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# Novel pathways in cholesterol metabolism to combat cardiometabolic diseases

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# General discussion and future perspectives

Cardiovascular diseases (CVD) are the number one cause of death worldwide [1] and the World Health Organization estimated that in 2016 about 17.9 million people died from CVD, representing 31% of total global deaths [2]. Atherosclerosis is the major underlying cause of CVD and elevated cholesterol or hypercholesterolemia is a well-documented risk factor for atherosclerosis. Strikingly, it has been estimated that over 50% of adults have raised total cholesterol levels [3]. Despite the effectiveness of cholesterol-lowering therapies, e.g. using statins, 55-75% of cardiovascular events still remain [4-6], highlighting the need to discover new cholesterol-lowering strategies. In 2009 metabolically active brown fat has been found to still be present in adult humans [7] and was shown to utilize glucose to produce heat [8, 9], and brown fat thus became an emerging target for the treatment of diabetes in humans [10]. Interestingly, our research group has previously shown that brown fat activation also reduces plasma cholesterol and prevents atherosclerosis development in *APOE*3-Leiden.CETP (E3L.CETP)* mice, a well-established model for human like cardiometabolic disease [11], suggesting that brown fat may also be a promising target to treat hypercholesterolemia and CVD in humans. In the first two sections of this Chapter, we will therefore discuss the role of brown fat in cholesterol metabolism and provide future perspectives for applying brown fat activation to prevent cardiometabolic disease in humans.

Besides hypercholesterolemia, non-alcoholic fatty liver disease (NAFLD) is another risk factor for CVD. NAFLD has a global prevalence of 25% and approximately 25% of NAFLD patients develop non-alcoholic steatohepatitis (NASH), a progressive liver disease that can potentially lead to cirrhosis, hepatocellular carcinoma, and even death [12, 13]. Although NAFLD/NASH causes a large economic burden and poor health-related quality of life, no Food and Drug Administration approved medications are currently available for the treatment of NASH. Liver X receptors (LXRs) are potential drug targets for NAFLD/NASH treatment due to their important roles in both lipid metabolism and inflammation [14, 15]. However, synthetic LXR agonists usually cause hyperlipidemia, which hampers their application in the clinic. In the third part of this Chapter, we will discuss a novel strategy to activate LXR by increasing desmosterol via inhibition of Δ24-dehydrocholesterol reductase (DHCR24) for the treatment of cardiometabolic diseases including NALFD/NASH.

## Role of brown fat in cholesterol metabolism

Brown fat is physiologically activated by cold exposure and as a consequence dissipates energy to produce heat, aimed at preventing against hypothermia and tissue damage induced by the cold. Although positron emission tomography-computed tomography (PET-CT) using the glucose tracer [$^{18}$F]fluorodeoxyglucose (FDG) currently is the most applied method for brown fat detection and quantification, activated brown fat in fact produces heat by combusting intracellular fatty acids (FAs), derived from intracellular lipid droplets, rather than glucose [16]. FAs released from circulating triglyceride (TG)-rich lipoproteins (TRL) during lipoprotein lipase (LPL)-mediated lipolysis within brown fat are the main source to refill intracellular TG stores upon brown fat activation [17, 18] (**Figure 1**). At the same time, such LPL-mediated lipolysis of TRL generates cholesterol-enriched TRL remnants in the circulation that are subsequently taken up by the liver [16]. As a consequence of increased energy expenditure resulting from activation of brown fat, mammalians usually compensate by raising their food intake. This may pose a problem when the food contains cholesterol, as increased cholesterol intake may result in accumulation of cholesterol-enriched remnants in the circulation to potentially induce cardiometabolic disease [19]. Interestingly, our group previously showed that brown fat activation via β3-adrenergic receptor (AR) agonism using the specific agonist

