Novel modulators of lipoprotein metabolism : implications for steatohepatitis and atherosclerosis

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GENERAL DISCUSSION AND FUTURE PERSPECTIVES

Cardiovascular disease (CVD), which is mainly due to atherosclerosis, is currently the leading cause of death in the Western world [1]. One of the most important risk factors for atherosclerosis is dyslipidemia, hallmarked by increased plasma levels of (V)LDL-cholesterol (C) and triglycerides (TG), and decreased plasma levels of HDL-C. Lipid-lowering agents (especially statins) improve dyslipidemia and have proven to reduce major cardiovascular events [2, 3]. However, since these drugs only partially improve the mortality and morbidity due to CVD, other drug targets to combat CVD are being explored. Since HDL-C is inversely correlated with cardiovascular risk [4], novel strategies to raise HDL-C levels are currently under development, aiming at further reduction of atherosclerosis. Because the cholesteryl ester transfer protein (CETP) plays a pivotal role in HDL-C metabolism [5], CETP has become one of the most important targets for development of HDL-raising strategies.

Another risk factor for CVD is non-alcoholic steatohepatitis (NASH), characterized by accumulation of triglycerides and immune cells including macrophages in the liver. The prevalence of NASH is increasing steadily [6]. In contrast to atherosclerosis, no established pharmacological agents have been identified thus far to treat NASH. Lifestyle modifications, such as weight loss, exercise, and restriction of nutrient intake, are still the mainstays for the treatment of NASH [7]. Additionally, the non-invasive diagnosis for NASH is cumbersome in routine clinical practice, and no easily accessible biomarker with sufficient sensitivity and specificity is available to detect increased hepatic macrophage content, a hallmark of NASH.

The studies described in this thesis 1) demonstrated the cellular origin of CETP expression and its implications for NASH and CVD, 2) elucidated the effects of pharmacological and dietary lipid-lowering interventions on plasma CETP levels, 3) investigated a novel target for treatment of atherosclerosis and NASH, and 4) evaluated the role of the brain in peripheral TG metabolism.

The cellular origin of CETP, and its implications for NASH and CVD

Previous studies have demonstrated that CETP mRNA is abundantly expressed in adipose tissue and liver of several mammalian species, including human, monkey, rabbit, pig and hamster [8]. In addition, CETP is expressed to a lower extent in the spleen, heart, small intestine, adrenal gland, and skeletal muscle [9-12]. A small human cohort study suggested that CETP expression in adipose tissue correlates with plasma CETP concentration [13]. In **Chapter 6**, our data showed much more prominent *CETP* expression in the liver as compared to adipose tissue. In addition, we observed no association between central obesity measured as waist circumference and plasma CETP level in a large cohort of the general population (n~1,500), implying that central adipose tissue does not correlate

with plasma CETP concentration. Rather, by analyzing liver biopsies from obese patients undergoing bariatric surgery, we found that plasma CETP concentration was strongly correlated with CETP expression in the liver but not with that in the adipose tissue, indicating that liver is the main site of *CETP* expression and is a determinant of the total plasma CETP pool in humans. Few studies suggested that changes in the degree of adiposity induced by body weight reduction reduced CETP expression and improved lipoprotein metabolism [14, 15]. However, we (**Chapter 4**) and others [16] showed that body weight reduction, in addition to reducing adiposity, significantly reduced hepatic lipid content and attenuated hepatosteatosis. Moreover, we also showed in this thesis that a decrease in hepatic lipid content was accompanied by a decrease in plasma CETP level (**Chapter 3** and **4**). It is thus tempting to speculate that bodyweight reduction via attenuation of hepatosteatosis reduces the production of CETP by the liver, and via this mechanism reduces plasma CETP concentration.

