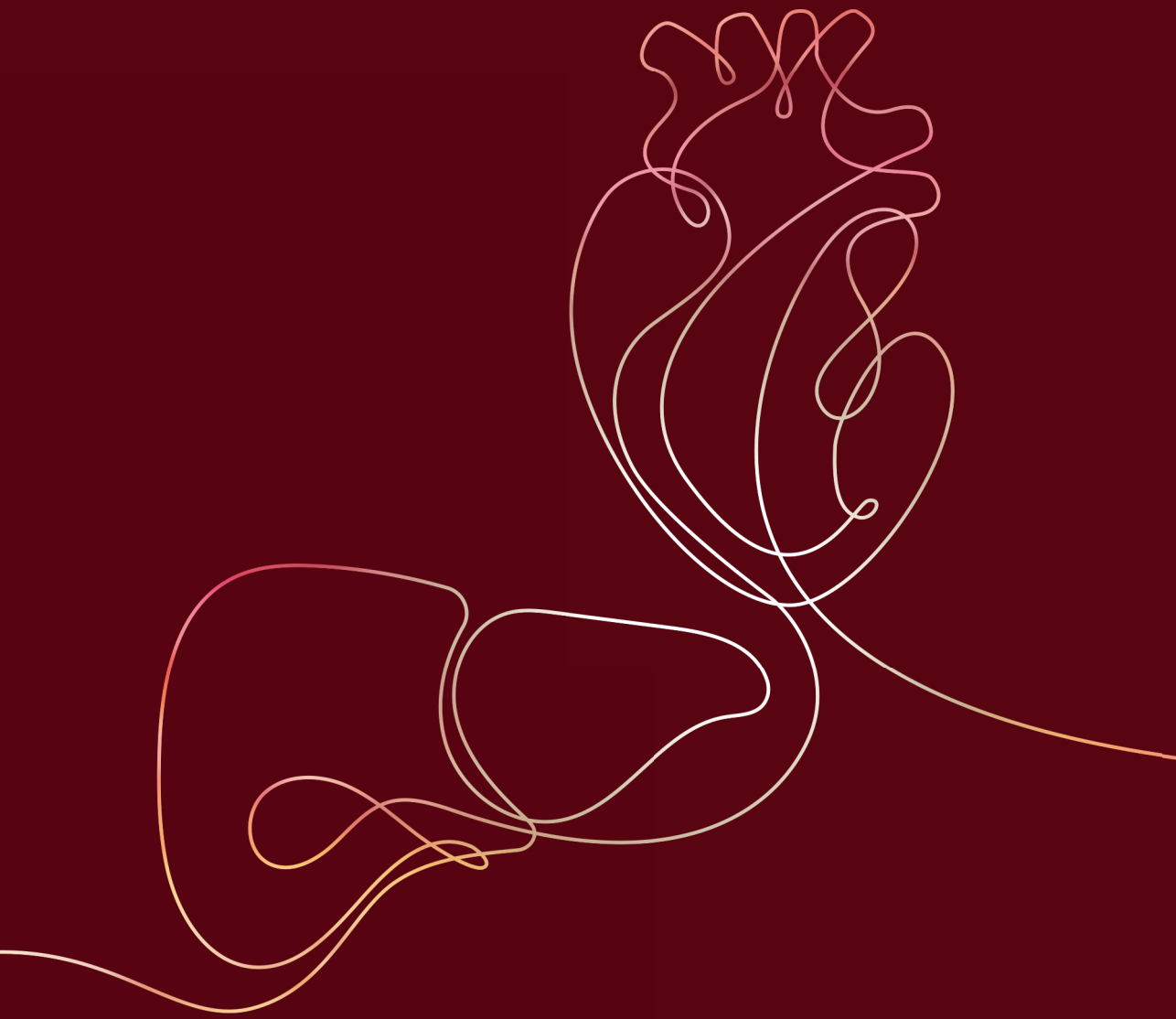
Interventions targeting hepatic and cardiovascular complications of metabolic syndrome

Inia, J.A.



1

General introduction

1.1 Metabolic syndrome

Metabolic syndrome refers to a cluster of interrelated conditions that often present simultaneously and that together significantly increase the risk of developing type 2 diabetes mellitus (T2DM), metabolic dysfunction-associated steatotic liver disease (MASLD) and cardiovascular diseases (CVD). One of these conditions is obesity, which results from a chronic imbalance between caloric intake and energy expenditure. Due to evolutionary pressure to store energy combined with continuous accessibility to food and prevalent sedentary lifestyles, obesity has reached epidemic proportions. It is estimated that 1.5 billion people have metabolic syndrome, accounting for about 20% of the global population[1]. Over the years, several definitions for metabolic syndrome have been proposed. In 2009, different organizations including the American Heart Association (AHA), International Diabetes Federation (IDF) and the National Institutes of Health (NIH) agreed on a harmonized definition of metabolic syndrome[2]. According to this consensus, the presence of any three out of five abnormalities qualifies a person for metabolic syndrome: decreased insulin sensitivity, hypertension, elevated triglycerides (TGs), reduced high density lipoprotein (HDL) cholesterol or abdominal obesity with visceral fat accumulation, often measured as increased waist circumference[2,3]. These risk factors increase the risk of developing complications, including T2DM, MASLD and CVD.

The first-line intervention for managing metabolic syndrome is lifestyle modification, particularly weight loss through dietary changes and increased physical activity. However, in clinical practice, implementing and sustaining these changes often proves challenging for many individuals. When lifestyle changes are insufficient, pharmacological interventions offer a valuable add-on or alternative for weight management and for mitigating or preventing metabolic complications.

1.2 Lipoprotein metabolism

Given the central role of lipid abnormalities in metabolic syndrome, understanding lipoprotein metabolism is essential to elucidate underlying mechanisms and potential therapeutic targets. Lipoprotein metabolism involves the transport and processing of lipids throughout the body[4]. Because of their hydrophobic nature, lipids require specialized transport mechanisms. They are carried within lipoprotein particles, which are lipid spheres made out of a phospholipid monolayer embedded with apolipoproteins that mediate structural integrity and receptor interactions. Lipoproteins are classified based on size, lipid composition and apolipoprotein content. In lipid metabolism, the exogenous pathway refers to dietary lipids absorbed by enterocytes in the intestine, while the endogenous pathway refers to lipids that are synthesized, primarily in the liver. Additionally, excess cholesterol is removed from peripheral tissues via reverse cholesterol transport, a process largely mediated by HDL. **Figure 1** shows an overview of the pathways involved in lipoprotein metabolism[5].

