figure 2. The basic pathways of cholesterol synthesis and excretion[7]. See text for explanation.

1.1.2 HDL and reverse cholesterol transport

The major protein of HDL is apolipoprotein A-I (apoA-I), which is synthesized in the liver and intestine. HDL metabolism consists of five main processes: (1) apoA-I synthesis and secretion into plasma as nascent HDL; (2) uptake of free cholesterol from the periphery; (3) maturation into large spherical particles with cholesterol esterification; (4) delivery of cholesteryl ester to the liver, steroidogenic organs, and apoB-containing lipoproteins; and (5) catabolism of apoA-I[9]. HDL serves an anti-atherogenic function because of its ability to mediate reverse cholesterol transport (RCT), which is a major protective system against atherosclerosis[10,11]. HDL can remove cholesterol from the periphery, allowing it to be cleared by the liver and then excreted into the bile[12]. Cholesterol efflux from macrophages to HDL is a crucial step in RCT and it occurs at all stages of atherosclerosis[13]. As shown in Figure 3, the liver secretes lipid-poor apoA-I, which quickly acquires cholesterol via the hepatocyte ABCA1 transporter. Lipid-poor apoA-I also promotes the efflux of free cholesterol from macrophages via ABCA1. LCAT esterifies free cholesterol to cholesteryl esters to form mature HDL, which promotes cholesterol efflux from macrophages via the ABCG1 transporter. In macrophages, both ABCA1 and ABCG1 are regulated by nuclear receptor LXR. Mature HDL can transfer its cholesterol to the liver directly via SR-BI or indirectly via CETP-mediated transfer to

apoB-containing lipoproteins, with subsequent uptake by the liver via the LDL-receptor[14]. Modulation of major macrophage mediators in RCT, such as ABCA1, ABCG1, and SR-B1 has been considered as promising strategies for the development of drugs aimed at the prevention of atherosclerosis[15,16,17].

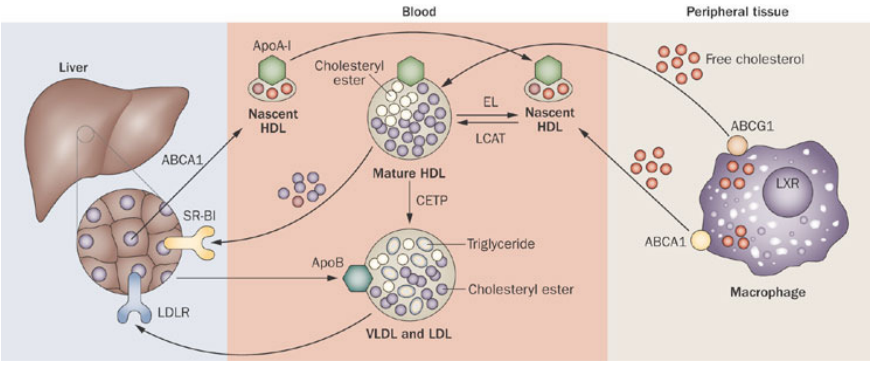

Figure 3. HDL metabolism and reverse cholesterol transport14. See text for explanation.

1.2 Hepatic lipid metabolism

Atherosclerosis is a liver disease of the heart[18]. Liver is considered as the major organ with significant therapeutic importance for the maintenance of metabolic homeostasis[19]. A common associated clinical feature of patients with non-alcoholic fatty liver disease (NAFLD) is atherogenic dyslipidaemia, i.e. high triacylglycerol, low HDL-cholesterol, and increased LDL-cholesterol levels[20]. Liver fat is highly significantly and linearly correlated with all components of the metabolic syndrome independent of obesity[21]. NAFLD is not merely a marker of atherosclerosis, but may also be actively involved in the pathogenesis of atherosclerosis[22,23].