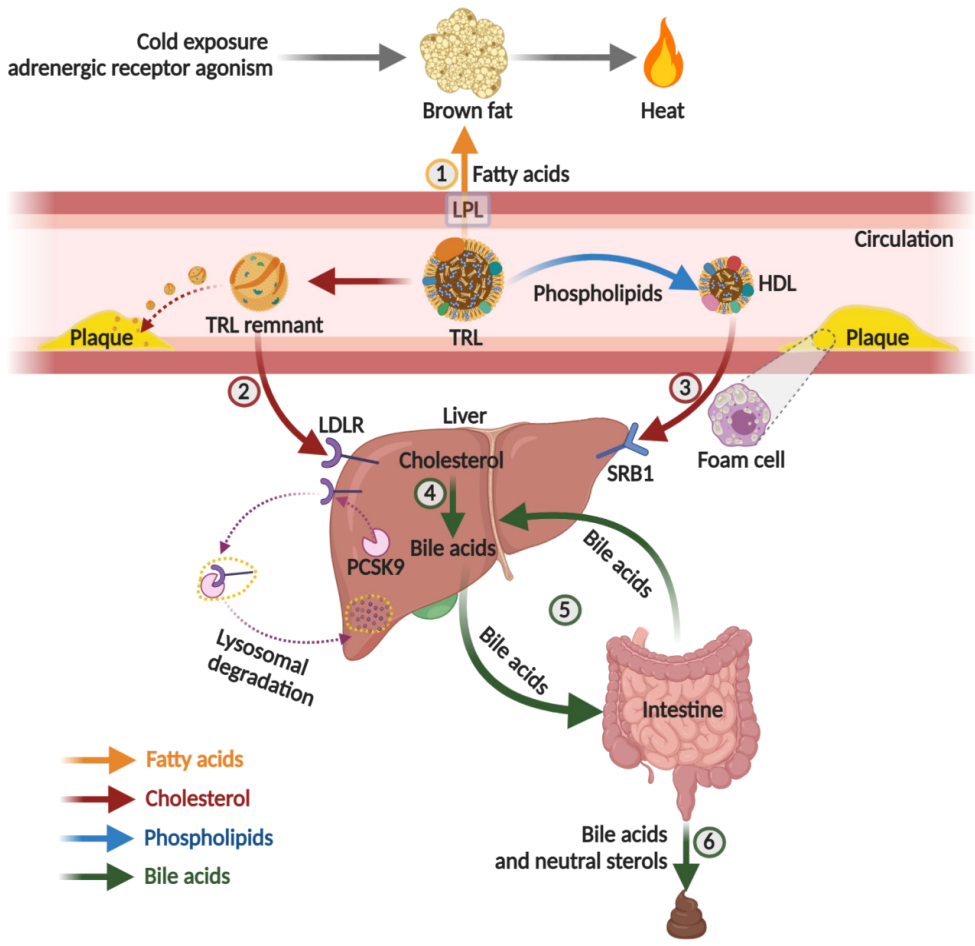


**Figure 1. Proposed metabolic pathways through which brown fat activation improves cholesterol metabolism and reduces atherosclerosis development.** Activated brown fat takes up triglyceride-rich lipoprotein (TRL)-derived fatty acids through lipoprotein lipase (LPL)-mediated lipolysis (**pathway 1**) for heat production leaving TRL core remnants and TRL surface remnants, mainly phospholipids, in the circulation. Core remnants are then efficiently taken up by hepatocytes of the liver after binding of ApoE on their particle surface to the LDL receptor (LDLR) (**pathway 2**), the levels being regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9), resulting in reduction of circulating cholesterol and therefore less cholesterol-driven atherosclerosis progression. The surface remnants are transferred to HDL, which improves HDL cholesterol efflux capacity e.g., from macrophages in the plaque (**pathway 3**), with subsequent uptake of the acquired cholesterol via scavenger receptor class B type 1 (SRB1) on hepatocytes. The hepatic uptake of cholesterol from TRL remnants and HDL as induced by brown fat activation drives cholesterol conversion into bile acids thus increasing bile acid synthesis (**pathway 4**), coupled to enterohepatic circulation of bile acids (**pathway 5**), with partial excretion of bile acids and neutral sterols within the feces (**pathway 6**). See text for more details.