The liver consists of multiple cell types including hepatocytes and non-parenchymal cells such as endothelial cells and macrophages (i.e. Kupffer cells). Therefore, we set out to evaluate the cell type responsible for the hepatic expression of CETP. In this thesis, we demonstrated that the expression of established macrophage markers (e.g. *Cd68*, *Abcg1* and *Marco*) in the liver strongly correlated with the hepatic expression of CETP both in human CETP transgenic (Tg) mice (**Chapter 5**) and in humans (**Chapter 6**). Moreover, CETP appeared to be specifically co-localized with $F480^+$ macrophages in the mouse liver and with $CD68^+$ macrophages in the human liver (**Chapter 6**). Mechanistic studies in *APOE*3-Leiden.CETP (E3L.CETP)* mice showed that depletion of macrophages from liver following administration of clodronate liposomes virtually abolished hepatic CETP expression and largely reduced plasma CETP level, fully corroborating our findings in humans that hepatic macrophages, rather than hepatocytes, are the main cellular origin of hepatic CETP expression and the plasma CETP pool.

Previously, hepatic expression of CETP has been attributed to both macrophages and hepatocytes. This dogma was mainly derived from studies assessing hepatic CETP expression 8 weeks after transplantation of bone marrow from wild-type (WT) littermates into human CETP Tg mice and vice versa, suggesting that hepatic macrophages contribute ~50% to total hepatic CETP expression [17]. However, it should be realized that the turnover of liver macrophages after bone marrow transplantation occurs slowly. In the same study, it was found that only 50% of Kupffer cells were replaced by the donor cells 8 weeks after bone marrow transplantation, accompanied by 50% reduction in plasma CETP as well as 2-fold lower hepatic *CETP* expression in WT → CETP Tg mice as compared to control transplanted CETP Tg → CETP Tg mice [17]. Interestingly, we found hepatic CETP expression to be decreased by approximately

-90% at 12 weeks after transplantation when more Kupffer cells had been replaced (*Berbée et al.* unpublished), again confirming that hepatic macrophages are the main predominant source of CETP expression.

In contrast to the liver (i.e. Kupffer cells), we could hardly detect CETP expression in extrahepatic macrophage-rich organs, including adipose tissue and spleen, or in isolated peritoneal macrophages. It has been reported that that CETP gene promoter contains a liver X receptor α (LXRα) responsive element [10], and CETP expression is regulated by the activation of LXRα [18], which is highly expressed in multiple organs. Recently, *Gautier et al.* [19] reported that hepatic CETP expression is also upregulated by activation of the farnesoid X receptor (FXR), for which bile acids are the natural ligands. In addition to an LXRα responsive element in the CETP gene promoter, the CETP gene was shown to contain an ER8 FXR response element in its first intron. Since FXR is highly expressed in the liver and its natural ligand bile acids are produced by hepatocytes, the specific liver environment may be essential for maintaining high expression of CETP in hepatic versus extrahepatic macrophages. Although treatment of CETP Tg mice with both LXR agonists [18] and FXR agonists [19] induces CETP expression in the liver, the leading regulator for CETP expression in hepatic macrophages *in vivo* is still unknown yet, which should be investigated by future studies.

Our findings that whole body CETP expression is predominantly derived from hepatic macrophages reveal that plasma CETP may be a biomarker for hepatic macrophage content, a hallmark of NASH. NASH is characterized by accumulation of fat (steatosis) in combination with inflammation (e.g. infiltrated macrophages) in the liver [20]. Although several imaging modalities have been advocated as non-invasive diagnostic method for liver steatosis, they are insufficient to distinguish NASH from simple non-inflammatory fatty liver disease (NAFLD). Liver biopsies are currently the golden standard methods for the diagnosis of NASH. However, there are several severe limitations to liver biopsies, such as sampling error, differences in histopathologic interpretation, as well as patient stress and discomfort, risk of bleeding and long hospitalizations. In this thesis, we showed that hepatic macrophages are the main cellular origin of the plasma CETP pool (**Chapter 6**), and that the plasma CETP level significantly correlates with hepatic macrophage content in CETP Tg mice (**Chapter 5**). More importantly, treatment of *E3L.CETP* mice with niacin (**Chapter 5**) and exendin-4 (**Chapter 10**) reduces hepatic macrophage content accompanied with the reduction in plasma CETP level. Taken together, these data suggest that measurement of the plasma CETP concentration can be developed as a diagnostic and predictive test for the hepatic macrophage content in clinical practice, which should be tested in large population cohorts.