The liver consists of different types of cells, including parenchymal cells, namely hepatocytes, and a variety of non-parenchymal cells (Figure 4). Non-parenchymal cells are comprised of mainly liver sinusoidal endothelial cells and Kupffer cells. Liver endothelial cells form a continuous but fenestrated lining of the hepatic sinusoids, while Kupffer cells are found in the sinusoidal lumen on top of or between endothelial cells[24]. Liver endothelial cells free the bloodstream from a variety of macromolecular waste products during inflammation[25]. Kupffer cells are a population of hepatic resident macrophages. They constitute 80-90% of the tissue macrophages present in the body[26]. Although non-parenchymal cells count for only 6.5% of the liver volume, they contain 55% of the lipid droplets in the liver and 43% of the lysosomes, and specific activities of enzymes are generally higher in non-parenchymal cells than in parenchymal cells [27, 28]. Parenchymal and non-parenchymal cells synchronize crucial roles in liver metabolic homeostasis as well as inflammation. The majority of studies upon liver has focused on the array of target genes and metabolic pathways within parenchymal cells[29,30]. However, non-parenchymal cells are also intimately involved in the pathogenesis of various liver metabolic diseases including steatohepatitis, non-alcoholic fatty liver, and liver fibrosis[31]. Previous studies have shown that diet-induced hypercholesterolemia results in marked changes in the hepatic distribution of LDL and significant accumulation of cholesteryl ester/lipid droplets in liver endothelial and Kupffer cells, suggesting a prominent role of liver non-parenchymal cells in removing modified

LDL from blood[32,33,34]. It has also been shown that cross-talk between Kupffer cells and hepatocytes regulates hepatic lipid storage[35].

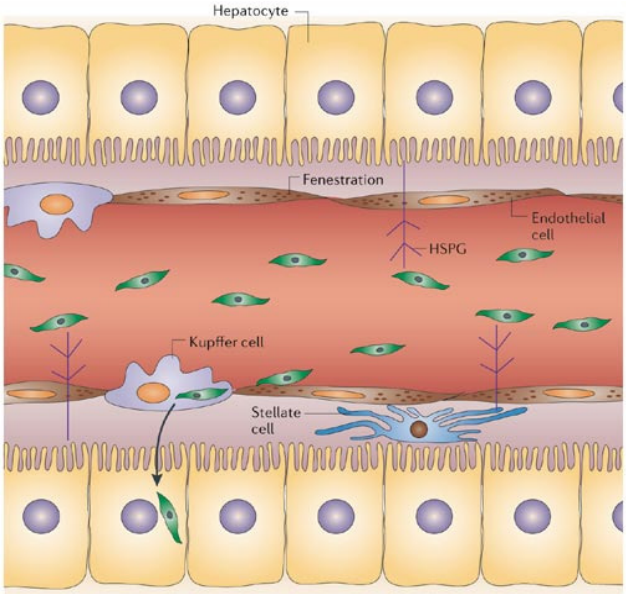

Figure 4. Cell composition of liver[36].

2. ATHEROSCLEROSIS

Cardiovascular diseases are the leading cause of morbidity and mortality in industrialized countries [37] . Atherosclerosis is the underlying cause of most cardiovascular diseases. Lipid accumulation leads to an inflammatory condition clinically causing occlusive vascular disease, myocardial infarction and stroke[38]. The atherosclerotic process is initiated when cholesterol-containing low-density lipoproteins accumulate in the intima and activate the endothelium.

2.1 Lesion progression

Macrophages contribute to the pathogenesis of atherosclerosis through their accumulation of cholesterol and development into foam cells[39]. Foam cells arise either from resident macrophages in the arterial wall or from blood monocytes that enter the wall at sites of endothelial damage[40]. Figure 5 illustrates the infiltration and inflammation of macrophages in arterial wall. Leukocyte adhesion molecules and chemokines promote recruitment of monocytes which differentiate into macrophages and up-regulate pattern recognition receptors on these cells, including scavenger receptors. Scavenger receptors mediate lipoprotein internalization, leading to foam-cell formation, inflammation, and, ultimately, to tissue damage[41,42]. Accumulation of cholesterol-loaded “foam cells” is the hallmark of the early atherosclerotic lesion[43].