1.2.1 Exogenous pathway

When dietary cholesterol and fats are absorbed by enterocytes in the small intestine, they are incorporated into chylomicrons, which are large lipoprotein particles rich in TGs containing apolipoprotein(apo)B48[4]. In the circulation, chylomicrons acquire apoC-II from HDL particles, which can activate lipoprotein lipase (LPL), that turn TGs from chylomicrons into free fatty acids (FFAAs). These FFAAs can then be taken up by peripheral tissues, such as skeletal muscle, the heart and adipose tissue, for energy consumption by fatty acid oxidation (burning) or storage by re-esterification into TGs. In addition to apoC-II, HDL particles donate apoE to chylomicrons, which allows chylomicron remnant particles to be cleared by the liver via remnant receptors. Concurrently, a portion of the cholesterol and phospholipids from chylomicrons is transferred to HDL particles (**Figure 1**).

1.2.2 Endogenous pathway

In a fasting state, the liver activates *de novo* cholesterol and TG synthesis, leading to the assembly and secretion of very low density lipoprotein (VLDL) particles[4]. VLDL particles contain apoB100 and, upon entering the circulation, acquire apoC-II and apoE from HDL particles. ApoC-II activates LPL, which hydrolyzes TGs within VLDL, releasing FFAAs and glycerol for uptake by peripheral tissues. This lipid exchange results in the formation of denser particles known as intermediate-density lipoproteins (IDLs).

IDLs undergo further TG hydrolysis, ultimately becoming LDL particles. These LDL particles can bind to LDL receptors on peripheral cell membranes, facilitating the delivery of free cholesterol to tissues. Free cholesterol refers to unesterified cholesterol molecules that retain their hydroxyl group, allowing them to integrate into cell membranes where they contribute to membrane fluidity and serve as precursors for steroid hormones, bile acids and vitamin D. Alternatively, LDL can be internalized by LDL receptors expressed by hepatocytes, a process that downregulates 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in hepatic cholesterol synthesis (**Figure 1**).

1.2.3 Reverse cholesterol transport

Nascent HDL particles are formed in both the liver and intestine[4]. HDL particles play a central role in reverse cholesterol transport by acquiring free (unesterified) cholesterol and phospholipids from peripheral tissues, a process mediated by the ATP-binding cassette transporter A1 (ABCA1). Once matured, HDL particles may be transported directly to the liver, where cholesterol is selectively taken up via the scavenger receptor class B type 1 (SR-B1). Alternatively, cholesteryl esters (CEs) within HDL particles, that are formed when free cholesterol is enzymatically esterified with a fatty acid, can be exchanged for TGs from apoB-containing lipoproteins (such as

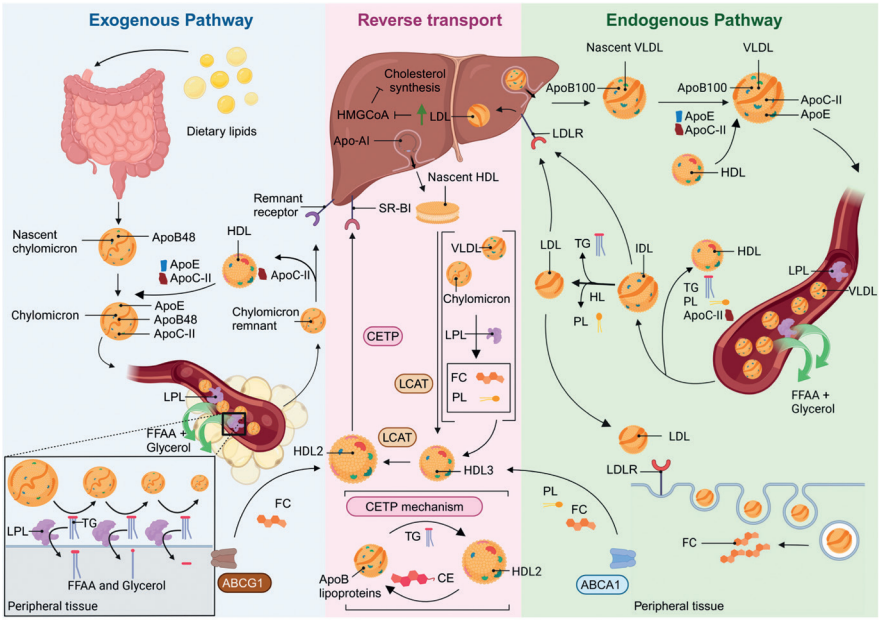


Figure 1. Lipoprotein metabolism. The exogenous pathway starts in the small intestine, where dietary lipids are absorbed and reassembled into nascent chylomicrons. These lipoproteins interact with HDL and acquire apoE and apoC-II. Mature chylomicrons transport TG and cholesterol to peripheral tissues, where LPL hydrolyses TG, releasing FFAA for energy or storage. Chylomicron remnants can interact with HDL and are taken up by the liver. The endogenous pathway is activated with the liver synthesizing VLDL to distribute TG and cholesterol. Nascent VLDL particles receive apoE and apoC-II from interactions with HDL. Mature VLDL is progressively metabolized into IDL and LDL, which return directly to the liver for degradation or deliver cholesterol to tissues by LDLR binding and endocytosis. HDL particles mediate reverse cholesterol transport, shuttling cholesterol from peripheral tissues to the liver for its excretion. ApoA-I is synthesized in the liver and acquires free cholesterol and PL as a result of LPL activity or via ABCA1 transporter from peripheral cells. LCAT esterifies free cholesterol on the HDL surface. As HDL matures, it engages with ABCG1 transporters from peripheral cells to acquire free cholesterol and interacts with CETP to transfer TG in exchange for CE with apoB-containing lipoproteins. Then, HDL can either deliver cholesterol directly to the liver via the SR-BI receptor or indirectly via CETP to apob-containing lipoproteins, which are then cleared by the liver. Apo: apolipoprotein; TG: triglycerides; FFAA: free fatty acid; VLDL: very-low-density lipoproteins; IDL: intermediate-density lipoproteins; LDL: low-density lipoproteins; HDL: high-density lipoproteins; LDLR: LDL receptor; PL: phospholipids; LPL: lipoprotein lipase; ABCA1: ATP-binding cassette protein A1; LCAT: lecithin–cholesterol acyltransferase; ABCG1: ATP-binding cassette protein G1; CETP: cholesteryl ester transfer protein; CE: cholesterol esters; SR-BI: scavenger receptor class B type I.[1]

1. Figure by Alcover et al.

VLDL and LDL), a process facilitated by cholesteryl ester transfer protein (CETP). In the liver, unesterified cholesterol from remnant particles and LDL can either be secreted into bile or converted into bile acids, contributing to cholesterol homeostasis (**Figure 1**).