In addition, our findings that CETP expression in the hepatic macrophages

determines the plasma CETP level as well as the metabolism of plasma lipoproteins sheds new light on the development of new strategies involving CETP inhibition for the treatment of dyslipidemia and CVD. Although a past CETP inhibitor (i.e. torceptrapib) and current CETP inhibitors (e.g. dalcetrapib, anacetrapib, evacetrapib) convincingly demonstrate HDL-raising effects, they show uncertain results for the treatment of CVD. For example, although torcetrapib [21] and dalcetrapib [22] increase the plasma HDL-C level effectively, both of them failed to reduce CVD outcome in trials. Torcetrapib even caused a marked increase in deaths [21], and dalcetrapib had no beneficial effects on carotid artery wall index, endothelial function or CVD outcomes [23-25]. Although the precise mechanism(s) by which torcetrapib and dalcetrapib inhibit CETP activity is not known, these CETP inhibitors directly act on the plasma CETP protein and induce tight binding of CETP to HDL particles [26]. However, the tight binding of CETP with HDL particles might compromise the function of HDL to generate large CE-rich HDL particles instead of small HDL particles and nascent discoidal HDL, as observed with torcetrapib [27].

The development of CETP inhibitors to raise HDL-C is based on epidemiological studies showing a strong inverse correlation of HDL-C level with CVD risk [4]. The classical "HDL cholesterol hypothesis" predicted that interventions to raise the HDL-C level will result in reduction of CVD risk. However, recent studies showed that HDL functionality perhaps is a more important consideration than the circulating HDL-C level for the treatment of CVD. Indeed, the reverse cholesterol transport capacity of HDL has been shown a much better predictor of CVD than the concentration of HDL-C [28, 29]. Therefore, within the field of HDL-targeting therapeutics a gradual transition takes places from the simple "HDL cholesterol hypothesis" to the "HDL functionality hypothesis" aimed at increasing the HDL particle number and improving HDL functionality for the treatment of CVD [28-30]. Based on this perspective, to avoid potentially adverse effects of the current CETP inhibitors on the function of HDL, strategies focusing on inhibiting CETP synthesis at its cellular origin may be a promising alternative.

Regulation of plasma CETP by pharmacological and dietary lipid-lowering interventions

Previously, we have shown that several classical lipid-lowering drugs including statins [31], fibrates [32] and niacin [33], increase the plasma level of HDL-C in addition to decreasing the plasma level of (V)LDL-C and TG by reducing hepatic CETP expression and decreasing plasma CETP activity in preclinical studies using *E3L.CETP* transgenic mice. In this thesis, we again demonstrated in **Chapter 6** that fenofibrate and niacin raise the HDL-C level accompanied by reduced hepatic CETP expression and plasma CETP level in *E3L.CETP* mice. In line with those classical lipid lowering drugs, in **Chapter 10** we observed that

exendin-4, a glucagon like peptide-1 (GLP-1) receptor agonist that was approved in 2005 for the treatment of T2DM, decreased plasma VLDL-C and increased HDL-C also accompanied with decreased hepatic *CETP* expression as well as plasma CETP level in *E3L.CETP* mice. Moreover, in **Chapter 3**, we found that although both pioglitazone (PPARγ agonists) and metformin decreased plasma TG and apoB level equivalently, only pioglitazone significantly increased the plasma HDL-C level associated with a reduction in hepatic triglyceride and plasma CETP level in patients with type 2 diabetes mellitus (T2DM). In addition to pharmacological interventions, in **Chapter 4**, prolonged caloric restriction markedly decreased the plasma CETP level and increased the plasma apoAI level in obese patients with T2DM. These collective results from human studies are in full accordance with the findings in *E3L.CETP* mice, suggesting that reduction of (liver-derived) CETP plays an important role in the HDL-raising effects of both pharmacological and dietary lipid-lowering interventions.

