Novel targets in the liver to treat cardiometabolic diseases

Ge, X.

CHAPTER 6

General Discussion and Future Perspectives

General discussion

Obesity has become a major global health problem, affecting more than 0.89 billion adults in 2022 [1,2] and with alarming projections of over 1.53 billion adults affected by 2035 [3]. The increase in obesity is strongly linked to a high incidence of a series of cardiometabolic diseases, e.g., steatotic liver disease, type 2 diabetes (T2D), cardiovascular diseases and other metabolic disorder-related diseases. Among these, metabolic dysfunction-associated steatotic liver disease (MASLD) and atherosclerotic cardiovascular disease (ASCVD) are among the most prevalent chronic diseases, substantially contributing to morbidity and mortality worldwide [4,5]. Although lifestyle interventions and pharmacological therapies are available, the latter mainly for ASCVD, limitations in their effectiveness and safety profile remain. AS the prevalence of these diseases is predicted to continue rising rapidly in the coming years, the search for new effective pharmacological therapies is thus warranted.

Given that the liver is the central organ to regulate lipid metabolism and systemic inflammation, strongly influencing the progression of both MASLD and ASCVD, this thesis aimed to explore novel hepatic therapeutic targets to prevent/treat these diseases through experimental studies in relevant mouse models. The results of the performed experiments and the implications, limitations as well as future perspectives of these novel therapeutic targets are discussed in this chapter.

1. DHCR24 as a novel target to prevent and treat MASLD and ASCVD

liver X receptor (LXR) activation has been considered as a therapeutic target for cardiometabolic diseases for over two decades due to its dual beneficial role in modulating cholesterol metabolism and suppressing inflammation. In macrophages, activation of LXR by cholesterol loading or synthetic agonists, promotes the expression of the cholesterol transporters ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) that cooperatively facilitate the efflux of cholesterol to high-density lipoprotein (HDL), which can transport cholesterol to the liver for excretion [6]. Efflux of excessive cholesterol from macrophages inhibiting its polarization toward a pro-inflammatory state is a critical step for preventing both MASLD and ASCVD progression [7,8]. However, currently available synthetic LXR agonists also activate the sterol regulatory element-binding protein 1c (SREBP-1c) pathway in hepatocytes, thereby inducing the upregulation of its downstream lipogenic genes and eventually causing hepatic steatosis and hyperlipidemia [9-11]. Due to these severe side effects, the clinical development of synthetic LXR agonists has been halted.

To circumvent induction of lipogenesis, the concepts of 'tissue-specific LXR activation' and 'selective LXR activation' emerged to achieve anti-inflammatory effects of LXR activation while minimizing its lipogenic side effects, e.g. by nanoparticle-based delivery of LXR agonists. Nanoparticles have been used in targeted drug delivery to specific cells/tissues for treating certain diseases in humans. The materials used for developing nanoparticles include liposomes, chitosan, poly(lactic-coglycolic acid) (PLGA), dextran, silica and metals [12]. Nanoparticles made from these materials can be selectively recognized and absorbed by target cells, e.g., endothelial cells, tumor cells and macrophages, depending on size, surface charge and cell-specific ligands [12]. For example, encapsulating the synthetic LXR agonist GW3965 in nanoparticles targeting macrophages increases cholesterol efflux and suppresses inflammation of the plaque via increasing ABCA1 and ABCG1 expression, thereby preventing atherosclerosis in mice [13]. Importantly, while free GW3965 induces lipogenesis, GW3965 encapsulated in nanoparticles actually reduces lipids in liver and plasma [13]. Similarly, encapsulation of the LXR agonist T0901317 into synthetic HDL nanoparticles targeting plaque-resident macrophages effectively reduces atherosclerosis in mice without affecting the expression of SREBP-1c target lipogenic genes in the liver [14]. Nanoparticles are now being widely used in the treatment of various diseases, e.g., cancer, autoimmune and neurological diseases, after several decades of technological developments. However, their application still faces limitations, including challenges in production complexity and scale-up consistency, individual variability and immune system interference, and high costs [15,16], all of which hinder the clinical application of nanoparticle-based delivery of synthetic LXR agonists.

To prevent induction of lipogenesis while circumventing the need for drug encapsulation, drugs that selectively activate LXR in immune cells but not in hepatocytes would be highly desirable. Very interestingly, in 2012 the research group of Christopher Glass has identified desmosterol as a selective and potent endogenous LXR ligand that accumulates in cholesterol-laden macrophages. Desmosterol is the last intermediate in the Bloch pathway of *de novo* cholesterol synthesis and is converted into cholesterol by the enzyme Δ24-dehydrocholesterol reductase (DHCR24). Loading of macrophages with cholesterol causes product inhibition of DHCR24, resulting in accumulation of desmosterol, which was initially shown to reprogram lipid metabolism and suppress inflammation by activating LXR, while suppressing the SREBP-1c pathway [17]. Later, desmosterol was further revealed to strongly activate LXR target genes in macrophages of both mice and humans, while it does not activate LXR in hepatocytes [18]. Following these promising findings, elevating desmosterol to selectively activate LXR has been considered a potential therapeutic strategy for cardiometabolic diseases.

In 2017, Christoph Müller and his team successfully generated SH42 as the first selective and potent DHCR24 inhibitor, and found that treatment of mice with SH42 remarkably increases circulating desmosterol levels [19]. Subsequently, SH42 was shown to increase the

production of anti-inflammatory and pro-resolving lipid mediators, thereby resolving peritonitis in mice [20]. Inspired by its potent anti-inflammatory effects and predicted inability to inhibit LXR in hepatocytes, we aimed to investigate the potential of SH42 in preventing the development of MASLD and ASCVD by using APOE*3-Leiden.CETP mice, a well-established humanized mouse model for the study of (cardio)metabolic diseases. In **Chapter 2**, we revealed that i.p. injection of SH42 increases desmosterol in liver and plasma to ameliorate high-fat, high-cholesterol diet-induced inflammation by preventing activation of resident macrophages in the liver (i.e., Kupffer cells), while even attenuating hepatic steatosis and without inducing hyperlipidemia. However, when evaluating the effect of SH42 on Western-type diet-induced atherosclerosis in **Chapter 3**, SH42 treatment again increases desmosterol without affecting plasma lipids, but does not attenuate atherosclerosis development. Considering that the primary effect of desmosterol is anti-inflammatory, we next tested the effect of SH42 in *Ldlr-deficient* (*Ldlr*$^{-/-}$) mice, a model for more inflammation-driven atherosclerosis. While more pronounced effects were observed on circulating monocyte populations in this mouse model, still no effect on atherosclerosis development was seen. Finally, a recent study in our lab showed that SH42 is also unable to accelerate plaque regression in APOE*3-Leiden.CETP mice after a switch from a Western-type diet to a regular chow diet (Miao et al., unpublished).