CL316243 improves hypercholesterolemia and reduces atherosclerosis development in *E3L.CETP* mice that were fed a cholesterol-containing Western-type diet [11]. In this thesis, we confirmed that brown fat activation by CL316243 decreases plasma total cholesterol as well as non-HDL cholesterol levels in *E3L.CETP* mice (**Chapters 2-4**). The cholesterol-lowering effects of brown fat activation is mainly caused by accelerated clearance of cholesterol-enriched TRL remnants by the liver and this process is strictly dependent on an intact ApoE/LDLR pathway that is present in *E3L.CETP* mice [11] (**Chapters 2-4**). In contrast, in $ApoE^{-/-}$ or $LDLR^{-/-}$ mice, the generated TRL-remnants upon brown fat activation by CL316243 cannot be cleared, so cholesterol levels are not reduced and no effects are observed on atherosclerosis development [11]. In fact, cold exposure to activate brown fat in $ApoE^{-/-}$ or $LDLR^{-/-}$ mice even aggravates hyperlipidemia as well as atherosclerosis development, as related to increased food intake [19]. Collectively, these date show that activated brown fat combusts FAs mainly derived from circulating TRL, which accelerates the formation of cholesterol-enriched TRL remnants that can subsequently be taken up via the hepatic ApoE/LDLR pathway, consequently reducing plasma total cholesterol levels, as explained by a reduction in non-HDL-C (**Figure 1**).

Given the importance of the ApoE/LDLR pathway in the beneficial effects on cholesterol metabolism and atherosclerosis development of brown fat accumulation, we reasoned that increasing hepatic TRL remnant uptake may add on the effects of brown fat activation on reducing atherosclerosis. Therefore, we initiated studies to evaluate the combination of brown fat activation using CL316243 and HMGCoA reductase inhibition using atorvastatin [20] and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibition using alirocumab (**Chapter 2**), both aimed at increasing hepatic LDL receptor levels, and indeed observed additive effects of combination treatment on plasma cholesterol levels and atherosclerosis. *Vice versa*, if the experiments in *E3L.CETP* mice have translational value for humans, these data may implicate that pharmacological brown fat activation may have additive effects in current lipid-lowering strategies using statins and PCSK9 inhibition in the clinical setting.

In addition to lowering non-HDL-C levels, we and others found that brown fat activation by CL316243 in *E3L.CETP* mice increases plasma HDL-C levels [21], an effect that was also observed in humans after cold exposure [22], and after treatment with the β3-adrenergic receptor agonist mirabegron [23]. In this thesis (**Chapters 2 and 3**), we investigated the mechanism underlying this phenomenon, and demonstrated that LPL-mediated hydrolysis of TRL upon brown fat activation generates redundant TRL surface remnants that constituted of mainly phospholipids, and which intercalate into the HDL pool (**Figure 1**). This mechanism explained why brown fat activation alters the phospholipid composition of HDL [21], and suggests that an increased surface-to-core ratio in HDL increases the capacity of HDL to pack more cholesterol resulting in the observed increase in HDL-C levels [11, 20]. Indeed, we previously showed that brown fat activation in *E3L.CETP* mice increases the cholesterol efflux capacity of HDL *in vitro* and promotes reverse cholesterol transport (RCT) from macrophages to feces *in vivo*, at least upon short term treatment [21]. Likewise, the increase in small HDL particles that results from brown fat activation by short-term cooling in humans were also shown to possess increased cholesterol efflux capacity *in vitro* [22].

Additional proof for a role of brown fat activation in RCT was provided by a report showing that 7 days of cold exposure of mice promotes hepatic conversion of cholesterol to bile acids by inducing cytochrome P450, family 7, subfamily b, polypeptide 1 (CYP7B1) within the alternative pathway of bile acid synthesis, which was accompanied by increased fecal excretion as well as plasma levels of bile acids [24]. Apparently, the increased flux of cholesterol within TRL core remnants to the liver increases the bile acid synthesis pathways to prevent