Although accumulating evidence indicates that lipid-lowering interventions exert HDL-raising capacity by reducing the plasma CETP level, the mechanisms underlying the CETP-reducing effects of those lipid-lowering interventions has thus far not been delineated. In **Chapter 6**, we demonstrated that hepatic macrophages are the main cellular origin of CETP expression, and that CETP expression in hepatic macrophages determines the plasma CETP level and modulates plasma lipoprotein metabolism. Thus, in **Chapter 5**, we set out to investigate the role of hepatic macrophages in the CETP-lowering effect of niacin. Interestingly, our observations *in vitro* showed that niacin at various concentrations did not reduce CETP expression in cultured macrophages derived from CETP Tg mice, indicating that niacin does not directly regulate the CETP expression *per se* in macrophages. Rather, we observed that niacin reduced the hepatic cholesterol content *in vivo*. More importantly, in line with attenuated liver cholesterol accumulation, we observed that niacin decreased hepatic mRNA expression of macrophage markers (e.g. *Cd68* and *Abcg1*) and the number of F4/80^{+} macrophages, as well as the hepatic expression of CETP. In fact, hepatic macrophage markers showed a high correlation and association with hepatic CETP expression. These data suggest that the reduction of hepatic CETP expression induced by niacin treatment is a direct consequence of reduced macrophage content in the liver. In addition to niacin, we demonstrated in *E3L.CETP* mice that fenofibrate (**Chapter 6**) and exendin-4 (**Chapter 10**) also reduce the hepatic lipid content and decreased the hepatic macrophage content thereby decreasing the hepatic expression and plasma level of CETP.

In humans, we are able to measure hepatic TG content by proton (^{1}H) magnetic resonance spectroscopy, although it is currently impossible to assess the hepatic macrophage content noninvasively. We observed that both pioglitazone (**Chapter 3**)

and prolonged caloric restriction (**Chapter 4**) decreased the plasma CETP level accompanied with a decrease in hepatic TG content in patients with T2DM. It has been reported that the reduction of hepatic TG content induced by e.g. weight loss is associated with a decrease in hepatic inflammation [34], and that the histological severity of inflammation is correlated with the number of $CD68^+$ macrophages in patients with NASH [35]. It is thus tempting to speculate that both pioglitazone (**Chapter 3**) and prolonged caloric restriction (**Chapter 4**) decrease not only the hepatic lipid content but also hepatic macrophage content in humans, thereby reducing hepatic expression and plasma level of CETP. This hypothesis needs to be evaluated in future studies, for which the assessment of macrophage number in the liver will ultimately be required.

As summarized in Figure 1, we propose the current mechanism how lipid-lowering interventions reduce hepatic CETP expression and plasma CETP level. Albeit through different actions, they all reduce hepatic lipid content (i.e. TG and cholesterol). This reduction in hepatic lipid content subsequently attenuates hepatic inflammation, which leads to less macrophage infiltration into and/or increased macrophage efflux/emigration out of the liver. Since liver macrophages are the main cellular origin of CETP expression, the decreased number of hepatic macrophages leads to an overall reduction in hepatic CETP expression, and, consequently, the plasma CETP level. Therefore, these lipid-lowering interventions induce a less atherogenic lipid phenotype, e.g. decreasing (V)LDL-C and TG, and increasing HDL-C.