Collectively, we thus identified SH42-induced inhibition of DHCR24 to raise desmosterol as a promising therapeutic strategy for prevention of MASLD, without triggering lipogenic effects associated with previous synthetic LXR agonists, but were unable to make a strong case for application in prevention or treatment of ASCVD. Interestingly, a synthetic LXR inverse agonist (SR9238) has been shown to reduce hepatic steatosis and inflammation in mice via suppressing SREBP-1c target genes [21], providing evidence that inhibiting LXR activation in hepatocytes may be beneficial in MASLD. However, in macrophages this LXR inverse agonist downregulates the expression ABCA1 and ABCG1 [22,23], which in turn impairs the cholesterol efflux from macrophages, potentially increasing the risk of atherosclerosis [24]. In fact, an siRNA targeting LXRα in hepatocytes has been developed for treating metabolic dysfunction-associated steatohepatitis (MASH) that reached a phase 1 clinical trial [25]. Although such a strategy will probably reduce liver steatosis, it evidently does not make use of the benefits of LXR activation in macrophages, and is less effective compared to SH42-induced DHCR24 downregulation.

So why would SH42 be very effective in peritonitis and MASLD but not ASCVD? Since peritonitis and diet-induced MASLD occur under much higher inflammatory conditions compared to the low-grade inflammatory conditions in diet-induced ASCVD, it is tempting to speculate that DHCR24 inhibition is especially useful to treat highly inflammatory diseases. Indeed, in our lab, SH42 was recently shown to be also effective in preventing septic death in mice (Li et al., unpublished) and inducing remyelination in a mouse model of multiple sclerosis (Feng et al., submitted). Nonetheless, it may be premature to conclude

that DHCR24 inhibition is not beneficial in ASCVD. While in diseases like peritonitis, MASH and sepsis immune cells, including macrophages, are easily reached by i.p. injected SH42, the ability of SH42 or desmosterol to reach macrophages within subendothelial arterial plaques may be compromised. In fact, depleting desmosterol in macrophages through conditional overexpression of DHCR24 in myeloid cells of *Ldlr*$^{-/-}$ mice increased lipid accumulation and inflammation by downregulating the expression of ABCA1 and ABCG1, thereby accelerating atherosclerosis development [26]. Therefore, knowing the efficacy of SH42 in increasing desmosterol levels in arterial macrophages, and how to improve that, is essential for understanding its potential effect in atherosclerosis. In addition, extensive evidence demonstrated that inflammatory responses display distinct characteristics at different stages of atherosclerosis progression [27-29]. Thus, it would be important to study whether the anti-inflammatory effect of SH42 exerts a stage-dependent effect/efficacy in atherosclerosis development.

Taken together, given the pronounced anti-inflammatory effects of SH42 by selectively activating LXR in macrophages without inducing hepatic steatosis and hyperlipidemia in preclinical studies, clinical development of DHCR24-inhibitory strategies in diseases associated with macrophage-driven inflammation is warranted. To this end, steroidal and non-steroidal DHCR24 inhibitors other than SH42 have been patented (WO2023172132A1) for therapeutic application in these diseases. Future research should focus on (1) optimizing DHCR24 inhibitors with respect to oral availability, (1) evaluating the safety and therapeutic efficacy of the long-treatment in preclinical as well as clinical studies; (2) enhancing organ/cell-specificity to maximize therapeutic outcomes; (3) improving the cost-effectiveness. If all these obstacles for human application have been taken, DHCR24 inhibitory compounds may be a breakthrough in the treatment of inflammatory diseases.

2. Hepatic lipase as a novel target to combat ASCVD

Over the past decades, the core strategy for combating ASCVD has been lowering circulating cholesterol levels. For example, current pharmacological management for ASCVD risk is primarily achieved via inhibiting endogenous cholesterol synthesis via hydroxymethylglutaryl-CoA reductase inhibition (i.e., statins), decreasing intestinal cholesterol absorption via Niemann Pick C1 like 1 inhibition (i.e., ezetimibe), and enhancing cholesterol clearance from the circulation via proprotein convertase subtilisin/kexin type 9 inhibition (i.e., alirocumab, evolocumab, inclisiran). However, substantial residual ASCVD risk persists, suggesting that additional therapeutic strategies targeting pathways in lipoprotein metabolism other than directly altering cholesterol metabolism are warranted. In recent years, enhancing hydrolysis of triglycerides (TG) and phospholipids (PL) within lipoproteins to accelerate remodeling their composition/structure and thus their catabolism

has been suggested as a novel therapeutic target for ASCVD. This led to increased interest in various lipases acting on circulating lipoproteins, such as lipoprotein lipase (LPL), endothelial lipase (EL) and hepatic lipase (HL), all of which exhibit both TG hydrolase activity and phospholipase activity [30,31].

The functions of LPL and EL in lipoprotein metabolism and atherosclerosis have been well-studied, mainly in preclinical animal studies. LPL has relatively high TG hydrolase activity compared to phospholipase activity, while EL has the highest phospholipase activity with low TG hydrolase activity [31]. By remodeling lipoproteins, LPL and EL promote the clearance of circulating TG-rich lipoprotein (TRL) remnants and low-density lipoproteins (LDL) [32,33]. The activity of both LPL and EL is regulated by angiopoietin-like protein 3 (ANGPTL3). Inhibiting ANGPTL3 to increase LPL and EL activities has been shown to attenuate hyperlipidemia and atherosclerosis in mice [34-36], via an LDLR-independent manner [37]. As such, the ANGPTL3 inhibitor evinacumab was approved by US Food and Drug Administration (FDA) and US Food and Drug Administration (FDA) for the treatment of adult patients with homozygous familial hypercholesterolemia (HoFH) to reduce ASCVD risk [38,39]. The safety and efficacy of evinacumab in pediatric patients are still under investigation [40]. In addition, as apolipoprotein C-III (APOC3) and angiopoietin-like protein 4 (ANGPTL4) are the other two endogenous inhibitors of LPL, they have also been investigated as therapeutic targets for improving lipid profiles. Liver-targeted inhibition of both APOC3 [41] and ANGPTL4 [42] demonstrated robust lipid-lowering and atheroprotective effects in preclinical studies. Several inhibitors of APOC3 (olezarsen [43,44]; plozasiran [45]) and ANGPTL4 (lipisense [46], MAR001 [47]) are currently in advanced clinical trials, showing profound lipid-lowering effects, to further evaluate their efficacy and safety in reducing ASCVD risk in humans.