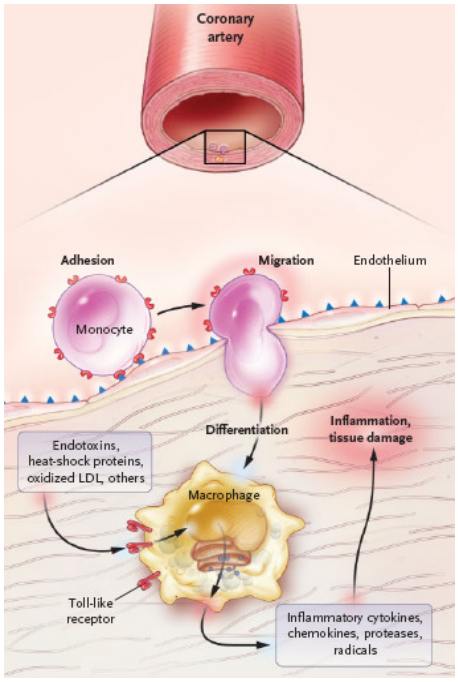

Figure 5. Illustration of macrophage infiltration and inflammation in arterial wall41. See text for explanation.

The atherosclerotic plaque is a dynamic tissue, where increases in cell number (driven by cell proliferation and migration) and decreases in cell number (driven by cell death and possibly emigration) are continuous processes [44]. Initial atherosclerotic lesions are primarily composed of lipid-loaded macrophages. Stable advanced lesion contains a macrophage core, a small necrotic core, if present at all, extracellular matrix and a firm fibrous cap of smooth muscle cells (SMCs)[45]. Instable advanced atherosclerotic lesions are characterized by a thin fibrous cap containing few SMCs and overlying a large necrotic core composed of dead cells, lipid deposits, and cellular debris[46].

2.2 Lesion regression

While numerous studies have been dedicated to inhibit the development and progression of atherosclerosis, recent attention has been drawn to the goal of reversing atherosclerosis, meaning regressing pre-existing atherosclerotic plaques. The first evidence of dramatic atherosclerotic regression in mice was achieved via robust surgical measures to rapidly improve plaque environment[47]. That study suggested that the essential prerequisite for promoting regression of atherosclerotic lesions is robust improvement of plasma lipoprotein profiles and plaque milieu, including large plasma reductions in atherogenic apoB-lipoproteins and brisk enhancements in efflux of cholesterol from plaques to the blood circulation and subsequently into the liver. Recently, the same group showed that the LXR agonist T0901317 promotes egress of monocyte-derived cells from mouse aortic plaques, indicating that LXR is required for maximal effects on plaque

macrophage egression during atherosclerosis regression in mice[48]. In another mouse model, apoE*3Leiden mice, LXR agonist was also shown to promote regression of moderate lesions[49]. Raffai *et al* for the first time addressed the apoE-mediated mechanisms of atherosclerosis regression[50]. They demonstrate that apoE promotes the regression of atherosclerosis independently of lowering plasma cholesterol levels. Potteaux *et al* also achieved regression of atherosclerosis after apoE complementation in ApoE$^{-/-}$ mice, suggesting that therapies to inhibit monocyte recruitment to plaques may constitute a viable strategy to reduce plaque macrophage burden than attempts to promote migratory egress[51].

3. ANIMAL MODELS IN ATHEROSCLEROSIS

Atherosclerosis is a complex and chronic inflammatory disease in which multiple modulating factors may play a role. Its chronicity and complexity make it very difficult to study the detailed mechanisms of atherogenesis in unregulated human populations. Therefore, animal models with a homogenous genetic background are useful for the study of the mechanism of this process[52]. With the development of genetic models of atherosclerosis the mouse has become a very accessible model, especially with the very large genetic data base about this species in relation to human biology that has become available[53].

3.1 C57BL/6 mice

The C57BL/6 mouse strain is used as a model for studies of diet-induced atherosclerosis[54, 55, 56]. C57BL/6 mice fed with a high-fat cholate-containing atherogenic diet have a hyperlipidemic response, develop fatty streak lesions, and form atheromatous plaques in the aorta and coronary arteries[57]. This murine model of atherogenesis represents an alternative to the use of genetically modified mice with impaired lipoprotein clearance, thus it may prove beneficial for the evaluation of new classes of anti-hyperlipidemic agents[58].