1.2.4 Lipoprotein metabolism in metabolic syndrome

In metabolic syndrome, dyslipidemia is characterized by elevated plasma TGs, an increase in apoB-containing lipoproteins, alongside reductions in HDL-C levels. These abnormalities arise from both increased production and impaired clearance of TGs and apoB-containing lipoproteins. Moreover, HDL particles often exhibit functional impairments, diminishing their atheroprotective roles such as reverse cholesterol transport. The imbalance in lipid metabolism is further exacerbated by chronic low-grade inflammation, which primarily originates from the adipose tissue, where hypertrophied adipocytes release pro-inflammatory cytokines that attract other immune cells, thereby further amplifying the inflammatory response[6,7]. This persistent inflammatory state disrupts normal lipoprotein metabolism by interfering with key enzymatic processes, impairing HDL maturation and function, reducing TG clearance, and promoting LDL formation[8]. Collectively, these metabolic disturbances not only increase CV risk but also accelerate the progression of liver dysfunction.

1.3 Metabolic dysfunction-associated fatty liver disease

The liver plays a central role in lipid metabolism. Dysregulation of lipoprotein metabolism contributes to lipid accumulation in the liver, which in turn drives the development of MASLD. Clinically, MASLD encompasses a spectrum of liver damage, usually beginning with hepatic fat accumulation, i.e. steatosis (**Figure 2**)[9]. When steatosis is accompanied by inflammation, MASLD progresses to metabolic dysfunction-associated steatohepatitis (MASH)[9]. Persistent MASH can result in the development of hepatic fibrosis and, in advanced stages in cirrhosis, hepatocellular carcinoma and end-stage liver disease (**Figure 2**)[9]. While MASLD is a liver-centered disease, its systemic implications are profound. The leading cause of mortality in patients with MASLD is CVD, highlighting the strong interconnection between hepatic lipid metabolism and CV risk[10,11].

Driven by the global rise in obesity and metabolic syndrome, the prevalence of MASLD has increased substantially. However, diagnosis of MASLD is difficult, since the current golden standard method is an invasive method, namely performing a biopsy followed by histological assessment. Prevalence estimates therefore often rely on less invasive methods, such as blood-based biomarkers (e.g. elevated alanine transaminase (ALT) and aspartate transaminase (AST) levels), ultrasound imaging or magnetic resonance imaging. While these approaches are safer and more accessible, they lack precision in assessing disease severity, particularly in distinguishing

between simple steatosis and progressive forms like MASH[12]. Moreover, MASLD often remains asymptomatic in the early stages with clinical signs typically emerging only after advanced fibrosis has developed, when therapeutic options are more limited and prognosis is poorer. As a consequence of the increased worldwide obesity prevalence, the clinical burden of MASLD has increased as well. Current estimates are that MASLD affects 38% of the adult population worldwide[13,14]. Due to difficulties in diagnosing MASLD, the prevalence of the disease may be even higher than the present estimations.

Despite the growing prevalence of MASLD, therapeutic progress has been modest. While several investigational compounds have shown efficacy in reducing hepatic steatosis and inflammation, most have failed to demonstrate meaningful improvements in hepatic fibrosis, the strongest predictor for long-term outcomes. Notably, several high-profile candidates, including the farnesoid X (FXR) agonist obeticholic acid and peroxisome proliferator-activated receptors (PPAR) agonists, showed promise in early-phase trials but ultimately failed to meet primary endpoints in phase 3[15,16]. These setbacks underscore the complexity of MASLD pathophysiology and the challenge of developing effective monotherapies. However, during the course of writing this thesis, several breakthroughs have been made and two compounds have now been approved for the treatment of MASLD and MASLD-related fibrosis. The thyroid hormone receptor β (THR-β) agonist resmetirom was shown in phase 3 (NCT03900429) to result in resolution of MASH and at least one stage improvement of fibrosis[17], resulting in its approval and therefore making it the first drug approved for the treatment of MASLD. Furthermore, the GLP-1 receptor agonist semaglutide, that will be discussed further in this thesis as well, was recently approved by the U.S. Food Drug Administration (FDA) for treatment of MASH in adults with moderate to advanced liver fibrosis, based on results of a phase 3 clinical trial (NCT04822181)[18].

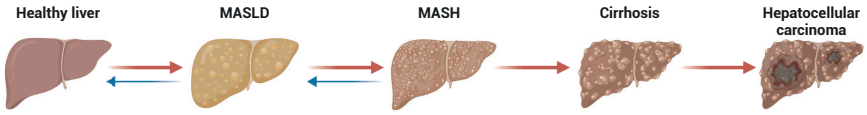


Figure 2. Stages of liver disease development. The healthy liver can accumulate fat due to metabolic overload, resulting in metabolic dysfunction-associated steatotic liver disease (MASLD). Continued lipid accumulation triggers liver inflammation, resulting in metabolic syndrome-associated steatohepatitis (MASH). If unresolved, MASH can progress to liver fibrosis, and in advanced stages cirrhosis and hepatocellular carcinoma. Red arrows indicate disease progression, blue arrows indicate stages of the disease that are reversible. Figure created with BioRender.com.

1.4 Atherosclerotic cardiovascular disease

The development of atherosclerotic lesions in the blood vessel wall begins in early childhood and progresses throughout life (**Figure 3**). In response to triggers such as dyslipidemia, hypertension, hyperglycemia or pro-inflammatory mediators, endothelial cells become activated and endothelial permeability increases[19]. This leads to the expression of leukocyte adhesion molecules on endothelial cells, facilitating the recruitment of circulating immune cells. LDL particles that have entered the intima layer of the vessel wall become oxidized (oxLDL), which further promotes attraction of monocytes from the circulation. Monocytes extravasate, after which they differentiate into macrophages that take up oxidized and aggregated LDL cholesterol, thereby transitioning into foam cells. The formation and increase in number of foam cells form the first steps in atherosclerosis development, classified by the AHA as type I 'early fatty streak' and type II 'regular fatty streak' lesions[20]. In the imbalanced dyslipidemic environment, smooth muscle cells (SMCs), that form the media layer of the arterial wall, migrate to the intima layer where they proliferate and produce extracellular matrix molecules. In doing so, a fibrous cap is formed that overlies the lipid-laden foam cells, known as type III 'mild' lesion. Ultimately, the accumulation of oxidized lipids, cholesterol and foam cells trigger macrophage cell death with release of proteolytic enzymes and cell debris, which in turn triggers more pro-inflammatory processes. A necrotic core is formed within the atherosclerotic lesion, which is classified as a type IV 'moderate' lesion. Further necrosis and formation of calcium