In contrast to the relatively consistent reports on the anti-atherosclerotic effect of LPL and EL, reports on HL have been conflicting. HL is primarily synthesized and secreted by hepatocytes and then anchored to the surface of hepatocytes and hepatic sinusoidal endothelial cells by binding to endothelial heparan sulfate proteoglycans (HSPGs) [48]. In contrast to LPL and EL, HL exerts balanced TG hydrolase activity and phospholipase activity. Although HL is suggested to contribute to an atherogenic lipid profile in humans [49] and HL deficiency attenuates atherosclerosis development in mice [50], low HL activity is found to be associated with increased cardiovascular risk in humans [51]. Despite these controversial reports, the recent identification in a French family of a novel rare variant in the gene encoding HL (i.e*., LIPC*) [52] has renewed interest in the potential of HL as a therapeutic target.

This *LIPC* variant encodes a gain-of-function form of HL, in which glutamate at position 97 is replaced by glycine (HL-E97G), and individuals who carry this variant have very low plasma lipid levels [52]. A study into the structure of HL-E97G found that this mutation disrupts a conserved salt bridge within the lid region of HL, leading to a conformational modification that might consequently alter substrate access to the catalytic site [52]. Compared to wild-

type HL, HL-E97G has altered substrate specificity, with increased phospholipase activity without altered TG hydrolase activity. Expression of HL-E97G in mice showed lipid profiles similar to those of human HL-E97G carriers, including decreased plasma PL, TG and cholesterol levels [52]. Of note, in both humans and mice, the cholesterol-lowering effect is reflected by a decrease in non-HDL-C as well as HDL-C levels [52]. Since a simultaneous decrease in all lipoproteins, including HDL led to uncertainties about the overall consequence of HL-E97G on ASCVD, we investigated the effect of HL-E97G on the development of atherosclerosis in **Chapter 4**. Our results showed that expressing HL-E97G in hepatocytes of APOE*3-Leiden.CETP mice remarkably decreases plasma PL, TG and total cholesterol (TC) levels. The decrease in TC by HL-E97G is mainly due to a decrease in non-HDL-C, but a clear decrease in HDL-C is also observed. Nonetheless, HL-E97G largely prevents the development of atherosclerosis, indicating that the decrease in HDL-C caused by HL-E97G at least does not counteract the atheroprotective effect mediated by lowering (V)LDL-C levels.

Mechanically, the (V)LDL-lowering effect of HL-E97G is not caused by decreased VLDL production [52]. Instead, our study showed HL-E97G enhances the clearance of circulating (V)LDL (remnants) by the liver and various extrahepatic tissues. We assume that the enhanced phospholipid lipase activity of HL-E97G can remodel (V)LDL to increase their affinity to receptors and/or internalizing binding sites on liver and other tissues. Notably, the enhanced clearance of (V)LDL (remnants) is likely independent of LDLR, given we also observed pronounced lipid-lowering and atheroprotective effects of HL-E97G in *Ldlr*$^{-/-}$mice. In fact, in addition to its lipolytic actions, HL may also travel with lipoproteins to act as a ligand to facilitate their uptake by interacting with HSPGs [53]. As mentioned above, HSPGs serve as HL receptors and anchor HL on the surface of cells, which is the basis for the role of HL in hydrolysis and clearance of lipoproteins [54]. Meanwhile, HL binding to HSPGs also stimulates the production of more HSPGs [55], and HSPGs have been reported to mediate the clearance of TRLs/LDL independently of LDLR [56,57]. Therefore, investigating whether HL-E97G can promote the clearance of TRLs/LDL through a pathway by interacting with HSPGs may provide new insights into its roles in lipoprotein metabolism and offer novel strategies for treating hyperlipidemia.

In addition to further elucidating the underlying mechanism by which HL-E97G exerts anti-atherogenic effects, further research needs to explore viable strategies to accelerate clinical translation of the functional advantage of HL-E97G in therapeutic applications for ASCVD. I foresee several approaches that may be considered:

(1) *Developing replacement protein therapy* by producing recombinant HL-E97G as a direct therapeutic agent targeting hyperlipidemia, similar to a recombinant lysosomal acid lipase (i.e., sebelipase alfa) that is clinically used to treat metabolic disorders in lysosomal acid lipase deficiency [58]. Of note, such therapy might be particularly suited for individuals with

HL deficiency or dysfunctional HL. In individuals with intact/functional HL, this therapy can raise safety concerns, including triggering immune responses or imbalanced (or excessive) enzymatic activity due to the coexistence of both forms of wild-type HL and HL-E97G.

(2) *Gene-editing therapy* aimed at modifying *LIPC*, the gene encoding HL, e.g., by CRISPR/Cas technology, to induce the amino acid conversion in HL to yield HL-E97G. In fact, the first CRISPR-based gene-editing therapy to improve the lipoprotein profile has entered clinical trials (i.e., VERVE-101), which aims at correcting the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene in hepatocytes to treat heterozygous FH [59]. Notably, even though gene-editing therapy offers the potential for a single intervention with a long-lasting effect, it faces substantial challenges, particularly with respect to safety, off-target effects, and ethical concerns. Hence, this approach is mainly considered appropriate for patients with severe hyperlipidemia or FH, not effectively responding to other conventional lipid-lowering treatments.

(3) *Developing allosteric activators* aimed to specifically modify the conformation of wild-type HL to mimic the functional effect of HL-E97G. A precedent example is ticagrelor, a negative allosteric modulator of the $P2Y_{12}$ receptor (a G-protein-coupled purinergic receptor on platelets) to inhibit platelet aggregation, which reached the clinic for treating acute coronary syndrome [60]. A major challenge in developing this approach is the need for substantial investment in structure-function studies. Nevertheless, if an allosteric inhibitor can be developed with a favorable safety profile, high efficacy and good oral bioavailability, it may be a promising application for many individuals with hyperlipidemia that are e.g., intolerant or non-responsive to current lipid-lowering strategies.

Taken together, several options are available to enhance the phospholipase activity of HL, which can potentially be developed as a lipid-lowering therapy for humans. Of these, the development of allosteric activators of HL to reduce ASCVD risk may be most realistic.