3.2 LDLr$^{-/-}$ mice

LDLr$^{-/-}$ mice are among the most widely used mouse models for characterization of atherosclerosis. Due to the absence of hepatic LDL receptors, LDLr$^{-/-}$ mice exhibit prolonged half life of plasma VLDL and LDL[59]. These mice display a modestly elevated plasma cholesterol level when maintained on a regular chow diet, whilst on potent cholesterol-rich diet, LDLr$^{-/-}$ mice show strongly elevated plasma cholesterol levels (hypercholesterolemic) and rapid development of atherosclerosis[60].

3.3 ApoE$^{-/-}$ mice

ApoE$^{-/-}$ mice with targeted deletion of the apoE gene show severe hypercholesterolemia. Therefore, ApoE$^{-/-}$ mice form one of the most common animal models to study atherogenesis. The most obvious phenotype of ApoE$^{-/-}$ mice is the spontaneous development of atherosclerotic lesions, even on a standard chow diet which is low in fat content and does not contain cholesterol. Lesions of ApoE$^{-/-}$ mice develop over time from initial fatty streaks to complex lesions, and this process can be strongly accelerated by a high-fat, high-cholesterol diet[61].

4. DRUG TARGETS AND PHARMACEUTICAL INTERVENTIONS

Lipid-lowering is established as a proven intervention to reduce atherosclerosis and its complications. The development of the HMG-CoA reductase inhibitors (statins) has lead to important advances in the management of cardiovascular disease[62]. However, statins reduce cardiovascular events by only about 20%-40%, and non-statin therapies (either as monotherapy or in addition to statins) to reduce LDL-cholesterol by mechanisms that do not involve inhibition of HMG-CoA reductase are also likely to be useful for patients in need of LDL reduction[63]. These therapies include drug targets such as squalene synthase, microsomal transfer protein (MTP), acyl-cholesterol acyl transferase (ACAT), cholesterol ester transfer protein (CETP), and peroxosimal proliferator activating receptors (PPARs)[64].

4.1 Nuclear receptor

The nuclear receptor (NR) superfamily describes a related but diverse array of ligand-activated transcription factors. NR binds DNA and translates physiological signals into gene regulation involved in biological processes. Figure 6 shows a higher-order network tying nuclear receptor function to reproduction, development, central, and basal metabolic functions, dietary-lipid metabolism, and energy homeostasis[65]. Several receptors including the peroxisome proliferator activated receptors (PPARs), the liver X receptors (LXRs), the farnesoid X receptor (FXR) and the retinoid-related orphan receptors (RORs) are called “metabolic receptors” and poised to sense and respond to small changes in the flux through the metabolic pathways that they control[66].

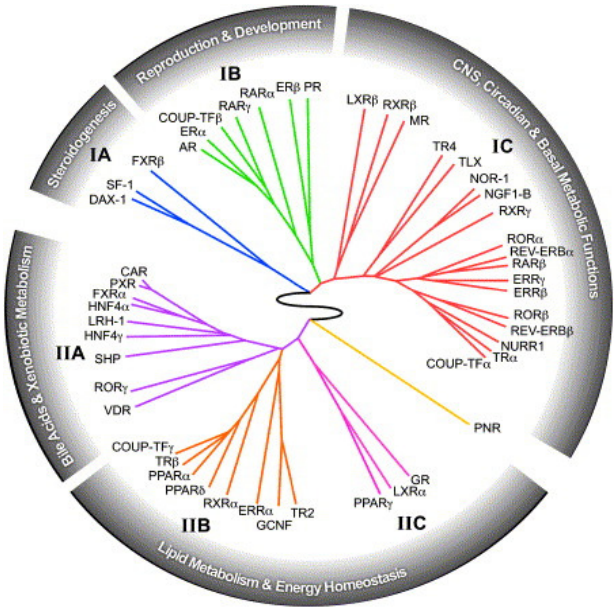

Figure 6. The nuclear receptor ring of physiology. The relationship between receptor expression, function, and physiology is depicted as a circular dendrogram65.