excessive hepatic cholesterol accumulation and thus maintain cholesterol homeostasis within the liver (**Figure 1**). However, in contrast to acute brown fat activation, prolonged brown fat activation for 9 weeks increased bile flow and plasma bile acid levels, while decreasing fecal bile acid excretion (**Chapter 4**). Bile acids are endogenous signaling molecules and bind to the farnesoid X receptor (FXR) thus inhibiting cytochrome P450, family 7, subfamily a, polypeptide 1 (CYP7A1) to reduce bile acid synthesis [25]. Therefore, the increased plasma bile acids after long-term brown fat activation probably reflect an increased flux of bile acids to the liver, where they induce an inhibitory feedback on bile acid synthesis, resulting in accumulation of cholesterol that is continuously being delivered to the liver as part of TLR core remnants that are generated during brown fat activation. Indeed, combination of brown fat activation with colesevelam treatment interrupted the enterohepatic circulation by largely increasing fecal bile acid excretion, resulting in efficient hepatic bile acid synthesis without accumulation of cholesterol in the liver, with efficient cholesterol uptake from the circulation as an additional beneficial effect. A such, colesevelam added to the cholesterol-lowering and anti-atherogenic effects of brown fat activation (**Chapter 4**). Consistent with our mouse study, the β3-AR agonist mirabegron increases gallbladder size in humans [26], which could be due to the increased bile flow upon brown fat activation. Together, acute brown fat activation promotes the uptake of cholesterol-enriched TRL remnants by the liver which drives bile acid synthesis, while prolonged brown fat activation increases bile acid reabsorption and decreases hepatic BA synthesis, which probably serves as a self-protecting mechanism to prevent too much bile acid loss from the body. On the other hand, bile acids such as chenodeoxycholic acid (CDCA) activate brown fat via the G-coupled protein receptor TGR5 to increase energy expenditure in both mice and humans [27, 28]. Whether the increased bile acids in plasma upon brown fat activation also function as a feed-forward mechanism to further increase brown fat activation is interesting to be further investigated. Also, considering the differences between rodents and humans *e.g.,* with respect to bile acid metabolism and composition and relative abundance of brown fat, it is also required to further investigate the interplay between brown fat activation and bile acid metabolism in humans.

The hepatic scavenger receptor class B type 1 (SRB1) is known to facilitate the selective uptake of cholesteryl esters derived from HDL as an intermediate step in RCT, thus facilitating the atheroprotective properties of HDL [29]. Therefore, we reasoned that the hepatic SRB1 pathway contributes to the atheroprotective effects of brown fat activation by CL316243. Therefore, we evaluated the effect of liver-specific knockdown of SRB1 in *E3L.CETP* mice using an AAV8-siRNA approach (**Chapter 3**). In contrast to our expectations, hepatic SRB1 knockdown, by approx. 50-60%, was demonstrated not to counteract, but even to further reduce atherosclerosis development on top of brown fat activation. In fact, hepatic SRB1 knockdown *per se* appeared to improve dyslipidemia and prevent atherosclerosis development in *E3L.CETP* mice (**Chapter 3**). This was quite counterintuitive as hepatic SRBI was generally assumed to protect against diet-induced atherosclerosis, at least in experimental models without functional ApoE or LDL receptor [30, 31]. However, these data are likely explained by facilitated LPL-mediated TLR processing due to more efficient delivery of TRL surface remnants to the larger HDL pool caused by partial hepatic SR-BI deficiency, again resulting in more efficient hepatic clearance of TRL core remnants by the hepatic ApoE/LDL receptor clearance pathway. Nevertheless, reduced hepatic SRBI expression certainly does not attenuate the effects of brown fat activation on cholesterol lowering and atherosclerosis development. Given that brown fat activation does not prevent atherosclerosis development in $ApoE^{-/-}$ or $LDLR^{-/-}$ mice [11], it is safe to assume that SRB1 does not contribute substantially, if any, to the atheroprotective effects of brown fat activation. Again, if these data can be translated to the human setting, we anticipate that brown fat activation can also be effective to treat CVD in

subjects with impaired SRB1 function.