Novel strategies for treatment of atherosclerosis and NASH

Given the fact that current strategies are insufficient to reduce CVD and no established pharmacological agents have shown adequate and convincing benefits in NASH outcomes, novel strategies for the treatment of those two diseases are eagerly warranted and under development. Emerging evidence indicates that gut hormones regulating energy homeostasis and food intake, could also beneficially affect lipid metabolism, thus have the potential to treat atherosclerosis and fatty liver disease. Glucagon like peptide-1 (GLP-1) is one of those incretin hormones produced by intestinal L-cells and the brain [36, 37], and released in response to food intake to stimulate glucose-dependent insulin production [38]. In addition, GLP-1 exerts multiple other functions, including inhibition of food intake [39], slowing the gastric emptying [40], inhibition of glucagon secretion [41], and improving glucose metabolism [41, 42]. In addition, we (***Chapter 10***) and others [43, 44] observed that GLP-1 receptor agonists decrease plasma TG and VLDL-C level, and increase HDL-C level. Thus, GLP-1 receptor agonism may be a valuable target for both atherosclerosis and NASH.

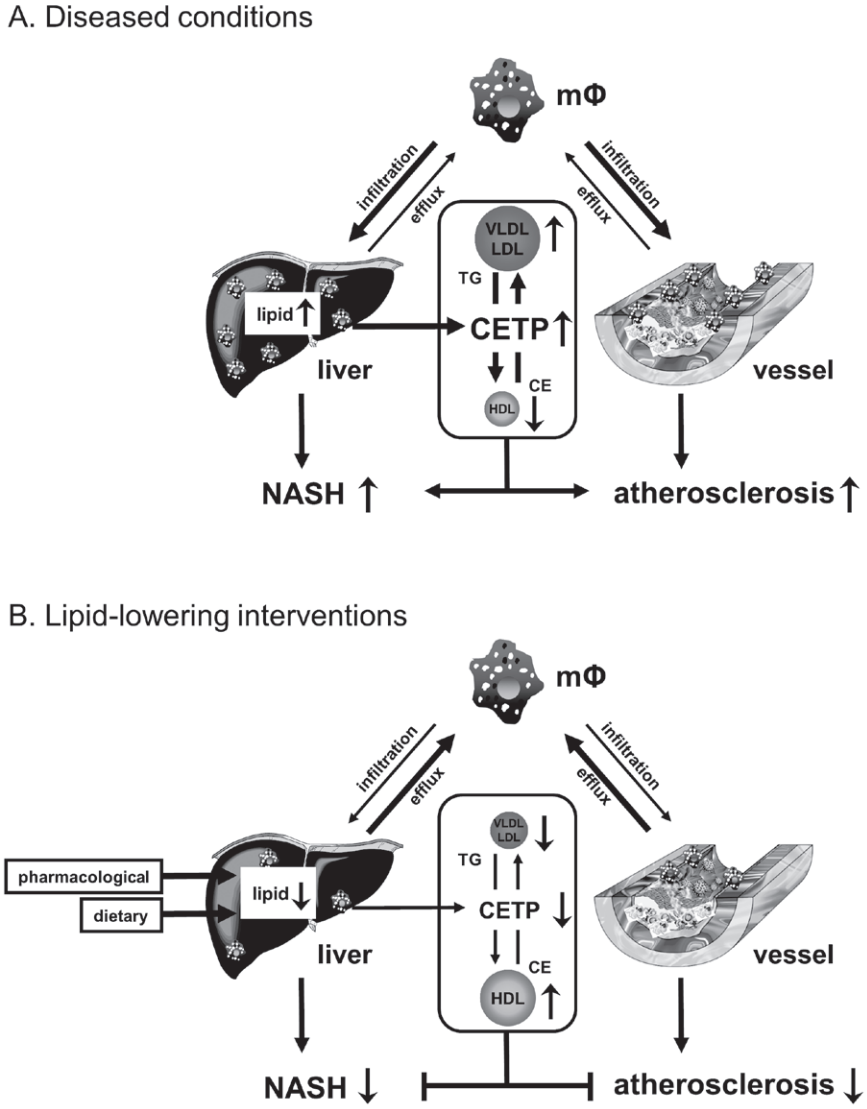

Figure 1. Proposed mechanism underlying the CETP-lowering effects of lipid-lowering interventions. (A) Under diseased conditions (e.g. NASH and atherosclerosis), macrophages infiltrate into both the vessel wall and the liver. In the liver, increased hepatic macrophages result in an overproduction of CETP, and consequently to an elevated plasma CETP pool. (B) Lipid-lowering interventions, including both pharmacological (e.g. fenofibrate, niacin, pioglitazone and exendin-4) and dietary (e.g. caloric restriction) interventions, reduce the hepatic lipid content and concomitantly attenuate macrophage infiltration into the liver. The decreased number of hepatic macrophages leads to a reduction in hepatic CETP expression and plasma CETP level, as a result, generating a less atherogenic lipid phenotype. See text for further explanation.

In **Chapter 8**, we first investigated the mechanisms underlying the beneficial effects of GLP-1 receptor agonism on liver TG metabolism in *E3L* mice fed a high fat diet (HFD). We found that GLP-1 receptor agonists decreased hepatic VLDL particle production and completely reversed HFD-induced hepatic steatosis reflected by largely decreasing the hepatic lipid content to the low levels observed in chow-fed control mice. GLP-1 receptor agonists decreased the expression of the nuclear transcription factor *Srebp-1c* and its targets *Fasn* and *Dgat1*, implying that GLP-1 receptor agonism primarily reduces