4.2 LXR and LXR agonists

Liver X receptors (LXRs) are sterol-responsive nuclear receptors that regulate expression of genes involved in cholesterol metabolism and homeostasis[67]. LXRs act as cholesterol sensors. When cellular oxysterols accumulate as a result of increasing concentrations of cholesterol, LXR induces the transcription of genes that protect cells from cholesterol overload[68]. LXR agonists have potent anti-atherogenic effects, shown in different hyperlipidemic mouse models. Several studies have demonstrated that activation of LXR with compounds, such as T0901317, significantly up-regulates cholesterol efflux activity and inhibits development of atherosclerosis, providing direct evidence for an atheroprotective effect of LXR agonists[69,70]. T0901317 treatment was associated with reduced cholesterol absorption and promoted biliary cholesterol excretion[71,72,73]. Furthermore, LXR agonist significantly increased the generation of HDL particles in plasma. Table 1 summarizes the studies of LXR agonists performed in mouse models to study atherosclerotic progression or regression.

4.3 PNPLA3

Patatin-like phospholipase domain-containing protein 3 (PNPLA3), also knows as adiponutrin (ADPN) or calcium-independent phospholipase A2-epsilon, is an enzyme that in humans is encoded by the PNPLA*3* gene[74,75]. High human PNPLA3 activity is associated with increased liver fat content and liver injury. Variation in PNPLA3 contributes to ancestry-related and inter-individual differences in hepatic fat content, risk of hepatic steatosis, and susceptibility to NAFLD[76,77].

4.4 CETP

Cholesteryl ester transfer protein (CETP) is a 74-kDa hydrophobic plasma glycoprotein that has an established role in mediation of neutral lipid transport among lipoproteins[78]. In humans, CETP mRNA is expressed predominantly in adipose tissue, liver, and spleen, with lower levels of expression in the small intestine, adrenal gland, kidney, skeletal muscle, and heart[79,80]. In addition, Van Eck *et al*[81] have demonstrated that bone marrow-derived cells, including macrohpages, are an important contributor to total serum CETP activity and mass in mice.

Cholesteryl ester transfer protein (CETP) is a key modulator not only of the intravascular metabolism of HDL and apoA-I but also of triglyceride-rich lipoproteins (TRL). CETP modifies the lipid composition of the plasma by mediating the transfer of cholesteryl esters from HDL to pro-atherogenic apoB-lipoproteins, with heterotransfer of TG mainly from very low-density lipoprotein to HDL, thereby decreasing plasma HDL-C concentrations and increasing the proportion of lipids present in the atherogenic LDL-C and VLDL-C fractions[82,83] (Figure 7). The overall effect of CETP is to promote a net mass transfer of CE from HDL to TRL and LDL and of TG from TRL to LDL and HDL. CETP plays a significant role in reverse cholesterol transport (RCT)[84]. Pharmacological inhibition of CETP in humans therefore presents a preferential target to improve the lipid profile of dyslipidemic patients, not only by increasing HDL-C levels but also by reducing LDL-C levels[85].

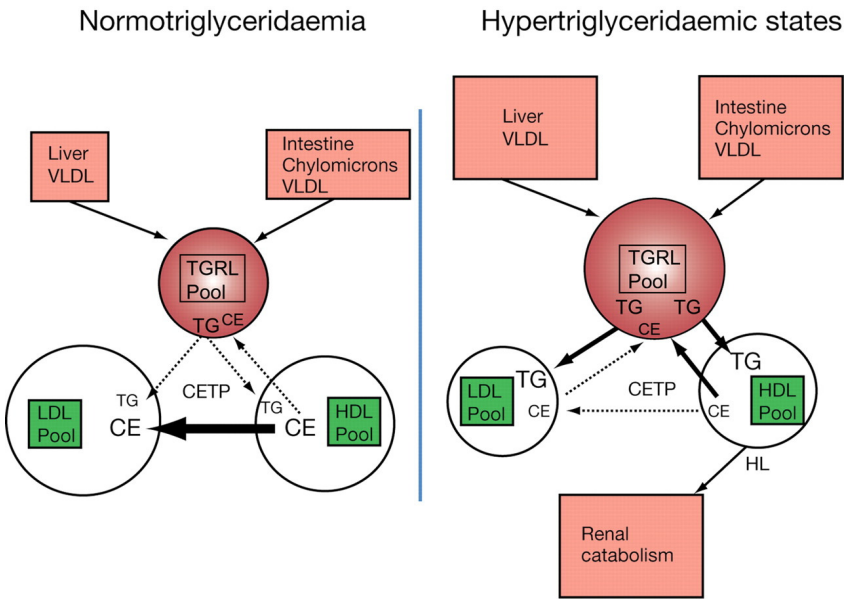

Figure 7. Role of CETP in plasma lipoprotein transport82.