## Brown fat as a target for the treatment of cardiometabolic disease in humans

Considering the marked beneficial effects of brown fat activation on cholesterol metabolism and atherosclerosis development in *E3L.CETP* mice (**Chapters 2-4**) [11], and the fact that this is a well-established mouse model for human like cardiometabolic disease, brown fat seems a promising tissue to target in the prevention of atherosclerotic CVD in the clinic. Indeed, human brown fat is metabolically active. Initially, brown fat was detected in humans from its ability to take up glucose, as evidenced from [$^{18}$F]FDG PET-CT scans [32]. Furthermore, brown fat activation has been shown to improve glucose homeostasis and insulin sensitivity [9, 33, 34], and short-term cold exposure has even shown to improve insulin sensitivity in individuals with T2D [35]. Besides modulating glucose metabolism, brown fat has also been demonstrated to take up FAs as demonstrated using [$^{18}$F]fluorothiaheptadecanoic acid ([$^{18}$F]FTHA) PET-CT analysis. In fact, while the uptake of [$^{18}$F]FDG by BAT decreases with increased age and body fat, neither the uptake of [$^{18}$F]FTHA nor oxidative metabolism are impaired in individuals with T2D [36]. Since our studies in mice consistently show that the uptake of lipids by brown fat is mainly derived from TLRs as it is highly dependent on LPL (**Chapters 2-4**) [11, 37], it is highly likely that also in a human setting brown fat mainly takes up FAs from TRLs, although an [$^{18}$F]triglyceride tracer still needs to be developed to prove this hypothesis. A role of human brown fat mainly in lipid metabolism is also demonstrated by the notion that cold exposure of human subjects increases the oxidation of FAs rather than glucose, as determined with indirect calorimetry [38].

Human brown fat is also likely to play a role in cholesterol metabolism. As pointed out in the previous section short term personalized cold exposure of human subjects to selectively activate human brown fat was shown to increase HDL [22], which likely results from generation of TRL surface remnants during LPL-mediated lipolysis as we showed in mice in **Chapter 3**. In fact, brown fat activation by means of chronic cold exposure reduces LDL-C levels in hypercholesterolemic patients [39], which would be expected from increased hepatic uptake of VLDL remnants following efficient LPL-mediated lipolysis. Previous studies have shown that high brown fat activity, at least based on [$^{18}$F]FDG uptake, is associated with less accumulation of visceral fat [40] and a reduced risk of CVD events [41]. Probably the most convincing evidence of a beneficial cardiometabolic effect of brown fat in humans was very recently presented in a very recent large study that examined as many as 139,224 [$^{18}$F]FDG-PET-CT scans of 53,475 individuals. This study showed that individuals with detectable brown fat have improved glucose, TG and HDL-C levels, and lower prevalence of cardiometabolic diseases including T2D and coronary artery disease [42]. These beneficial effects were observed in all BMI categories, albeit more pronounced in individuals with overweight and obesity. Taken these data together implies that targeting brown fat is a promising strategy to improve energy metabolism to combat cardiometabolic disease in humans, even with overweight and obesity, probably by similar mechanisms as we and others have elucidated in translational mouse models.

However, many hurdles obviously still have to be taken. First, the abundance of brown fat in humans (approx. 0.1% of body mass) is relatively smaller than that in mice (> 0.5% of body mass), implying that the relative benefit of brown fat activation may be more limited in humans compared to mice. Nevertheless, it has been estimated that human brown fat

potentially burns approx. 20% of the basal caloric need [43], and brown fat has been shown to be plastic as it can be recruited, e.g. by cold acclimation, and even in obese individuals [44]. It should also be realized that human white fat has remarkable plasticity and via a process called 'browning' develops beige/brite adipocytes with thermogenic properties [45-47]. In fact, a switch in white to brown fat increases energy expenditure in cancer-associated cachexia [48] as well as pheochromocytoma and paraganglioma [49, 50]. Taken together, these data confirm the potential of human brown fat to substantially contribute to energy metabolism, and thus TRL turnover, but this should still be confirmed once [$^{18}$F]TG tracers will become available. Ideally, such a tracer should be incorporated into the TRL-like particles that our group has previously developed [51] and that we have used in many studies described in this thesis to trace the effect of brown fat activation on TRL metabolism in the context of cardiometabolic disease. An initial attempt to endogenously synthesize such a tracer in humans *in vivo* by oral administration of [$^{18}$F]FTHA, resulting in incorporation into TG within the core of chylomicrons, was not very effective to trace brown fat [52], which may indicate that chylomicrons are poor substrates for brown fat. Indeed, it should be realized that under physiological conditions cold exposure increases sympathetic outflow to white fat, liver and brown fat, resulting in liberation of FA from white fat to be packed into TG within the liver and secreted as VLDL to deliver FA to brown fat [22, 53]. As such, VLDL sized particles, as used in this thesis, are probably more effective compared with chylomicron sized particles to visualize and quantify human brown fat, which would be interesting to investigate in future human studies.