3. Hepatic ABCA6 as a novel target to combat ASCVD

ABC transporters are involved in the ATP-powered transport of various substrates across cellular membranes, playing crucial roles in various diseases, such as cancer, neurodegenerative diseases, and cardiometabolic diseases. Due to their function in transporting cholesterol, the role of several ABC transporters in development and treatment of MASLD and ASCVD has been well-studied, including ABCA1, ABCG1 and the heterodimer ABCG5/ABCG8 [61]. These ABC transporters act as a floppase to facilitate cholesterol efflux by transporting cholesterol within plasma membranes from the cytosolic side (inner leaflet) to the exoplasmic side (outer leaflet) [61]. Enhancing cholesterol efflux from cells, coupled with reverse cholesterol transport towards feces not only reduces cholesterol levels in blood, but

also suppresses the cholesterol overload-induced inflammation, both of which are key in preventing the development of MASLD and ASCVD. In addition to these extensively studied ABC transporters, emerging evidence indicates that additional ABC transporters may be involved in the transport of cholesterol. For example, ABCA6 has been suggested as a novel target for regulating cholesterol metabolism and related diseases.

In 2001, ABCA6 was cloned and given its name due to the structural similarity to ABCA1, and it was initially assumed to be mainly responsible for the cholesterol efflux from macrophages, similar to ABCA1 [62]. However, this concept was challenged by the more recent discovery that ABCA6 is highly expressed in liver, and specifically in hepatocytes [63,64]. ABCA6 has not been associated with circulating lipids in initial large genome-wide association studies (GWAS). However, a GWAS in a Dutch population reported a rare missense *ABCA6* variant to be strongly associated with high circulating TC as well as LDL-C [65], which is the first direct evidence demonstrating a link between ABCA6 and cholesterol metabolism. Inducing this *ABCA6* variant in wild-type mice and hamsters, leading to a pronounced loss in functional ABCA6 protein in the liver, did not markedly affect plasma cholesterol levels [63]. However, this could be explained by relatively low circulating (V)LDL-C, at least in wild-type mice.

For that reason, in **Chapter 5**, we aimed to investigate the physiological function of ABCA6 in cholesterol metabolism and its potential in the prevention/treatment of ASCVD by using APOE*3-Leiden.CETP mice as a well-established model of human lipoprotein metabolism. As ABCA6 is mainly expressed by hepatocytes within the liver, we specifically disrupted *Abca6* in hepatocytes by AAV-CRISPR technology and evaluated its effects on Western-type induced hypercholesterolemia and atherosclerosis development. Using this experimental strategy, we demonstrated that approx. 65% reduction in hepatic ABCA6 protein levels increases plasma TC, reflected by the increase of (V)LDL-C levels. These findings are not only consistent with the GWAS in the Dutch population, showing that a loss-of-function mutant of *ABCA6* increases LDL-C, but in addition, reveal that reduction of functional hepatic ABCA6 protein increases circulating VLDL (remnants), which we could confirm for the rare *ABCA6* mutant in UK Biobank. Mechanistically, we found no direct role of ABCA6 in the secretion of cholesterol from hepatocytes, which would have been similar to ABCA1. Instead, hepatic *Abca6* disruption appeared to increase circulating cholesterol as dependent on the presence of the LDLR. In fact, our results suggested that partial disruption of *Abca6* in hepatocytes increases cholesterol levels within the plasma membrane, accompanied by a reduced LDLR protein level within the plasma membrane, thereby reducing the hepatic LDLR-mediated uptake of (V)LDL (remnants) by liver.

Taken together, these data underscore the value of the APOE*3-Leiden.CETP mice as a valuable experimental model to study the effect of modulation of gene function in the liver on circulating lipids over other experimental models, including wild-type mice [66]. However, we cannot exclude that He et al. have missed a potential cholesterol-increasing effect of

ABCA6 by the strategy of global induction of the ABCA6 mutant, as ABCA6 expression in other cells of organs may potentially have counteracted the increase in circulating cholesterol [63]. In this respect, it is interesting that while ABCA1 within hepatocytes and enterocytes mainly contributes to the generation of HDL precursors [67], ABCA1 within macrophages is involved in promoting cholesterol efflux and exerting an anti-inflammatory effect [68,69]. Interestingly, while macrophage-specific inactivation of ABCA1 in mice markedly increases atherosclerosis, global ABCA1 depletion does not affect atherosclerosis development [70]. Another example of a protein that has different effects depending on its location is LXR as discussed in section 1. While LXR activation in hepatocytes induces lipogenesis, activation of LXR in macrophages prevents inflammation [9,71]. Although ABCA6 is primarily expressed in hepatocytes in liver, it has also been detected in e.g., brain, spleen, lung, and peripheral leukocytes [62]. Thus, global loss of functional ABCA6 protein as in the study of He et al. [63] may have triggered effects in extrahepatic organs/cells, which possibly counterbalances effects on circulating cholesterol resulting from the knockdown in hepatocytes. Nonetheless, the GWAS in the Dutch population identified the loss-of-function *ABCA6* mutant, which is globally expressed, to be strongly associated with increased TC and LDL-C levels [65]. These collective findings suggest that the abundance and function of ABCA6 might vary across tissues/cells and between species, which needs to be uncovered in the future.

Our study not only indicates that functional hepatic ABCA6 attenuates circulating (V)LDL-C, but also protects against the development of atherosclerosis. Of note, the anti-atherosclerotic effect of ABCA6 likely involves a mechanism distinct from that of ABCA1 and ABCG1. While ABCA1 and ABCG1 primarily promote cholesterol efflux and inhibit inflammation in macrophages, ABCA6 likely mainly regulates cholesterol homeostasis of the plasma membrane in hepatocytes, which is essential for maintaining the abundance/function of transmembrane LDLR. Future research should further unravel the exact sequence of events leading to a reduction in LDLR within the plasma membrane of hepatocytes, and investigate the function of ABCA6 in other organs and cells, including macrophages. Also, based on our findings, we predict that increasing the levels and/or function of ABCA6 in hepatocytes may prevent the development of atherosclerosis, provided that such a strategy increases the LDLR abundance in the plasma membrane, potentially via a different mechanism compared to PCSK9 inhibition. If validated in future research, increasing ABCA6 protein abundance/activity would be a promising strategy for the prevention or treatment of ASCVD. In theory, high-throughput approaches such as genome-wide transcription factor profiling, enhancer mapping and CRISPR-based functional screens, can be used to identify transcriptional regulators or enhancers of ABCA6. In fact, ABCA6 expression has recently been found to be regulated by forkhead box O (FoxO), a transcription factor family that also regulates hormone balance and lipid/glucose metabolism [72]. As the glucose-lowering metformin [73,74] and antioxidant supplement

resveratrol [75,76] have been shown to activate FoxO, it would be of interest to investigate whether these agents may exert cholesterol-lowering and/or atheroprotective effects, at least partially through modulation of the FoxO-ABCA6 pathway.