4.5 GPR109A, niacin, and niacin derivatives

The G protein-coupled receptor GPR109A, also known as PUMA-G in mouse and HM74A in humans, has been identified as a high-affinity receptor for nicotinic acid, also known as niacin[86,87]. GPR109A is dominantly expressed in adipose tissue and spleen[88,89]. It is also highly expressed in macrophages and other immune cells, such as lymphocytes[90,91,92,93]. The lipid-lowering effect of niacin on plasma (V)LDL-TG is the consequence of a direct interaction between niacin and its receptor GPR109A in adipose tissue[94].

Niacin is the most effective agent currently available to treat dyslipidaemic disorders [95] . It lowers plasma levels of pro-atherogenic lipids, including chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and triglycerides (TG) in normolipidemic as well as hypercholesterolemic subjects[96]. Several clinical trials have shown that nicotinic acid reduces cardiovascular disease and myocardial infarction incidence, providing a solid rationale for the use of nicotinic acid in the treatment of atherosclerosis[97,98]. The effects of niacin on lipid metabolism in human have been summarized in Figure 8. Recently, we showed that niacin also reduces the hepatic expression and plasma levels of the pro-atherogenic cholesteryl ester transfer protein (CETP) in mouse models [99]. A summary of studies on effects of niacin on lipid metabolism and CETP regulation in mouse models are shown in Table 2.

LIPOPROTEIN	EFFECT
Chylomicrons	↓
VLDL	↓
β-VLDL	↓
IDL	↓
LDL	↓
sdLDL	↓
HDL	↑
HDL_2	↑
LP(a)	↓

Figure 8. Summary of the effects of niacin on plasma lipoprotein classes in human[100].

Despite its established cardiovascular benefits, the clinical use of niacin has been limited due to the cutaneous flushing, a well-recognized adverse skin effect from nicotinic acid therapy[101]. The niacin receptor GPR109A expressed in the skin is a critical mediator of nicotinic acid-induced flushing[102]. Nicotinic acid stimulates GPR109A in epidermal Langerhans cells and keratinocytes, causing the cells to produce vasodilatory prostaglandin D_2 (PGD_2) and prostaglandin E_2 (PGE_2), which leads to cutaneous vasodilation[103,104,105,106]. For the past decade, the pharmacology of GPR109A has been studied and its full or partial agonists, including acipimox, acifran, 3-pyridine-acetic acid, 5-methylnicotinic acid, pyridazine-4-carboxylic acid, and pyrazine-2-carboxylic acid, have been developed in an attempt to achieve the beneficial effects of nicotinic acid while avoiding the unwanted flushing side effect[107,108,109].

5. THESIS OUTLINE

Atherosclerosis is a chronic disease causing many cardiovascular complications, among which atherosclerotic coronary heart disease is the leading cause of morbidity and mortality worldwide. Atherosclerosis is a progressive inflammatory disease which has an onset by vascular obstruction from the deposits of plaque, subsequently developing into athero-thrombosis and abnormal blood flow. The evidence to support a cholesterol-atherosclerosis link has been revealed in the past three decades. There is a growing consensus that therapeutic lowering of plasma cholesterol level will reduce the risk of cardiovascular incidence. In **Chapter 1**, established mechanisms underlying lipid metabolism and atherosclerotic pathology are reviewed, including the important players involved in plasma / hepatic lipoprotein metabolism pathways and atherosclerotic plaque progression / regression process.