Since cold acclimation is probably a brown fat activating strategy that is hard to adhere to for many individuals, a next challenge will be to find selective and effective drugs to activate brown fat in humans. The β3-AR is main adrenergic receptor involved in brown fat activation in mice, and an initial study showed that acute treatment with the β3-AR agonist mirabegron also increases energy expenditure and [$^{18}$F]FDG uptake by brown fat in humans [54]. Furthermore, chronic mirabegron treatment was recently shown to increase brown fat, HDL-C and insulin sensitivity in humans [23]. However, these effects have been observed with a dose of 200 mg, which is well above the pharmacological dose of 50 mg that is used to treat hyperactive bladder. Given that β-AR agonists often show some degree of cross-reaction between the various β-ARs, it is likely that mirabegron at 200 mg evokes effects on β1-AR and/or β2-AR. This would be consistent with the observed increase in heart rate that likely results from β1-AR activation [54]. Interestingly, in collaboration with researchers from Vancouver and Copenhagen, our group recently revealed that mirabegron at 50 mg does not activate brown fat, and that the β3-AR is virtually absent from human brown fat. In fact, human brown fat was shown to mainly express the β2-AR, and *in vitro* studies demonstrated that the stimulating effects of mirabegron and noradrenalin on brown adipocyte activity are blocked by selective β2-AR antagonism [55]. These data thus strongly suggest that the β2-AR is responsible for (physiological) brown fat activation in humans, and strongly warrant studies to evaluate the effect of selective β2-AR agonism on human brown fat activity, eventually in the context of cholesterol metabolism and cardiometabolic disease. Since our preclinical studies in mice have shown that brown fat can also be activated by FDA-approved drugs including salsalate [56], metformin [57] and GLP-1 receptor agonism [58], it would be highly interesting to study the contribution of brown fat in the beneficial effects of these drugs on lipid metabolism, body weight and cardiometabolic disease in humans.

Irrespective of the ideal drug candidate that in the end will (selectively) activate human brown fat, which may well be a brown fat-targeted β2-AR agonist, it is reasonable to speculate that such brown fat activation can be a strategy to replace or complement current lipid-lowering

therapeutic strategies to combat atherosclerotic CVD. Currently, hypercholesterolemic patients who cannot reach their cholesterol goal using a low dose of statins, may receive a higher drug dose, which however usually causes adverse effects [59]. Therefore, combination therapy is usually applied in the clinic by simultaneously interfering with multiple major pathways within cholesterol metabolism. Since brown fat activation accelerates the LPL-mediated generation of TRL remnants, its mechanism may well be complementary to that of statins and PCSK9 inhibitors that increase LDL receptor-mediated hepatic TLR remnant uptake. Indeed, the experimental studies in *E3L.CETP* mice including those described in this thesis revealed that brown fat activation adds to the cholesterol-lowering and anti-atherosclerotic effects of both atorvastatin [20], the PCSK9 antibody alirocumab (**Chapter 2**) and even the bile acid sequestrant colesevelam (**Chapter 4**), yielding promise that such combination interventions may ultimately also be effective in humans. Besides reducing side effects of e.g. high-dose statin therapy, such combination therapies could potentially also reduce the financial burden associated with e.g. PCSK9 antibody therapy.