Finally, it should be noted that ABCA6 may have functions other than regulating plasma cholesterol levels. Interestingly, Pasello et al. found that ABCA6 can also act as a crucial intracellular cholesterol transporter to inhibit IGF1R/AKT/MDM2 signaling, thereby suppressing the development of Ewing sarcoma in humans [77]. Potential therapeutic applications of modulating ABCA6 function may thus be much broader than ASCVD.

Conclusions and Future Perspectives

In the past decades, the prevalence of obesity has dramatically increased. Consequently, the incidence of obesity-induced cardiometabolic diseases has markedly increased, which severely impacts the quality of life and threatens long-term health outcomes worldwide, despite current lifestyle and pharmacological interventions. Lifestyle intervention, including dietary and physical exercise intervention, is still recommended as the first-line prevention/treatment for ASCVD and MASLD by numerous clinical guidelines, and current research focuses on evaluating the optimal timing or time windows of lifestyle interventions, e.g., eating and exercise, aimed to maximize the benefits of lifestyle interventions. Many studies in both mice and humans have shown that time-restricted eating [78-80] and late exercise [81-83] improve metabolic profiles, thereby reducing the risks of MASLD and ASCVD. However, despite these efforts, there is still a need to discover additional pharmacological options. Therefore, in this thesis, I explored several novel therapeutic targets, primarily within the liver, to combat cardiometabolic diseases, mainly focused on MASLD and ASCVD.

First, we identified DHCR24 inhibition as a therapeutic strategy to increase desmosterol in macrophages where it acts as a selective LXR ligand to suppress inflammation. As such, we discovered that the DHCR24 inhibitor SH42 largely suppresses inflammation to prevent development of MASLD in a human-relevant mouse model, and in an LXR-dependent manner. Importantly, SH42 does not induce hepatosteatosis or hyperlipidemia, common side effects of synthetic LXR agonists. To date, rezdiffra, approved (conditionally) by the FDA in 2024 [78], remains the first and only specific pharmacological therapy for MASLD. Therefore, we identified DHCR24 inhibition as a promising additional therapeutic option for MASLD. In sharp contrast, we revealed that DHCR24 inhibition by SH42 treatment does not prevent atherosclerosis development in mice, possibly related to insufficient availability of systemically administered SH42 to macrophages within atherosclerotic plaques to exert anti-inflammatory/anti-atherosclerotic effects. This may be addressed by developing lesion macrophage-targeted DHCR24 inhibition in future studies.

Second, we demonstrated that hepatic expression of HL-E97G, an HL variant with enhanced phospholipase activity due to induction of a conformational change [52], markedly reduces plasma lipids, thereby preventing atherosclerosis development in a human-relevant mouse model. Importantly, these beneficial effects of HL-E97G are LDLR-independent, suggesting its potential as a target to lower ASCVD risk in patients with FH, in addition to e.g. ANGPTL3 inhibiting stragegies. Future research is needed to explore approaches to modify HL conformation to mimic the function of HL-E97G, probably by designing allosteric activators.

Third, we observed that hepatic *Abca6* disruption accelerates the progression of atherosclerosis by increasing circulating VLDL (remnant) levels in a human-relevant mouse model, likely by increasing cholesterol content within liver plasma membrane to decrease the abundance of LDLR in plasma membrane. As such, our study is the first to demonstrate the crucial role of ABCA6 in cholesterol metabolism and atherosclerosis development. Given that we also found a loss-of function variant of ABCA6 to be associated with elevated (V)LDL in the UK Biobank, Future efforts should be on assessing whether increasing the abundance/activity of hepatic ABCA6 exerts atheroprotective effects. If so, this may led to a novel therapeutic strategy for ASCVD.

Apart from these potential targets investigated in my thesis, other strategies for combating cardiometabolic diseases are currently being actively explored. For example, several drugs originally approved for treating other conditions have recently been reported to have therapeutic effects in MASLD and ASCVD, among which glucagon-like peptide-1 receptor (GLP-1R) agonist semaglutide and the GLP-1R/glucose-dependent insulinotropic polypeptide receptor (GIPR) dual agonist tirzepatide, initially approved for treatment of T2D and obesity. In 2024, semaglutide was additionally approved by FDA for the treatment of serious CVD in adults with obesity/overweight [84] and recently its effects on reducing adverse cardiovascular events in individuals with T2D and ASCVD have also been shown [85]. Meanwhile, the efficacy and long-term safety of semaglutide for the treatment of MASH is currently being investigated in a phase 3 clinical trial [86]. Similarly, tirzepatide has been reported to lower the risk of ASCVD in patients with T2D [87] and to reduce liver fibrosis in patients with MASH [88] in recent clinical trials. Repurposing these approved drugs for treating MASLD and ASCVD substantially reduces the required time and cost of development of new drugs due to established safety and pharmacokinetic profiles and accelerates translational research. Taken together, many novel pharmacological strategies to treat cardiometabolic diseases are in the pipeline, which will likely greatly benefit those individuals affected by these diseases.

In the future, additional research is needed to further investigate the mechanism(s) of action of the therapeutic targets investigated in my thesis, to develop pharmacological strategies to exploit these targets, and evaluate their safety and efficacy in clinical trials. Besides, it would be meaningful to study the effects of multiple-target therapies on MASLD and ASCVD,

such as by combining lifestyle intervention and pharmacological therapies, combining hepatocyte-targeted with macrophage-targeted strategies, and/or combining lipid-lowering and anti-inflammatory strategies, aimed at enhancing the efficacy of individual therapies.