The first part of the thesis focuses on the hepatic lipid metabolism and the pharmaceutical interventions in the liver. In **Chapter 2**, we have composed the hepatic cell type-specific expression profile of NRs to provide the most complete quantitative assessment of NRs distribution in liver reported to date. We have identified several liver-enriched orphan NRs as potential novel targets for pharmaceutical interventions in liver. In **Chapter 3**, we have determined the hepatic expression profile of NAFLD-related gene PNPLA3 and its metabolic effects. PNPLA3 is highly responsive to metabolic changes in hepatocytes within the liver and its relative change in expression level suggests an essential function in lipogenesis. In **Chapter 4** the mechanism underlying the hepatic and plasma CETP-lowering effect of niacin in mice is investigated. The clinical use of niacin has been limited due to the cutaneous flushing effect, which is mediated by the nicotinic acid receptor GPR109A. Therefore, in **Chapter 5**, we assessed the properties of two partial agonists for GPR109A compared to niacin. We showed that these two partial agonists are promising drug candidates to achieve the beneficial lipid-lowering effects while successfully avoiding the unwanted flushing side effect.

The second part of the thesis focuses on the concept of atherosclerotic lesion regression, shedding insights in the roles of LXR activation and application of mouse models in regression studies. In both **Chapter 6** and **Chapter 7** it is examined whether rapidly improved plasma lipoprotein profiles combined with LXR activation can lead to atherosclerotic lesion regression. In **Chapter 6** $LDLr^{-/-}$ mice are used and it is shown that intact LDL receptor function is crucial to overcome LXR-induced hyperlipidemia. In **Chapter 7** we used $ApoE^{-/-}$ mice reconstituted with bone marrow from C57BL/6 mice as a novel mouse model with chow diet feeding, providing an alternative model to investigate atherosclerotic plaque regression without robust surgical measures. Both of these two chapters demonstrate that rapidly optimized plasma lipoprotein profiles combined with LXR agonist induce favorable gene expression profiles leading to regression of pre-existing atherosclerotic plaques.

In **Chapter 8**, the results obtained from all the experiments mentioned above are summarized and discussed with respect to the implications of these studies for future investigations.

Table 1. Summary of studies of LXR agonists on atherosclerotic progression and regression in mouse models

Name	Mouse model	Diet during treatment	Treatment duration	Plasma TC	Plasma (V)LDL	Plasma TG	Plasma HDL	Lesion size (aortic root)	Lesion size (en face)	Reference
T0901317	C57BL/6	Chow	7 days	↑	↑	↑	↑			110
T0901317	LDLr–/–	AD	8 weeks	→	↓	→ (transient increase)	↑	Inhibited progression		111
T0901317	C57BL/6	Chow	3 days	↑		→	↑			112
T0901317	C57BL/6	Chow	6 days	↑	↑	↑	↑			113
T0901317	LDLR–/–	WTD	Baseline: 8 weeks WTD Regression study: 6weeks WTD±T0901317	↓	↓	↑	→	Inhibited progression. Macrophage content ↓ Collagen content ↑	Regression	114
T0901317	LDLr–/–	WTD	12 weeks	↑	↑	↑	↓	Inhibited progression Macrophage content ↓ Collagen content ↑	Inhibited progression Macrophage content ↑	115
T0901317	ApoE–/–	AD	Baseline: AD 8 weeks Regression study: 6weeks AD±T0901317	↑	→	↑	↑	Regression Macrophage content ↓	Regression	116,117
T0901317	ApoE*3Leiden		Baseline: WTD 18 weeks Regression study: 8weeks Chow±T0901317	→	↑	↑	→	Regression		49
T0901317	C57BL/6	Chow	4 days	Hepatic TC →		Hepatic TG ↑				118
T0901317	LXR*α*–/–;LDLr–/–	WTD	12 weeks	→		→		Inhibited progression	→	119
T0901317	LXR*β*–/–;LDLr–/–	WTD	12 weeks	→		↑		Inhibited progression	Inhibited progression	119
T0901317	LDLr–/–	WTD	12 weeks	→		↑		Inhibited progression	Inhibited progression	119
T0901317	C57BL/6	Chow	4 weeks	↑	↓	↓	↑			120
T0901317	LDLr–/–	WTD	4 weeks	↑	↑	↑	→			120
T0901317	ApoE–/–	AD	6 weeks	↑		↑	↑	Inhibited progression	Inhibited progression	121
GW3965	LDLr–/–	AD	12 weeks	↓		→	→	Inhibited progression	Inhibited progression	69
GW3965	ApoE–/–	Chow	12 weeks	→	↓	↑	→	Inhibited progression		69