Given that activated brown fat contributes to plasma lipoprotein metabolism by initially generating TRL remnants through LPL-mediated extraction of FAs for combustion it would also be interesting to investigate the interaction of brown fat activation with experimental strategies that target LPL, such as angiopoietin-like 3 (ANGPTL3) [60] and potentially also angiopoietin-like 4 (ANGPTL4) [61]. Both ANGPTL3 and ANGPTL4 are an endogenous inhibitors of LPL activity, and mouse studies demonstrated that cold exposure reduces ANGPTL4 in brown fat to facilitate TRL-derived FA uptake by the tissue for subsequent oxidation [62]. Likewise, strategies to increase LPL activity, *e.g.,* by increasing ApoC2 and ApoA5 or lowering ApoC1 and ApoC3, although not clinically available yet, could be interesting to further experimentally investigate for combination treatment with brown fat activation to improve lipid metabolism and reduce atherosclerosis, preferentially in *E3L.CETP* mice. Collectively, optimal management of CVD disease probably requires a combination of complementary approaches, of with activation of brown fat may well be one in the future.

## Targeting DHCR24 to activate LXR by raising desmosterol for the treatment of cardiometabolic disease

The interest in LXR as a drug target for NAFLD and atherosclerotic CVD stems from the fact that LXR activation promotes secretion of excess cholesterol from the body [63] as well as has anti-inflammatory effects in immune cells [64]. However, most of synthetic drugs targeting LXR thus far failed, which is mainly due to unfavorable effects of pharmacological LXR activation on sterol regulatory element-binding proteins (SREBPs)-induced lipogenesis in hepatocytes, resulting in elevated pro-atherogenic LDL-cholesterol and triglycerides [65, 66]. To avoid the occurrence of such hyperlipidemia, macrophage-selective LXR activation to specifically target inflammation could be an option to combat NAFLD and atherosclerosis since macrophages play an essential role in the development of both atherosclerosis (i.e., foam cells) and NAFLD (i.e., Kupffer cells), as well as in the overall immune response [64]. Interestingly, in 2018 desmosterol was reported to strongly activate LXR target genes in macrophages of both mice and humans, while it does not elicit LXR activation in hepatocytes from both species [67]. The desmosterol-induced LXR activation was shown to promote cholesterol efflux and inhibit activation of inflammatory responses in foam cells [68]. Collectively, these findings provide a molecular basis for desmosterol-induced LXR activation for the treatment of cardiometabolic disease, which would probably occur without causing hyperlipidemia. An antisense approach to reduce the expression of *DHCR24*, the

enzyme responsible for conversion of desmosterol into cholesterol, failed to induce LXR target genes in macrophages both *in vitro* and *in vivo*, probably due to an insufficient increase in desmosterol levels [67]. In addition, triparanol was developed as a DHCR24 inhibitor, but unfortunately had to be withdrawn from clinical application due to severe adverse effects, such as nausea and vomiting, cataracts, and skin disorders [69].

This prompted researchers including our collaborators in Munich to design selective and potent small molecule DHCR24 inhibitors to increase intracellular desmosterol and activate LXR, specifically in macrophages, and without unfavorable lipogenic effects. Our collaborators indeed succeeded in synthesizing SH42 as a selective and potent DHCR24 inhibitor. SH42 is a much more effective DHCR24 inhibitor ($IC_{50}$ < 10 nM) than triparanol ($IC_{50}$ =14 μM) as shown in a cellular assay [70, 71]. In fact, the high efficiency of SH42 to inhibit DHCR24 translated into a marked increase in the levels of desmosterol that was able to efficiently induce expression of LXR target genes in macrophages [70]. Via LXR activation, SH42 not only increased *Abaca* and *Abcg1*, but also promoted the generation of long chain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid and docosahexaenoic acid. Through these collective actions, we were able to show that administration of SH42 caused resolution of inflammation in a murine peritonitis model (**Chapter 5**). Therefore, we next evaluated DHCR24 inhibition also in a metabolic context, by assessing the consequence of SH42 treatment on NAFLD/NASH in *E3L.CETP* mice fed a diet rich in both fat and cholesterol. Under these conditions, we observed that SH42 suppresses diet-induced Kupffer cell activation, which subsequently alleviates immune cell infiltration in the liver as well as hepatic steatosis. Importantly, these benefits of SH42 were achieved without induction of hyperlipidemia, nor other overt adverse effects (**Chapter 6**). Interestingly, a recent genetic study demonstrated that the rs588709 variant near the *DHCR24* locus presents metabolic benefits with respect to lowering circulating TG-rich VLDL particles [72], which potentially extends the role of DHCR24 in human lipid metabolism. Taken together, we reveal that targeting DHCR24 by the synthetic agonist SH42 is a promising way to induce LXR activation specifically in macrophages for the treatment of inflammatory and metabolic liver disease without inducing hyperlipidemia.