References

1. World Health Organization. Obesity and overweight. https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight.
2. Lingvay I., Cohen R. V., le Roux C. W.*, et al.* Obesity in adults. *The Lancet* **404**, 972–987 (2024).
3. World Obesity Atlas 2024: No area of the world is unaffected by the consequences of obesity. https://www.worldobesity.org/news/world-obesity-atlas-2024.
4. Younossi Z. M., Golabi P., Paik J. M.*, et al.* The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH): a systematic review. *Hepatology* **77**, 1335–1347 (2023).
5. Barquera S., Pedroza-Tobías A., Medina C.*, et al.* Global Overview of the Epidemiology of Atherosclerotic Cardiovascular Disease. *Arch. Med. Res.* **46**, 328–338 (2015).
6. Naik S. U., Wang X., Da Silva J. S.*, et al.* Pharmacological activation of liver X receptors promotes reverse cholesterol transport in vivo. *Circulation* **113**, 90–97 (2006).
7. Ioannou G. N. The Role of Cholesterol in the Pathogenesis of NASH. *Trends Endocrinol. Metab.* **27**, 84–95 (2016).
8. Cuchel M., Rader D. J. Macrophage reverse cholesterol transport: key to the regression of atherosclerosis? *Circulation* **113**, 2548–2555 (2006).
9. Yuan W., Yu B., Yu M.*, et al.* Synthetic high-density lipoproteins delivering liver X receptor agonist prevent atherogenesis by enhancing reverse cholesterol transport. *J. Control. Release* **329**, 361–371 (2021).
10. Archer A., Stolarczyk E., Doria M. L.*, et al.* LXR activation by GW3965 alters fat tissue distribution and adipose tissue inflammation in ob/ob female mice. *J. Lipid Res.* **54**, 1300–1311 (2013).
11. Grefhorst A., Elzinga B. M., Voshol P. J.*, et al.* Stimulation of lipogenesis by pharmacological activation of the liver X receptor leads to production of large, triglyceride-rich very low density lipoprotein particles. *J. Biol. Chem.* **277**, 34182–34190 (2002).
12. Hu G., Guo M., Xu J.*, et al.* Nanoparticles Targeting Macrophages as Potential Clinical Therapeutic Agents Against Cancer and Inflammation. *Front. Immunol.* **10**, 1998 (2019).
13. Yu M., Amengual J., Menon A.*, et al.* Targeted Nanotherapeutics Encapsulating Liver X Receptor Agonist GW3965 Enhance Antiatherogenic Effects without Adverse Effects on Hepatic Lipid Metabolism in Ldlr(-/-) Mice. *Adv Healthc Mater* **6**, (2017).
14. Guo Y., Yuan W., Yu B.*, et al.* Synthetic High-Density Lipoprotein-Mediated Targeted Delivery of Liver X Receptors Agonist Promotes Atherosclerosis Regression. *EBioMedicine* **28**, 225–233 (2018).
15. Gavas S., Quazi S., Karpiński T. M. Nanoparticles for Cancer Therapy: Current Progress and Challenges. *Nanoscale Res Lett* **16**, 173 (2021).
16. Anik M. I., Hossain M. K., Hossain I.*, et al.* Recent progress of magnetic nanoparticles in biomedical applications: A review. *Nano Select* **2**, 1146–1186 (2021).
17. Spann N. J., Garmire L. X., McDonald J. G.*, et al.* Regulated accumulation of desmosterol integrates macrophage lipid metabolism and inflammatory responses. *Cell* **151**, 138–152 (2012).
18. Muse E. D., Yu S., Edillor C. R.*, et al.* Cell-specific discrimination of desmosterol and desmosterol mimetics confers selective regulation of LXR and SREBP in macrophages. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E4680–e4689 (2018).
19. Müller C., Hemmers S., Bartl N.*, et al.* New chemotype of selective and potent inhibitors of human delta 24-dehydrocholesterol reductase. *Eur. J. Med. Chem.* **140**, 305–320 (2017).

20. Körner A., Zhou E., Müller C.*, et al.* Inhibition of Δ24-dehydrocholesterol reductase activates pro-resolving lipid mediator biosynthesis and inflammation resolution. *Proc. Natl. Acad. Sci. U. S. A.* **116**, 20623–20634 (2019).
21. Griffett K., Welch R. D., Flaveny C. A.*, et al.* The LXR inverse agonist SR9238 suppresses fibrosis in a model of non-alcoholic steatohepatitis. *Molecular metabolism* **4**, 353–357 (2015).
22. Hou L., Feng X., Zhu Z.*, et al.* IGFBPL1 inhibits macrophage lipid accumulation by enhancing the activation of IGR1R/LXRα/ABCG1 pathway. *Aging (Albany N. Y.)* **15**, 14791–14802 (2023).
23. Hwang J., Wang Y., Fikrig E. Inhibition of Chikungunya Virus Replication in Primary Human Fibroblasts by Liver X Receptor Agonist. *Antimicrob. Agents Chemother.* **63**, (2019).
24. Welch R. D., Billon C., Losby M.*, et al.* Emerging Role of Nuclear Receptors for the Treatment of NAFLD and NASH. *Metabolites* **12**, (2022).
25. R&D pipeline: An siRNA targeting LXRα for subcutaneous treatment. https://www.novonordisk.com/science-and-technology/r-d-pipeline.html.
26. Zhang X., McDonald J. G., Aryal B.*, et al.* Desmosterol suppresses macrophage inflammasome activation and protects against vascular inflammation and atherosclerosis. *Proc. Natl. Acad. Sci. U. S. A.* **118**, e2107682118 (2021).
27. Song B., Bie Y., Feng H.*, et al.* Inflammatory Factors Driving Atherosclerotic Plaque Progression New Insights. *J Transl Int Med* **10**, 36–47 (2022).
28. Ajoolabady A., Pratico D., Lin L.*, et al.* Inflammation in atherosclerosis: pathophysiology and mechanisms. *Cell Death Dis.* **15**, 817 (2024).
29. Moore K. J., Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell* **145**, 341–355 (2011).
30. Kurtovic I., Marshall S. N., Zhao X.*, et al.* Lipases from mammals and fishes. *Reviews in Fisheries Science* **17**, 18–40 (2009).
31. McCoy M. G., Sun G. S., Marchadier D.*, et al.* Characterization of the lipolytic activity of endothelial lipase. *J. Lipid Res.* **43**, 921–929 (2002).
32. Zheng C., Murdoch S. J., Brunzell J. D.*, et al.* Lipoprotein lipase bound to apolipoprotein B lipoproteins accelerates clearance of postprandial lipoproteins in humans. *Arterioscler. Thromb. Vasc. Biol.* **26**, 891–896 (2006).
33. Khetarpal S. A., Vitali C., Levin M. G.*, et al.* Endothelial lipase mediates efficient lipolysis of triglyceride-rich lipoproteins. *PLoS Genet.* **17**, e1009802 (2021).
34. Dewey F. E., Gusarova V., Dunbar R. L.*, et al.* Genetic and Pharmacologic Inactivation of ANGPTL3 and Cardiovascular Disease. *N. Engl. J. Med.* **377**, 211–221 (2017).
35. Graham M. J., Lee R. G., Brandt T. A.*, et al.* Cardiovascular and Metabolic Effects of ANGPTL3 Antisense Oligonucleotides. *N. Engl. J. Med.* **377**, 222–232 (2017).
36. Gusarova V., Alexa C. A., Wang Y.*, et al.* ANGPTL3 blockade with a human monoclonal antibody reduces plasma lipids in dyslipidemic mice and monkeys. *J. Lipid Res.* **56**, 1308–1317 (2015).
37. Wu L., Soundarapandian M. M., Castoreno A. B.*, et al.* LDL-Cholesterol Reduction by ANGPTL3 Inhibition in Mice Is Dependent on Endothelial Lipase. *Circ. Res.* **127**, 1112–1114 (2020).
38. Raal F. J., Rosenson R. S., Reeskamp L. F.*, et al.* The Long-Term Efficacy and Safety of Evinacumab in Patients With Homozygous Familial Hypercholesterolemia. *JACC Adv* **2**, 100648 (2023).
39. Rosenson R. S., Rader D. J., Ali S.*, et al.* Evinacumab Reduces Triglyceride-Rich Lipoproteins in Patients with Hyperlipidemia: A Post-Hoc Analysis of Three Randomized Clinical Trials. *Cardiovasc. Drugs Ther.*, (2024).
40. Wiegman A., Greber-Platzer S., Ali S.*, et al.* Evinacumab for Pediatric Patients With Homozygous Familial Hypercholesterolemia. *Circulation* **149**, 343–353 (2024).