GW3965	C57BL/6	Chow	3 days	→		→	↑			112
GW3965	C57BL/6	Chow	6 days	↑	↑	↑	↑			113
GW3965	C57BL/6	Chow	12 days	↑	↑		↑			122
GW3965	ApoB/CETP Tg	Chow	12 days	↑	↑		↑			122
GW3965	LXR–/–;ApoE–/–	Chow	12 days	↑	→		→			122
GW3965	LXRα–/–;ApoE–/–	WTD	11 weeks	↓	→	→	↑	Inhibited progression	Inhibited progression	123
GW3965	ApoE–/–	WTD	11 weeks	↓		↑	↑	Inhibited progression	Inhibited progression	123
GW3965	LDLr-/-	AD	8 weeks	→		→			Inhibited progression	124
GW3965	C57BL/6	Chow	10 days	↑			↑			125
GW3965	LDLr–/–	Chow	10 days	↑			↑			70
GW3965	APOE*3Leiden	AD	20 weeks	→		↑		inhibited progression Collagen content ↓		126
ATI-111	LDLr–/–	AD	8 weeks	↓	↓	↓	→	inhibited progression	inhibited progression	70
AZ-876	APOE*3Leiden	AD	20 weeks	Low dose: → High dose: ↓		Low dose: → High dose: ↑		Inhibited progression upon low/high dose Lesion composition →		126
ATI-829	LDLr–/–	WTD	12 weeks	→	→	→	→	Inhibited progression Macrophage content ↓ Collagen content ↑	Inhibited progression	127
DMHCA	ApoE–/–	WTD	11 weeks	↓	↓	↓	→	Inhibited progression	Inhibited progression	118
DMHCA	C57BL/6	Chow	15 days	↓		→				118
DMHCA	C57BL/6	WTD	15 days	→		→				118
MHEC	ApoE–/–	AD	6 weeks	→		→	↑	Inhibited progression	Inhibited progression	121

↑: increased; ↓: decreased; →: unchanged.
WTD: Western-type diet; AD: Atherogenic diet; HFD: High-fat Diet; Tg: transgenic

Table 2. Summary of effects of niacin on lipid metabolism and CETP regulation in mouse models

Mouse model	Diet during treatment	Treatment duration (weeks)	Plasma TC	Plasma (V)LDL	Plasma HDL	Plasma TG	Plasma FFA	Effects on liver	Lesion size (aortic root)	Lesion size (en face)	Reference
C57BL/6	AD	4	→		→	↓					128
C57BL/6	WTD	12				↓	↓				129
C57BL/6	Chow	30 min after i.p. injection					↓				93
C57BL/6	Chow	3	↓	↓	↓			cAMP ↓ ABCA1 ↓			130
C57BL/6;Gpr109a–/–	Chow	3	→	→	→			cAMP → ABCA1 →			130
C57BL/6	Chow	2	↓	→	→	↓					131
Human CETP Tg	Chow	2	→	↓	↑	↓					131
Human apo B100 Tg	Chow	2	↓	↓	↓	→					131
Human CETP/apoB100 Tg	Chow	2	↓	↓	↑	↓					131
E3L mice	WTD	3	↓	↓	→	↓					99
E3L.CETP mice	WTD	3	↓	↓	↑	↓		TG, TC, FC, CE ↓ Plasma CETP ↓ Hepatic CETP ↓			99
LDLr–/–;Gpr109a+/+	HFD	10	→	→	→	At 2 weeks: ↓ At 10 weeks: →	→		↓	↓	132
LDLr–/–;Gpr109a–/–	HFD	10	→	→	→	At 2 weeks: ↓ At 10 weeks: →	→		→	→	132
ApoE–/–	AD	14	→		→	→			→		128

↑: increased; ↓: decreased; →: unchanged.
WTD: Western-type diet; AD: Atherogenic diet; HFD: High-fat Diet; Tg: transgenic

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