Raising desmosterol via DHCR24 inhibition to activate LXR may also have therapeutic benefits beyond NAFLD/NASH, as pharmacological LXR agonists such as T0901317 have been shown to combat *e.g.* atherosclerosis [73, 74], obesity and insulin resistance [75-77], cancer development [78, 79] and bacterial pathogens [80] in mice, despite of induction of hyperlipidemia. Therefore, SH42 raises desmosterol levels without inducing hyperlipidemia, suggesting DHCR24 inhibition has wide therapeutic applications, and warrants investigation of its effects on these diverse diseases. Hurdles that should be taken for clinical development of SH42 for treatment of CVD would at least include evaluation of the effects of SH42 on atherosclerosis development in *E3L.CETP* mice and extensive safety studies and pharmacokinetic/ pharmacodynamic (PK/PD) modeling in humans. If safe and effective in humans, SH42 could present a true revolution in the treatment of cardiometabolic diseases by targeting inflammation in macrophages, which would largely add to current strategies that mainly focus on lowering of lipids.



## Concluding remarks

The commonly used lipid-lowering strategies to prevent CVD in the clinic, *i.e.,* statins, only prevent about 25-45% of total cardiovascular events, which encouraged us to search for additional innovative therapeutic strategies including brown fat activation and LXR agonism.

Brown fat has recently been revealed as a promising potential target for the treatment of atherosclerotic CVD in humans. A first aim of this thesis was to further elucidate the mechanisms underlying the atherosclerosis-protective effects of brown fat activation by **β**3-AR agonism on lipid metabolism and atherosclerosis development in *E3L.CETP* mice, a well-established model for human-like cardiometabolic disease. These studies revealed that the hepatic ApoE/LDL receptor pathway for cholesterol clearance through the uptake of TRL remnants way is more important than the hepatic SR-B1 receptor pathway for the cholesterol clearance through the uptake of HDL-derived cholesteryl esters. In addition, these studies revealed that, in contrast to current dogma, lowering of hepatic SR-B1 attenuates rather than aggravates atherosclerosis development, at least in *E3L.CETP* mice, related to more efficient TRL remnant generation. Secondly, we aimed to evaluate whether brown fat activation would add to current lipid-lowering strategies to attenuate atherosclerosis progression and revealed that brown fat activation indeed adds to the anti-atherogenic effects of PCSK9 inhibition and bile acid sequestration. As such, the studies of this thesis provided further experimental proof for the anti-atherogenic effects of brown fat activation. Evidently, these studies should now be translated into clinical practice, which may be facilitated by the very recent discovery that the **β2**-AR is responsible for physiological brown fat activation in humans.

LXR has been regarded as a promising target to combat cardiometabolic diseases including atherosclerotic CVD and NAFLD/NASH, because of regulating cholesterol efflux and dampening inflammation. However, LXR activation using synthetic LXR agonists induce hyperlipidemia by increasing lipogenesis in the liver, which hampered their clinical development. Therefore, we explored the possibility to develop a potent and selective inhibitor of DHCR24 which increases the generation of desmosterol that serves as the endogenous activator of LXR only in macrophages to attenuate inflammation, and not in hepatocytes thus preventing hyperlipidemia. Indeed, our studies showed that the selective and potent DHCR24 inhibitor SH42 causes resolution of inflammation in a murine peritonitis model, and suppresses hepatic inflammation and steatosis during the progression of NAFLD/NASH in *E3L.CETP* mice, without inducing hyperlipidemia or overt other adverse effects. Future studies will have to confirm a similar protective effect of SH42 on atherosclerosis development, and show that SH42 is safe and effective in humans. If so, SH42 could present a true revolution in the treatment of cardiometabolic diseases by targeting inflammation in macrophages, which would largely add to current strategies that mainly focus on lowering of lipids.

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