41. Ramms B., Patel S., Sun X.*, et al.* Interventional hepatic apoC-III knockdown improves atherosclerotic plaque stability and remodeling by triglyceride lowering. *JCI Insight* **7**, (2022).
42. Modder M., het Panhuis W. I., Li M.*, et al.* Liver-targeted Angptl4 silencing by antisense oligonucleotide treatment attenuates hyperlipidaemia and atherosclerosis development in APOE*3-Leiden.CETP mice. *Cardiovasc. Res.*, (2024).
43. Bergmark B. A., Marston N. A., Prohaska T. A.*, et al.* Olezarsen in patients with hypertriglyceridemia at high cardiovascular risk: Rationale and design of the Essence-TIMI 73b trial. *Am. Heart J.* **286**, 116–124 (2025).
44. Bergmark B. A., Marston N. A., Prohaska T. A.*, et al.* Olezarsen for hypertriglyceridemia in patients at high cardiovascular risk. *N. Engl. J. Med.* **390**, 1770–1780 (2024).
45. Phase 3 Study of Plozasiran in Adults With Hypertriglyceridemia (MUIR-3). https://ctv.veeva.com/study/phase-3-study-of-plozasiran-in-adults-with-hypertriglyceridemia.
46. Lipisense® progressing to Phase II - what comes next? https://www.lipigon.se/en/investors/press-releases/?slug=lipisense-r-progressing-to-phase-ii-what-comes-next-93718.
47. Cummings B. B., Joing M. P., Bouchard P. R.*, et al.* Safety and efficacy of a novel ANGPTL4 inhibitory antibody for lipid lowering: results from phase 1 and phase 1b/2a clinical studies. *Lancet* **405**, 1923–1934 (2025).
48. Perret B., Mabile L., Martinez L.*, et al.* Hepatic lipase: structure/function relationship, synthesis, and regulation. *J. Lipid Res.* **43**, 1163–1169 (2002).
49. Carr M. C., Ayyobi A. F., Murdoch S. J.*, et al.* Contribution of hepatic lipase, lipoprotein lipase, and cholesteryl ester transfer protein to LDL and HDL heterogeneity in healthy women. *Arterioscler. Thromb. Vasc. Biol.* **22**, 667–673 (2002).
50. Mezdour H., Jones R., Dengremont C.*, et al.* Hepatic lipase deficiency increases plasma cholesterol but reduces susceptibility to atherosclerosis in apolipoprotein E-deficient mice. *J. Biol. Chem.* **272**, 13570–13575 (1997).
51. Silbernagel G., Scharnagl H., Kleber M. E.*, et al.* LDL triglycerides, hepatic lipase activity, and coronary artery disease: An epidemiologic and Mendelian randomization study. *Atherosclerosis* **282**, 37–44 (2019).
52. Dijk W., Di Filippo M., Kooijman S.*, et al.* Identification of a Gain-of-Function LIPC Variant as a Novel Cause of Familial Combined Hypocholesterolemia. *Circulation* **146**, 724–739 (2022).
53. Dichek H. L., Johnson S. M., Akeefe H.*, et al.* Hepatic lipase overexpression lowers remnant and LDL levels by a noncatalytic mechanism in LDL receptor-deficient mice. *J. Lipid Res.* **42**, 201–210 (2001).
54. Ji Z. S., Dichek H. L., Miranda R. D.*, et al.* Heparan sulfate proteoglycans participate in hepatic lipaseand apolipoprotein E-mediated binding and uptake of plasma lipoproteins, including high density lipoproteins. *J. Biol. Chem.* **272**, 31285–31292 (1997).
55. Lee S. J., Kadambi S., David C.*, et al.* Association of hepatic lipase with proteoglycans stimulates the production of proteoglycans in vivo and in vitro. *J. Lipid Res.* **45**, 1266–1271 (2004).
56. MacArthur J. M., Bishop J. R., Stanford K. I.*, et al.* Liver heparan sulfate proteoglycans mediate clearance of triglyceride-rich lipoproteins independently of LDL receptor family members. *J. Clin. Invest.* **117**, 153–164 (2007).
57. Foley E. M., Gordts P., Stanford K. I.*, et al.* Hepatic remnant lipoprotein clearance by heparan sulfate proteoglycans and low-density lipoprotein receptors depend on dietary conditions in mice. *Arterioscler. Thromb. Vasc. Biol.* **33**, 2065–2074 (2013).
58. Shirley M. Sebelipase alfa: first global approval. *Drugs* **75**, 1935–1940 (2015).