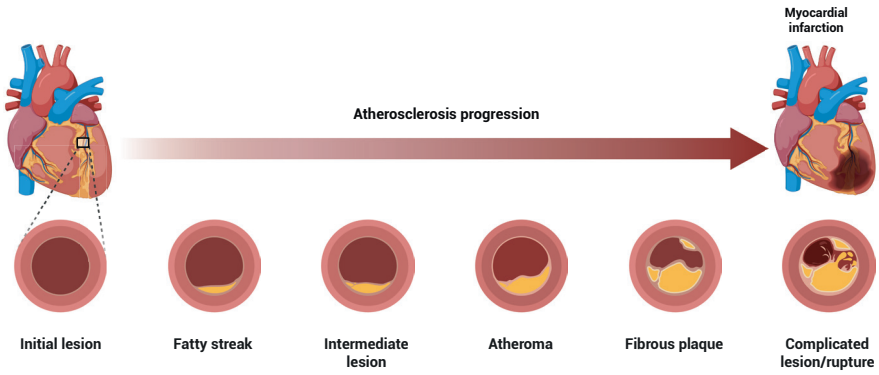


Figure 3. Stages of atherosclerosis development. The process of atherosclerosis starts with endothelial dysfunction, which may be caused by metabolic overload. Lipids accumulate in the arterial wall, which triggers the recruitment of immune cells, giving rise to fatty streaks. Over time, these fatty streaks evolve into atheromatous plaques, composed of lipids, inflammatory cells and fibrous tissue. Plaque progression may result in calcification, vascular remodeling, and narrowing of the arterial lumen, impairing blood flow. In advanced stages, plaque rupture can occur, potentially leading to thrombosis, myocardial infarction, or stroke. Figure created with BioRender.com.

deposits indicate that and the media layer is severely affected, classified as a type V 'severe' lesion. The severe type IV and V lesions are prone to rupturing, thereby releasing their necrotic content and highly thrombotic material into the lumen, which triggers the acute formation of a thrombus (type VI lesion). Atherosclerosis is a major contributor to CVD, the leading cause of death worldwide[21].

In 2022, CVDs accounted for approximately 32% of all global deaths, with heart attacks and strokes responsible for 85% of these fatalities[22]. As mentioned previously, risk factors include high blood pressure, hyperlipidemia, T2DM, obesity but also smoking, sedentary lifestyles and genetic predisposition. Despite the availability of effective medications such as statins, many patients fail to reach recommended lipid targets[23]. This highlights the importance for more effective and combination pharmacological strategies to better support patients in achieving lipid targets and improving long-term CV outcomes.

1.5 Intervention strategies for metabolic complications

Weight loss has been consistently shown to improve outcomes in both MASLD and CVD. As such, lifestyle changes with the goal of losing weight remain the first-line strategy for managing complications resulting from the metabolic syndrome. However, it is important to note that certain components of metabolic syndrome, like hyperlipidemia, can independently contribute to CVD risk, even in individuals without obesity. Therefore, while weight loss is beneficial, it may not fully address all risk factors. In cases where lifestyle changes prove insufficient, pharmacologic therapy becomes essential in accordance with European[24] and American[25] guidelines. Despite the multifactorial nature of metabolic syndrome, current standards of care tend to address its individual components, including hypertension, dyslipidemia, and hyperglycemia.

For management of blood pressure, beta-blockers, angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers are preferred[26] while in case of dyslipidemia, statins remain the first-line therapy for lowering lipid levels and reducing CV risk[27]. Fibrates or omega-3 fatty acids, on the other hand, may be included for patients with hypertriglyceridemia[24] and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are considered in high-risk individuals who do not achieve lipid targets with statins alone[24]. Bempedoic acid, an oral ATP-citrate lyase inhibitor, targets cholesterol synthesis upstream of HMG-CoA reductase[28]. It has emerged as an alternative for patients intolerant to statins or requiring additional LDL-C reduction, where it shows promise in reducing CV events[28]. In recent years, there is a growing emphasis on glucose-lowering therapies that offer CV and renal benefits. In patients with T2DM, metformin is usually prescribed while sodium glucose co-transporter 2 (SGLT2) inhibitors (empagliflozin, dapagliflozin) are increasingly used for their ability to improve glycemic control while also reducing risk of heart failure

and chronic kidney disease[29]. More recently, glucagon-like peptide-1 (GLP-1) receptor agonists (exenatide, liraglutide, semaglutide, tirzepatide (dual glucose-dependent insulinotropic polypeptide (GIP)/GLP-1 receptor agonist)) have gained attention for their benefits in improving metabolic parameters and promoting weight loss, making them a valuable therapeutic option even beyond glycemic control. Several of these existing, as well as emerging pharmacological therapies have been further studied in this thesis and will be discussed in short below.

1.5.2 Lipid-lowering interventions

1.5.2.1 Statins

Statins are inhibitors of HMG-CoA reductase, the rate-limiting enzyme in the mevalonate pathway responsible for hepatic cholesterol synthesis. Inhibiting this pathway leads to a reduction in intracellular cholesterol levels, which in turn upregulates LDL receptor expression of hepatocytes. This enhances LDL-C clearance from the circulation, resulting in a 20-50% reduction in plasma LDL-C levels depending on statin type and dosage[30]. Statins are the first-line approach for both primary and secondary CVD prevention. However, due to large inter-patient variability in response to statins or more extremely elevated cholesterol levels, a substantial proportion of patients fail to achieve target lipid levels or report adverse effects. To further reduce residual CV risk and achieve better lipid control, alternative approaches or combination therapies are increasingly being considered.

1.5.2.2 Ezetimibe

The cholesterol absorption inhibitor ezetimibe works by blocking Niemann-Pick C1-like 1, responsible for cholesterol uptake in the small intestine. Ezetimibe decreases absorption of biliary and dietary cholesterol without interfering with absorption of fat-soluble vitamins, TGs or bile acids. In accordance with European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines[24], ezetimibe is prescribed in combination with statins when statins alone do not result in a sufficient reduction in LDL-C. Adding ezetimibe to statin intervention further reduces circulating LDL-C levels by about 15-25%[31], thereby contributing to improved CV outcomes.