hepatic lipogenesis, thereby causing a reduction in hepatic lipid content. Taken together with the concomitantly reduced *Apob* expression, GLP-1 receptor agonism lowers the hepatic availability of TG, thereby reducing the production of VLDL particles. In addition to decreasing hepatic lipogenesis and increasing fatty acid oxidation, GLP-1 receptor agonists activate AMP-activated protein kinase (AMPK) and SIRT (sirtuin) dependent on GLP-1 receptor expressed in hepatocytes [45], thereby improving hepatic glucose and lipid metabolism and relieving NAFLD. Recently, several clinical trials have confirmed that GLP-1 receptor agonists largely reduce the hepatic TG content in obese patients with T2DM [45, 46], indicating that the GLP-1 receptor is a promising novel target for treatment of NAFLD.

Moreover, in **Chapter 10**, we observe that only 4 weeks of exendin-4 infusion markedly decreases total atherosclerotic lesion area, accompanied by a reduction in plaque macrophage content. Notably, in contrast to classical lipid-lowering compounds that reduce atherosclerosis mainly by improving dyslipidemia, exendin-4 only slightly decreased (V)LDL-C and TG, and increased HDL-C. Furthermore, exendin-4 also decreased hepatic inflammation reflected by reduced expression of inflammatory markers (e.g. TNFα, IL-1β and Il-6), as well as hepatic macrophage content. The GLP-1 receptor is thus not only a promising target for NAFLD, but also NASH and atherosclerosis. It is interesting to note that atherosclerosis and NASH are strongly associated and share common etiologies, involving monocyte recruitment and macrophage foam cell formation [47]. We demonstrated a reduced number of both adhering monocytes to the vessel wall and infiltrated macrophages into the liver after the treatment of exendin-4. In addition, exendin-4, via the GLP-1 receptor, reduced the uptake of oxLDL by macrophages, which may implicate reduced foam cell formation in the vessel wall and in the liver. Taken together, these data corroborate the hypothesis that exendin-4 reduces the development of atherosclerosis and NASH simultaneously by acting directly on monocyte/macrophage recruitment/maturation into both the vessel wall and liver.

So far, exendin-4 has been approved for the treatment of T2DM in the clinical practice. We show that GLP-1 receptor agonism not only suppresses diet-induced NAFLD, but also reduces the development of NASH and atherosclerosis, at least when administered

chronically by using an osmotic minipump. Therefore, we propose that GLP-1 receptor agonism is a novel strategy for treatment of atherosclerosis and NASH in addition to T2DM, in particular in patients who display a combination of these diseases.