59. Horie T., Ono K. VERVE-101: a promising CRISPR-based gene editing therapy that reduces LDL-C and PCSK9 levels in HeFH patients. *Eur Heart J Cardiovasc Pharmacother* **10**, 89–90 (2024).
60. Angiolillo D. J., Rollini F., Storey R. F.*, et al.* International Expert Consensus on Switching Platelet P2Y(12) Receptor-Inhibiting Therapies. *Circulation* **136**, 1955–1975 (2017).
61. Oram J. F., Vaughan A. M. ATP-Binding cassette cholesterol transporters and cardiovascular disease. *Circ. Res.* **99**, 1031–1043 (2006).
62. Kaminski W. E., Wenzel J. J., Piehler A.*, et al.* ABCA6, a novel a subclass ABC transporter. *Biochem. Biophys. Res. Commun.* **285**, 1295–1301 (2001).
63. He B., Kang S., Chen Z.*, et al.* Hypercholesterolemia risk associated Abca6 does not regulate lipoprotein metabolism in mice or hamster. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **1866**, 159006 (2021).
64. Ye D., Hoekstra M., Out R.*, et al.* Hepatic cell-specific ATP-binding cassette (ABC) transporter profiling identifies putative novel candidates for lipid homeostasis in mice. *Atherosclerosis* **196**, 650–658 (2008).
65. van Leeuwen E. M., Karssen L. C., Deelen J.*, et al.* Genome of The Netherlands population-specific imputations identify an ABCA6 variant associated with cholesterol levels. *Nat Commun* **6**, 6065 (2015).
66. McGonigle P., Ruggeri B. Animal models of human disease: challenges in enabling translation. *Biochem. Pharmacol.* **87**, 162–171 (2014).
67. Ji A., Wroblewski J. M., Cai L.*, et al.* Nascent HDL formation in hepatocytes and role of ABCA1, ABCG1, and SR-BI. *J. Lipid Res.* **53**, 446–455 (2012).
68. Rosenson R. S., Brewer H. B., Jr., Davidson W. S.*, et al.* Cholesterol efflux and atheroprotection: advancing the concept of reverse cholesterol transport. *Circulation* **125**, 1905–1919 (2012).
69. Singaraja R. R., Van Eck M., Bissada N.*, et al.* Both hepatic and extrahepatic ABCA1 have discrete and essential functions in the maintenance of plasma high-density lipoprotein cholesterol levels in vivo. *Circulation* **114**, 1301–1309 (2006).
70. Aiello R. J., Brees D., Bourassa P. A.*, et al.* Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. *Arterioscler. Thromb. Vasc. Biol.* **22**, 630–637 (2002).
71. Kirchgessner T. G., Sleph P., Ostrowski J.*, et al.* Beneficial and Adverse Effects of an LXR Agonist on Human Lipid and Lipoprotein Metabolism and Circulating Neutrophils. *Cell Metab.* **24**, 223–233 (2016).
72. Gai J., Ji M., Shi C.*, et al.* FoxO regulates expression of ABCA6, an intracellular ATP-binding-cassette transporter responsive to cholesterol. *Int. J. Biochem. Cell Biol.* **45**, 2651–2659 (2013).
73. Ren H., Shao Y., Wu C.*, et al.* Metformin alleviates oxidative stress and enhances autophagy in diabetic kidney disease via AMPK/SIRT1-FoxO1 pathway. *Mol. Cell. Endocrinol.* **500**, 110628 (2020).
74. Hou X., Song J., Li X.-N.*, et al.* Metformin reduces intracellular reactive oxygen species levels by upregulating expression of the antioxidant thioredoxin via the AMPK-FOXO3 pathway. *Biochem. Biophys. Res. Commun.* **396**, 199–205 (2010).
75. Sin T. K., Yung B. Y., Siu P. M. Modulation of SIRT1-Foxo1 signaling axis by resveratrol: implications in skeletal muscle aging and insulin resistance. *Cell. Physiol. Biochem.* **35**, 541–552 (2015).
76. Asadi S., Rahimi Z., Saidijam M.*, et al.* Effects of resveratrol on FOXO1 and FOXO3a genes expression in adipose tissue, serum insulin, insulin resistance and serum SOD activity in type 2 diabetic rats. *International Journal of Molecular and Cellular Medicine* **7**, 176 (2018).
77. Pasello M., Giudice A. M., Cristalli C.*, et al.* ABCA6 affects the malignancy of Ewing sarcoma cells via cholesterol-guided inhibition of the IGF1R/AKT/MDM2 axis. *Cell. Oncol. (Dordr.)* **45**, 1237–1251 (2022).

78. Xia J., Guo W., Hu M*., et al.* Resynchronized rhythmic oscillations of gut microbiota drive time-restricted feeding induced nonalcoholic steatohepatitis alleviation. *Gut Microbes* **15**, 2221450 (2023).
79. In Het Panhuis W., Schönke M., Modder M*., et al.* Time-restricted feeding attenuates hypercholesterolaemia and atherosclerosis development during circadian disturbance in APOE∗3-Leiden.CETP mice. *EBioMedicine* **93**, 104680 (2023).
80. Wei X., Lin B., Huang Y*., et al.* Effects of Time-Restricted Eating on Nonalcoholic Fatty Liver Disease: The TREATY-FLD Randomized Clinical Trial. *JAMA Netw Open* **6**, e233513 (2023).
81. Kovynev A., Ying Z., Zhang S*., et al.* Timing Matters: Late, but Not Early, Exercise Training Ameliorates MASLD in Part by Modulating the Gut-Liver Axis in Mice. *J. Pineal Res.* **76**, e70003 (2024).
82. Schönke M., Ying Z., Kovynev A*., et al.* Time to run: Late rather than early exercise training in mice remodels the gut microbiome and reduces atherosclerosis development. *FASEB J.* **37**, e22719 (2023).
83. Moholdt T., Parr E. B., Devlin B. L*., et al.* The effect of morning vs evening exercise training on glycaemic control and serum metabolites in overweight/obese men: a randomised trial. *Diabetologia* **64**, 2061–2076 (2021).
84. FDA Approves First Treatment to Reduce Risk of Serious Heart Problems Specifically in Adults with Obesity or Overweight. https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-reduce-risk-serious-heart-problems-specifically-adults-obesity-or.
85. McGuire D. K., Marx N., Mulvagh S. L*., et al.* Oral Semaglutide and Cardiovascular Outcomes in High-Risk Type 2 Diabetes. *N. Engl. J. Med.*, (2025).
86. Sanyal A. J., Newsome P. N., Kliers I*., et al.* Phase 3 Trial of Semaglutide in Metabolic Dysfunction-Associated Steatohepatitis. *N. Engl. J. Med.*, (2025).
87. Hamidi H., Bagheri M., Benzing T*., et al.* Effect of tirzepatide on the progression of coronary atherosclerosis using MDCT: Rationale and design of the tirzepatide treatment on coronary atherosclerosis progression: The (T-Plaque) randomized-controlled trial design. *Am. Heart J.* **278**, 24–32 (2024).
88. Loomba R., Hartman M. L., Lawitz E. J*., et al.* Tirzepatide for Metabolic Dysfunction-Associated Steatohepatitis with Liver Fibrosis. *N. Engl. J. Med.* **391**, 299–310 (2024).