1.5.2.3 PCSK9 inhibitors

A different class of lipid-lowering interventions are PCSK9 inhibitors. PCSK9 binds to LDL receptors on hepatocytes, which promotes their degradation. This process reduces the number of LDL receptors available to clear LDL-C from the circulation. By inhibiting PCSK9, the recycling and surface expression of LDL receptors is increased, which enhances LDL-C clearance. Monoclonal antibodies such as evolocumab and alirocumab reduce LDL-C by up to 60%, offering substantial CV risk reduction,

especially when given on top of statins[32–36]. Inclisiran, a small interfering RNA, also targets PCSK9 but works by silencing its hepatic synthesis, allowing for sustained LDL-C reduction[37]. However, the widespread use of PCSK9 inhibitors is limited by their high production costs, making them less accessible to a larger patient population.

1.5.2.4 ANGPTL3 inhibitors

Angiopoietin-like 3 (ANGPTL3) is a dual inhibitor of LPL and endothelial lipase (EL), enzymes responsible for hydrolysis of TGs in circulating lipoproteins. Inhibition of this process, e.g. by the ANGPTL3 inhibitor evinacumab, enhances LPL and EL activity, which in turn increases TG lipolysis and improves lipid clearance. Evinacumab reduces LDL-C levels by 47-50%[38] and is prescribed in patients in whom lipid lowering is not sufficiently reached using conventional lipid lowering strategies such as with statins and ezetimibe.

1.5.2.5 CETP inhibitors

CETP inhibitors are a class of compounds that modulate lipid metabolism by targeting CETP, a protein that facilitates the transfer of CEs in exchange for TGs from HDL-C to apoB-containing lipoproteins. By inhibiting this process, there is a shift towards more HDL-C and less LDL-C, thereby enhancing reverse cholesterol transport and reducing CV risk. Several CETP inhibitors have been developed in the past, that all failed in clinical stages of drug development due to adverse effects or lack of efficacy. Nevertheless, research on CETP inhibitors continues and promising results are being presented with the novel CETP inhibitor obicetrapib, that shows reductions in LDL-C of 35-50%[39,40].

1.5.3 Modulators of energy metabolism

1.5.3.1 GLP-1 receptor agonists

Recently, incretin-based therapies have emerged as approach to intervene in obesity. GLP-1 is an incretin hormone that induces the release of insulin and therefore GLP-1 agonists such as semaglutide have been developed to improve glycemic control in patients with T2DM. Beyond their intended glucose-lowering effects, GLP-1 receptor agonists demonstrated benefits on satiety and gastric emptying, resulting in reduced food intake and lower body weight[41,42]. Consequently, GLP-1 receptor agonists are now the first pharmacological approach for the treatment of obesity[43].

1.5.3.2 FGF21 analogues

Analogues of the fibroblast growth factor 21 (FGF21) form a promising new class of therapeutic agents due to the regulatory effects of FGF21 on glucose and lipid metabolism[44]. Because of the short half-life of endogenous FGF21, its beneficial effects on metabolism are limited[45]. Clinical data on FGF21 analogues that mimic endogenous

FGF21 signaling have been shown to improve insulin sensitivity and reduce hepatic steatosis and are therefore promising targets for treating MASLD[46,47].

1.6 Experimental MASLD and atherosclerosis

To develop and evaluate efficacy of (novel) strategies aimed at reducing plasma lipids, alleviating CV burden and managing MASLD, several pharmacological approaches have been explored. Ideally, these approaches would be immediately tested in human patient populations, which is ethically unacceptable and practically impossible. While *in vitro* strategies have been and are being used to replicate certain aspects of metabolic syndrome, they are limited by their inability to capture the systemic nature of the disease. Consequently, *in vivo* experimental models remain essential for a comprehensive and holistic investigation of metabolic syndrome and its potential therapeutic interventions. In this thesis, both existing and emerging therapeutic strategies were evaluated in mouse models that closely recapitulate features of human metabolic syndrome or lipoprotein metabolism, and consequently develop metabolic complications such as MASLD and atherosclerosis.

1.6.1 Metabolic differences in mouse versus man

Wildtype mice predominantly transport cholesterol in HDL particles with minimal distribution in the atherogenic (V)LDL particles[48]. In these mice, clearance of apoB-containing lipoproteins is fast and efficient, mediated through the apoE-LDL receptor pathway. Compared to mice, humans exhibit delayed clearance of apoB-containing lipoproteins, a process that is further influenced by CETP, which facilitates the transfer of cholesterol from HDL to apoB-containing lipoproteins, thereby contributing to a more atherogenic lipid profile[48]. Due to these species-specific differences in lipoprotein metabolism, wildtype mice do not develop the dyslipidemia characteristic of human metabolic syndrome and therefore fail to accurately replicate human lipoprotein metabolism and its associated pathophysiology, including MASLD and atherosclerosis.

1.6.2 Ldlr-/-.Leiden mice

The Ldlr-/-.Leiden mouse model has emerged as a more physiologically relevant model for studying aspects of the metabolic syndrome and MASLD. These mice are derived from a cross between Ldlr-/- mice on a C57BL/6 background and SV129 mice, resulting in a unique genetic background distinct from conventional Ldlr-/- mice. This results in the expression of a truncated and therefore non-functional LDL receptor protein that is unable to bind LDL lipoproteins[49]. The hepatic clearance of apoB-containing lipoproteins is therefore strongly reduced, shifting the lipoprotein profile in these mice towards a more hyperlipidemic pattern. When fed a diet high in fat and sugar content, these mice develop obesity, insulin resistance, adipose tissue

inflammation and MASH with bridging fibrosis (stage F3)[11,50,51]. Moreover, extensive transcriptomics analysis revealed that a very high percentage of differentially expressed pathways that characterize MASH patients is recapitulated in this mouse model[52]. The Ldlr-/-.Leiden mouse model is therefore a good model to study mechanisms and interventions related to metabolic dysfunction, as these mice develop dyslipidemia and consequently MASLD and atherosclerosis.

1.6.3 APOE*3-Leiden mice and APOE*3-Leiden.CETP mice

The APOE*3-Leiden mouse model was developed to express the human ApoE*3-Leiden variant and ApoC1 genes[53,54]. The ApoE*3-Leiden variant is a naturally occurring variant of the ApoE*3 allele. It differs from the standard APOE3 variant by altered receptor-binding properties, specifically showing reduced affinity for the LDL receptor, which impairs hepatic clearance of TG-rich lipoprotein remnants such as chylomicron and VLDL remnants. While multiple ApoE*3 variants exist, the Leiden variant is particularly relevant because it impairs remnant clearance, thereby mimicking human-like dyslipidemia. In humans, ApoE*3-Leiden is associated with a rare dominantly inherited form of familial hyperlipoproteinemia[55]. In APOE*3-Leiden.CETP mice, APOC1 is dominantly expressed, which further contributes to dyslipidemia by inhibiting lipolysis and further delaying clearance of apoB-containing lipoproteins. As such, when fed a diet high in sugar and fat content, these mice become dyslipidemic and develop atherosclerosis.