The role of brain in triglyceride metabolism

The brain plays an important role in maintaining energy homeostasis, with the hypothalamus being its key regulator [48]. Two major neuronal populations within the hypothalamic arcuate nucleus are involved in the regulation of energy intake, including pro-opiomelanocortin/cocaine and amphetamine-regulated transcript-expressing neurons and neuropeptide Y (NPY)/agouti-related protein-expressing neurons. Although the role of brain in the regulation of glucose metabolism has been firmly established [49], only a few studies have focused on its function in maintaining TG homeostasis. Recently, accumulating evidence have suggested that various neuronal populations, such as NPY expressing neurons and the melanocortin (MC) expressing neurons, modulate sympathetic outflow from the hypothalamus towards target organs involved in TG metabolism, such as liver, white adipose tissue (WAT) and brown adipose tissue (BAT), and thereby modulate peripheral TG metabolism. For example, a recent study in rats showed that central administration of NPY acutely increases hepatic VLDL-TG production [50]. Also, *Bruinstroop et al.* [51] further confirmed that hypothalamic NPY regulates hepatic VLDL secretion in rats via the sympathetic outflow, as selective sympathetic denervation of the liver abolished the effect of central NPY administration. In contrast to NPY signaling, central administration of melanocortin receptor (MC) receptor agonists decrease hepatic lipogenic gene expression in diabetic mice [52] and decrease hepatic TG content in rats [53].

Hypertriglyceridemia, associated with increased hepatic VLDL-TG production and/or decreased VLDL-TG clearance, is an important risk factor for CVD [54, 55]. Since atherosclerosis is generally studied in hyperlipidemic mice rather than in rats, in **Chapter 7**, we set out to validate the effect of central NPY signaling on hepatic VLDL-TG production in mice, with the ultimate goal to investigate whether NPY, by affecting VLDL-TG synthesis, contributes to the development of atherosclerosis. Although we confirmed that central administration of NPY acutely increases food intake in mice, similarly as in rats [56], surprisingly we were unable to detect any increase in hepatic VLDL-TG production in mice after central NPY infusion. Likewise, antagonizing central NPY signaling by either PYY_{3-36} or Y1 receptor antagonism did not affect VLDL production in mice. Apparently, central NPY signaling exerts different effects on TG metabolism in rats versus mice. One potential explanation is that the hepatic VLDL metabolism in itself is differentially regulated in rats versus mice, as rats display lower basal hepatic VLDL-TG

production rates [50, 51] when compared to that in mice. Secondly, the expression of the several NPY receptors in rats versus mice is different. Both rats and mice express similar Y1-Y5 receptors [57], while only mice express the Y6 receptor [58]. Although the exact function of Y6 receptor in appetite regulation remains elusive, if activation of this receptor by NPY would exert an opposing effect on hepatic VLDL production, this might explain our negative findings in mice. Notably, genetic association studies in humans have reported conflicting results on the association of polymorphisms in the NPY gene with plasma TG levels [59, 60]. Collectively, these data emphasize the requirement of further research, particularly in humans, on the role of hypothalamic NPY in peripheral TG metabolism.

In addition to hypothalamic NPY, gut hormones regulate energy homeostasis via activation of their receptors expressed in the brain. The GLP-1 receptor is abundantly expressed in various tissues [61], not only in the gastrointestinal tract, pancreatic islands, kidneys, and heart, but also in the central nervous system [62]. After showing that GLP-1 receptor agonism decreased hepatic VLDL-TG production and hepatic lipid content in mice (**Chapter 8**), we next investigated whether the effects of GLP-1 receptor agonism on lipid metabolism would be dependent on central GLP-1 receptor signaling. We found that chronic central administration of the GLP-1 receptor antagonist exendin-9 did not counteract the peripherally administered exendin-4-induced decrease in hepatic VLDL-TG production, suggesting that the beneficial effects of exendin-4 on hepatic lipid metabolism is not mediated by the central GLP-1 receptor signaling (unpublished). In contrast to our findings, *Panjwani et al.* [63] recently showed that acute central administration of exendin-4 rapidly decreased hepatic VLDL-TG production, indicating that central GLP-1 signaling might regulate hepatic lipid metabolism. There are several possible explanations for the distinct results obtained from our study and Panjwani's study: (1) the dosage of exendin-9 used in our study is insufficient to block the effects of central GLP-1 receptor activation on hepatic lipid production, and (2) acute activation of the central GLP-1 signaling plays a more important role in hepatic VLDL production than chronic activation. Experiments combining central and/or peripheral GLP-1 administration with hepatic denervations might prove an effective strategy to elucidate the exact role of central GLP-1 signaling in the regulation of hepatic TG metabolism.