By introducing the human CETP gene into the APOE*3-Leiden mouse model, the resulting transgenic mice replicate human metabolism even further[56]. This modification slows the clearance of apoB-containing lipoproteins, mimicking the delayed lipoprotein clearance observed in humans and thereby providing a more physiologically relevant model for studying dyslipidemia and CV risk.

Both APOE*3-Leiden and APOE*3-Leiden.CETP mice respond well to lipid-lowering interventions, including clinically used compounds such as statins[57–60], ezetimibe[58], PCSK9 inhibitors[59,61,62], ANGPTL3 inhibitors[38,60,63], HDL-raising treatments[64–66], and CETP inhibitors[67–69]. These models are therefore highly suitable for studying dyslipidemia and associated metabolic complications such as atherosclerosis, as well as for evaluating therapeutic interventions aimed at modulating these complications.

1.7 Aims and outline of this thesis

1.7.1 Aims

With the rising prevalence of obesity and metabolic syndrome-related complications, there is an urgent need for complementary approaches to lifestyle interventions. Currently, there is a lack of therapeutic approaches that target the metabolic syndrome holistically, aiming to relieve not only the CV burden but MASLD and

other complications as well. Using the Ldlr-/-.Leiden and APOE*3-Leiden(.CETP) mouse models, we studied the therapeutic potential and metabolic impact of existing and novel pharmacological interventions in the context of metabolic syndrome.

1.7.2 Outline

In **Chapter 2**, we evaluated how repeated weight cycling, a phenomenon often observed in patients that try to lose weight, affected metabolic complications in diet-induced obese mice. In the past, many compounds that have been developed for MASH-related fibrosis and were proven successful in preclinical stages, have ultimately failed in the clinic. To address this translational gap, we developed a rapid screening method based on a fibrogenic gene signature to predict the long-term efficacy of these drugs on MASH-related fibrosis. The results of this study are presented in **Chapter 3**.

In Chapters 4 through 7, the effects of several pharmacological compounds on metabolic complications were evaluated, with a focus on MASLD. The effects of a novel FGF21 analogue bFKB1 are described in **Chapter 4**. The effects of the GLP-1 receptor agonist semaglutide are described in **Chapter 5**. Further elaborating upon the data found in the study described in Chapter 5, we describe how semaglutide affects metabolic syndrome when combined with exercise in **Chapter 6**. **Chapter 7** describes the effects of atorvastatin, a well-established lipid-lowering agent, on MASLD. The following two chapters shift the focus toward atherosclerosis. **Chapter 8** describes the effects of a novel PCSK9 inhibitor alone and in combination with the ANGPTL3 antibody evinacumab on atherosclerosis development. In **Chapter 9**, we investigate the mechanisms of action of obicetrapib, alone and in combination with ezetimibe, and evaluate their effects on both the progression and regression of atherosclerosis. The results obtained in these studies and future perspectives are discussed in **Chapter 10**.

1.8 Acknowledgements

Figures 2 and 3 were made using BioRender.com, accessed September 2025.

2 References

1. Ferrari CKB. Epidemiology of metabolic syndrome: global scenario. In: *Metabolic Syndrome: From Mechanisms to Interventions*. ; 2024:59-71.
2. Alberti KGMM, Eckel RH, Grundy SM, et al. Harmonizing the Metabolic Syndrome. *Circulation*. 2009;120(16):1640-1645.
3. Nilsson PM, Tuomilehto J, Rydén L. The metabolic syndrome – What is it and how should it be managed? *Eur J Prev Cardiol*. 2019;26(2_suppl):33-46.
4. Feingold KR, Grunfeld C. *Introduction to Lipids and Lipoproteins*. MDText.com, Inc.; 2024. https://www.ncbi.nlm.nih.gov/books/NBK305896/
5. Alcover S, Ramos-Regalado L, Girón G, Muñoz-García N, Vilahur G. HDL-Cholesterol and Triglycerides Dynamics: Essential Players in Metabolic Syndrome. *Antioxidants*. 2025;14(4):434.
6. Khanna D, Khanna S, Khanna P, Kahar P, Patel BM. Obesity: A Chronic Low-Grade Inflammation and Its Markers. *Cureus*. 2022;14(2):e22711.
7. Kawai T, Autieri M V., Scalia R. Adipose tissue inflammation and metabolic dysfunction in obesity. *Am J Physiol - Cell Physiol*. 2020;320(3):C375. doi:10.1152/AJPCELL.00379.2020
8. Feingold KR, Grunfeld C. *Effect of Inflammation and Infection on Lipids and Lipoproteins*. MDText.com, Inc.; 2025.
9. Targher G, Valenti L, Byrne CD. Metabolic Dysfunction–Associated Steatotic Liver Disease. *N Engl J Med*. 2025;393(7):683-698.
10. Gutiérrez-Cuevas J, Santos A, Armendariz-Borunda J. Pathophysiological Molecular Mechanisms of Obesity: A Link between MAFLD and NASH with Cardiovascular Diseases. *Int J Mol Sci*. 2021;22(21).
11. Martínez-Arranz I, Bruzzone C, Noureddin M, et al. Metabolic subtypes of patients with NAFLD exhibit distinctive cardiovascular risk profiles. *Hepatology*. 2022;76(4):1121-1134.
12. Neuberger J, Cain O. The Need for Alternatives to Liver Biopsies: Non-Invasive Analytics and Diagnostics. *Hepatic Med Evid Res*. 2021;13:59.
13. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology*. 2023;77(4):1335.
14. Younossi ZM, Zelber-Sagi S, Lazarus JV, et al. Global Consensus Recommendations for Metabolic Dysfunction-Associated Steatotic Liver Disease and Steatohepatitis. *Gastroenterology*. Published online 2025.
15. Younossi ZM, Ratziu V, Loomba R, et al. Obeticholic acid for the treatment of non-alcoholic steatohepatitis: interim analysis from a multicentre, randomised, placebo-controlled phase 3 trial. *Lancet (London, England)*. 2019;394(10215):2184-2196.
16. Genfit. Phase 3 Study to Evaluate the Efficacy and Safety of Elafibranor Versus Placebo in Patients With Nonalcoholic Steatohepatitis (NASH) (RESOLVE-IT). ClinicalTrials.gov. Published 2022. https://clinicaltrials.gov/study/NCT02704403
17. Harrison SA, Bedossa P, Guy CD, et al. A Phase 3, Randomized, Controlled Trial of Resmetirom in NASH with Liver Fibrosis. *N Engl J Med*. 2024;390(6):497-509.
18. Sanyal AJ, Newsome PN, Kliers I, et al. Phase 3 Trial of Semaglutide in Metabolic Dysfunction–Associated Steatohepatitis. *N Engl J Med*. 2025;392(21):2089-2099.
19. Linton MF, Yancey PG, Davies SS, et al. The Role of Lipids and Lipoproteins in Atherosclerosis. *Science (80-)*. 2019;111(2877):166-186. Accessed October 26, 2025. https://www.ncbi.nlm.nih.gov/books/NBK343489/
20. Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb Vasc Biol*. 1995;15(9):1512-1531.
21. Di Cesare M, McGhie DV, Perel P, et al. The Heart of the World. *Glob Heart*. 2024;19(1):11.
22. Mensah GA, Fuster V, Murray CJL, et al. Global Burden of Cardiovascular Diseases and Risks, 1990-2022. *J Am Coll Cardiol*. 2023;82(25):2350-2473.
23. Ray KK, Molemans B, Schoonen WM, et al. EU-Wide Cross-Sectional Observational Study of Lipid-Modifying Therapy Use in Secondary and Primary Care: the DA VINCI study. *Eur J Prev Cardiol*. 2021;28(11):1279-1289.

24. Mach F, Koskinas KC, Roeters van Lennep JE, Tokgözoğlu L, Badimon L, Baigent C. 2025 Focused Update of the 2019 ESC/EAS Guidelines for the management of dyslipidaemias: Developed by the task force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J*. 2025;44(39):4043-4140.
25. Rao SV, O'Donoghue ML, Ruel M, et al. 2025 ACC/AHA/ACEP/NAEMSP/SCAI Guideline for the Management of Patients With Acute Coronary Syndromes: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation*. Published online 2025.
26. Johnson HM, Shimbo D, Abdalla M, et al. 2025 AHA/ACC/AANP/AAPA/ABC/ACCP/ACPM/AGS/AMA/ASPC/NMA/PCNA/SGIM Guideline for the Prevention, Detection, Evaluation and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Joint Committee. *Hypertension*. 2025;82(10):e212-e316.
27. Feingold KR. *Approach to the Patient with Dyslipidemia*. MDText.com, Inc.; 2025. doi:10.1530/endoabs.37.mte10
28. Ray KK, Nicholls SJ, Li N, et al. Efficacy and safety of bempedoic acid among patients with and without diabetes: prespecified analysis of the CLEAR Outcomes randomised trial. *Lancet Diabetes Endocrinol*. 2024;12(1):19-28.
29. Marx N, Federici M, Schütt K, et al. 2023 ESC Guidelines for the management of cardiovascular disease in patients with diabetes. *Eur Heart J*. 2023;44(39):4043-4140. doi:10.1093/EURHEARTJ/EHAD192
30. Collins R, Reith C, Emberson J, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. *Lancet*. 2016;388(10059):2532-2561.
31. Cannon CP, Blazing MA, Giugliano RP, et al. Ezetimibe Added to Statin Therapy after Acute Coronary Syndromes. *N Engl J Med*. 2015;372(25):2387-2397.
32. Bays H, Gaudet D, Weiss R, et al. Alirocumab as add-on to atorvastatin versus other lipid treatment strategies: ODYSSEY OPTIONS I randomized trial. *J Clin Endocrinol Metab*. 2015;100(8):3140-3148.
33. Robinson JG, Farnier M, Krempf M, et al. Efficacy and Safety of Alirocumab in Reducing Lipids and Cardiovascular Events. *N Engl J Med*. 2015;372(16):1489-1499.
34. Moreno PR. The high-risk thin-cap fibroatheroma: a new kid on the block. *Circ Cardiovasc Interv*. 2009;2(6):500-502.
35. Sabatine MS, Giugliano RP, Wiviott SD, et al. Efficacy and Safety of Evolocumab in Reducing Lipids and Cardiovascular Events. *N Engl J Med*. 2015;372(16):1500-1509.
36. Schwartz GG, Steg PG, Szarek M, et al. Alirocumab and Cardiovascular Outcomes after Acute Coronary Syndrome. *N Engl J Med*. 2018;379(22):2097-2107.
37. Ray KK, Wright RS, Kallend D, et al. Two Phase 3 Trials of Inclisiran in Patients with Elevated LDL Cholesterol. *N Engl J Med*. 2020;382(16):1507-1519.
38. Dewey FE, Gusarova V, Dunbar RL, et al. Genetic and Pharmacologic Inactivation of ANGPTL3 and Cardiovascular Disease. *N Engl J Med*. 2017;377(3):211-221.
39. Ray KK, Nicholls S, Ditmarsch M, et al. Obicetrapib lowers LDL-C in patients on high intensity statins: Results from the rose trial (NCT04753606). *Atherosclerosis*. 2022;355:11-12.
40. Bhatti MI, Safiullah M, Farhan K, et al. Obicetrapib and its impact on lipid parameters: A comprehensive meta-analysis of the latest evidence. *J Clin Lipidol*. Published online 2025.
41. Ahrén B, Dimas AL, Miossec P, Saubadu S, Aronson R. Efficacy and safety of lixisenatide once-daily morning or evening injections in type 2 diabetes inadequately controlled on metformin (GetGoal-M). *Diabetes Care*. 2013;36(9):2543-2550.
42. Wysham C, Blevins T, Arakaki R, et al. Efficacy and safety of dulaglutide added onto pioglitazone and metformin versus exenatide in type 2 diabetes in a randomized controlled trial (AWARD-1). *Diabetes Care*. 2014;37(8):2159-2167.
43. Knudsen LB, Lau J. The discovery and development of liraglutide and semaglutide. *Front Endocrinol (Lausanne)*. 2019;10(APR):155.
44. Goetz R, Beenken A, Ibrahimi OA, et al. Molecular insights into the klotho-dependent, endocrine mode of action of fibroblast growth factor 19 subfamily members. *Mol Cell Biol*. 2007;27(9):3417-3428.
45. Spann RA, Morrison CD, Den Hartigh LJ. The Nuanced Metabolic Functions of Endogenous FGF21 Depend on the Nature of the Stimulus, Tissue Source, and Experimental Model. *Front Endocrinol (Lausanne)*. 2022;12:802541.