The hypothalamus not only regulates, via the modulation of sympathetic outflow, TG metabolism in the liver but also in white adipose tissue (WAT). For example, chronic central NPY infusion promoted lipogenesis in WAT, independent of its effects on food intake [64]. Likewise, inhibition of central MC signaling induced the expression of lipogenic genes in WAT [65]. In contrast, activation of central MC signaling by chronic infusion of an MC3/4 receptor agonist, increased the expression of lipolytic genes in WAT of rats. Additionally, central GLP-1 was implicated to regulate TG metabolism in WAT, as a mouse

study showed that central infusion of GLP-1 decreased TG content in WAT [66].

Unlike WAT, BAT combusts TG in the process of thermogenesis. Recently, evidence has accumulated to suggest that hypothalamic signaling also influence BAT thermogenesis. We and others showed that central infusion of NPY decreased BAT activity and thermogenesis, both in mice (unpublished) and in rats [67]. Also, central infusion of MC4R antagonist resulted in decreased BAT thermogenesis in rats [68]. Since those studies investigating the effect of neuroendocrine factors on BAT thermogenesis have neither focused on lipid metabolism nor performed TG clearance experiments, future studies should therefore emphasize the effect of central signaling on TG clearance by BAT.

Taken together, the brain, in particular the hypothalamus is an important regulator of peripheral TG metabolism. However, the exact role of specific neuroendocrine factors that mediate TG metabolism in liver and BAT needs to be determined by future research.

Concluding remarks

The current strategies for the treatment of atherosclerosis, i.e. lipid-lowering strategies, are insufficient, and at the start of the studies described in this thesis no pharmacological agents had been identified thus far to treat NASH. Therefore, novel strategies for the treatment of those two diseases were eagerly warranted and currently under investigation. In addition to classical lipid-lowering agents, HDL-raising strategies, e.g. CETP inhibitors, are currently still considered as promising methods to treat dyslipidemia and ultimately CVD. However, current CETP inhibitors may affect the functionality of HDL. Therefore, other ways to reduce CETP levels may be advantageous.

In this thesis, we demonstrated CETP to be involved in the HDL-raising effects of both lipid-lowering agents (i.e. fenofibrate, niacin, pioglitazone and the GLP-1 receptor agonist exendin-4), as well as dietary lipid-lowering interventions (i.e. caloric restriction). In fact, we found that all of these interventions reduced plasma CETP level accompanied by reduction in hepatic lipid content. More mechanistic studies revealed that CETP is predominantly expressed by hepatic macrophages, and that reducing the hepatic macrophage content by lipid-lowering strategies reduces the hepatic CETP expression and plasma CETP level. Therefore, targeting the hepatic macrophage may be a promising alternative for CETP inhibitors to reduce the plasma CETP level and increase the HDL level. In addition, the fact that plasma CETP is mainly derived from hepatic macrophages, a hallmark of NASH, implies that measuring plasma CETP concentration may provide a useful relatively non-invasive biomarker for the hepatic macrophage content in NASH in clinical practice. In addition, we identified, by using *E3L.CETP* mice, the GLP-1 receptor as a novel target for the treatment of atherosclerosis and NASH in addition to T2DM, which need to be confirmed in future human studies.

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