46. Loomba R, Sanyal AJ, Kowdley KV, et al. Randomized, Controlled Trial of the FGF21 Analogue Pegozafermin in NASH. *N Engl J Med*. 2023;389(11):998-1008.
47. Harrison SA, Ruane PJ, Freilich BL, et al. Efruxifermin in non-alcoholic steatohepatitis: a randomized, double-blind, placebo-controlled, phase 2a trial. *Nat Med*. 2021;27(7):1262-1271.
48. Dietschy JM, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans - PubMed. *J Lipid Res*. 1993;34(10):1637-1659.
49. Powell-Braxton L, Véniant M, Latvala RD, et al. A mouse model of human familial hypercholesterolemia: Markedly elevated low density lipoprotein cholesterol levels and severe atherosclerosis on a low-fat chow diet. *Nat Med*. 1998;4(8):934-938.
50. Morrison MC, Kleemann R, Van Koppen A, Hanemaaijer R, Verschuren L. Key Inflammatory Processes in Human NASH Are Reflected in Ldlr -/-.Leiden Mice: A Translational Gene Profiling Study. *Front Physiol*. 2018;9(FEB):132.
51. Morrison MC, Verschuren L, Salic K, et al. Obeticholic Acid Modulates Serum Metabolites and Gene Signatures Characteristic of Human NASH and Attenuates Inflammation and Fibrosis Progression in Ldlr-/-.Leiden Mice. *Hepatol Commun*. 2018;2(12):1513-1532.
52. Van den Hoek AM, Verschuren L, Worms N, et al. A Translational Mouse Model for NASH with Advanced Fibrosis and Atherosclerosis Expressing Key Pathways of Human Pathology. *Cells*. 2020;9(9).
53. Van den Maagdenberg AMJM, Hofker MH, Krimpenfort PJA, et al. Transgenic mice carrying the apolipoprotein E3-Leiden gene exhibit hyperlipoproteinemia. *J Biol Chem*. 1993;268(14):10540-10545. doi:10.1016/S0021-9258(18)82232-3
54. Van Vlijmen BJM, Van 't Hof HB, Mol MJ, et al. Modulation of very low density lipoprotein production and clearance contributes to age- and gender- dependent hyperlipoproteinemia in apolipoprotein E3-Leiden transgenic mice. *J Clin Invest*. 1996;97(5):1184-1192.
55. Havekes L, De Wit E, Leuven JG, et al. Apolipoprotein E3-Leiden. A new variant of human apolipoprotein E associated with familial type III hyperlipoproteinemia. *Hum Genet*. 1986;73(2):157-163.
56. Westerterp M, Van der Hoogt CC, De Haan W, et al. Cholesteryl ester transfer protein decreases high-density lipoprotein and severely aggravates atherosclerosis in APOE*3-Leiden mice. *Arterioscler Thromb Vasc Biol*. 2006;26(11):2552-2559.
57. Van de Poll SWE, Römer TJ, Volger OL, et al. Raman Spectroscopic Evaluation of the Effects of Diet and Lipid-Lowering Therapy on Atherosclerotic Plaque Development in Mice. *Arterioscler Thromb Vasc Biol*. 2001;21(10):1630-1635.
58. Gierman LM, Kühnast S, Koudijs A, et al. Osteoarthritis development is induced by increased dietary cholesterol and can be inhibited by atorvastatin in APOE*3Leiden.CETP mice-a translational model for atherosclerosis. *Ann Rheum Dis*. 2014;73(5):921-927.
59. Kühnast S, Van Der Hoorn JWA, Pieterman EJ, et al. Alirocumab inhibits atherosclerosis, improves the plaque morphology, and enhances the effects of a statin. *J Lipid Res*. 2014;55(10):2103-2112.
60. Pouwer MG, Pieterman EJ, Worms N, et al. Alirocumab, evinacumab, and atorvastatin triple therapy regresses plaque lesions and improves lesion composition in mice. *J Lipid Res*. 2020;61(3):365.
61. Landlinger C, Pouwer MG, Juno C, et al. The AT04A vaccine against proprotein convertase subtilisin/kexin type 9 reduces total cholesterol, vascular inflammation, and atherosclerosis in APOE*3Leiden.CETP mice. *Eur Heart J*. 2017;38(32):2499-2507.
62. Suchowerska AK, Stokman G, Palmer JT, et al. A Novel, Orally Bioavailable, Small-Molecule Inhibitor of PCSK9 With Significant Cholesterol-Lowering Properties In Vivo. *J Lipid Res*. 2022;63(11).
63. Zancanella V, Vallès A, Liefhebber JMP, et al. Proof-of-concept study for liver-directed miQURE technology in a dyslipidemic mouse model. *Mol Ther Nucleic Acids*. 2023;32:454-467.
64. Kühnast S, Louwe MC, Heemskerk MM, et al. Niacin Reduces Atherosclerosis Development in APOE*3Leiden.CETP Mice Mainly by Reducing NonHDL-Cholesterol. *PLoS One*. 2013;8(6):66467. doi:10.1371/JOURNAL.PONE.0066467
65. Van den Hoek AM, Van der Hoorn JWA, Maas AC, et al. APOE*3Leiden.CETP transgenic mice as model for pharmaceutical treatment of the metabolic syndrome. *Diabetes Obes Metab*. 2014;16(6):537-544.

66. Kühnast S, Fiocco M, Van der Hoorn JWA, Princen HMG, Jukema JW. Innovative pharmaceutical interventions in cardiovascular disease: Focusing on the contribution of non-HDL-C/LDL-C-lowering versus HDL-C-raising: A systematic review and meta-analysis of relevant preclinical studies and clinical trials. *Eur J Pharmacol*. 2015;763:48-63.
67. Kühnast S, Van der Tuin SJL, Van der Hoorn JWA, et al. Anacetrapib reduces progression of atherosclerosis, mainly by reducing non-HDL-cholesterol, improves lesion stability and adds to the beneficial effects of atorvastatin. *Eur Heart J*. 2015;36(1):39-48.
68. Simic B, Mocharla P, Crucet M, et al. Anacetrapib, but not evacetrapib, impairs endothelial function in CETP-transgenic mice in spite of marked HDL-C increase. *Atherosclerosis*. 2017;257:186-194.
69. De Haan W, De Vries-van der Weij J, Van der Hoorn JWA, et al. Torcetrapib does not reduce atherosclerosis beyond atorvastatin and induces more proinflammatory lesions than atorvastatin. *Circulation*. 2008;117(19):2515-2522.