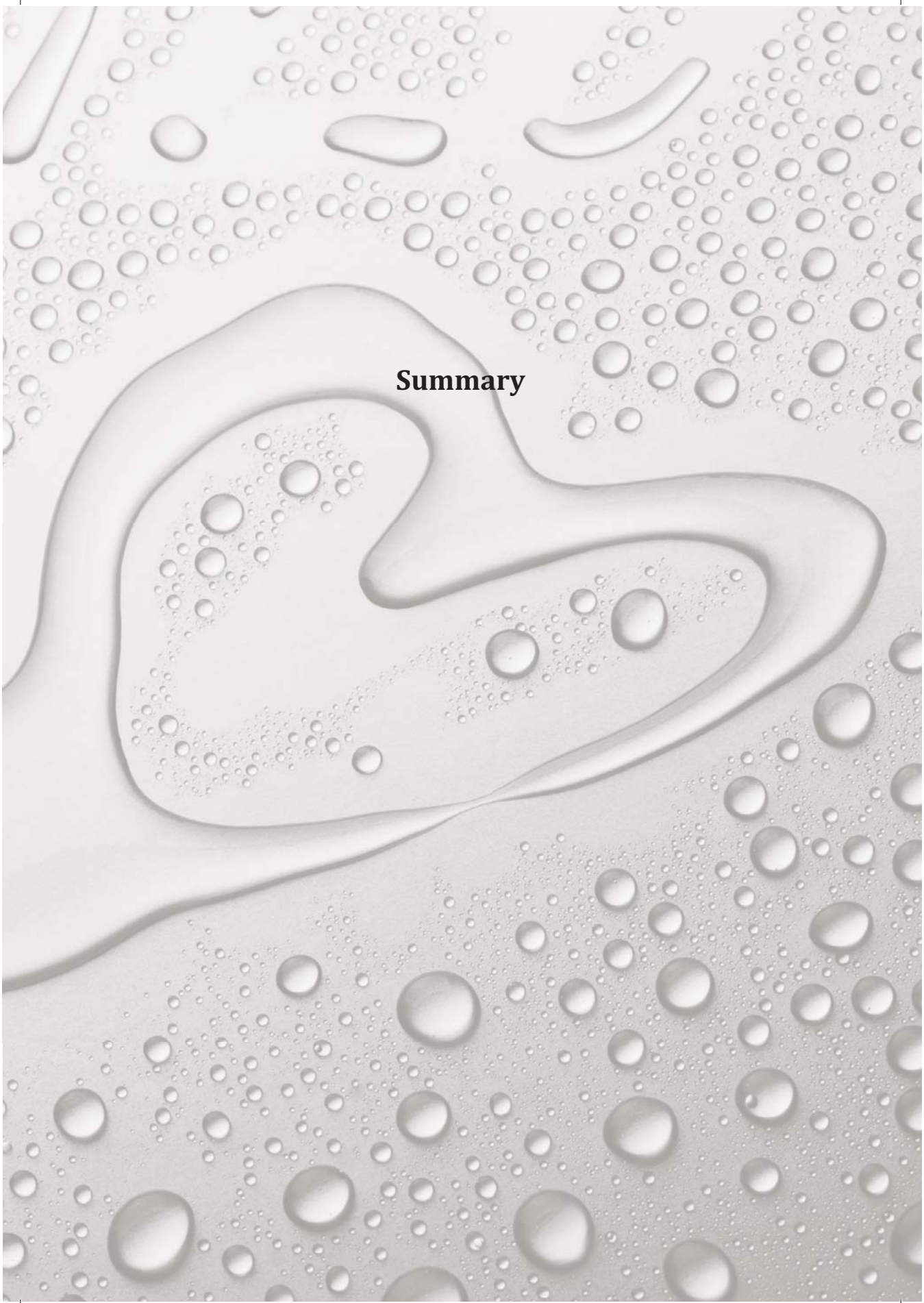
CETP and Inflammation in lipid metabolism and atherosclerosis

Vries-van der Weij, A.J. de



Summary

Cardiovascular disease (CVD) is a major cause of morbidity and mortality in the western world and is mainly caused by atherosclerosis. One of the major risk factors for atherosclerosis development, high levels of low density lipoprotein (LDL)-cholesterol (C), can be efficiently treated with cholesterol-lowering drugs such as statins. However, treatment with statins prevents only about 30% of all cardiovascular events, thus a significant residual risk remains. Therefore, therapeutic strategies targeting other risk factors than high LDL-C levels may be useful to further reduce CVD. Two other risk factors for CVD have been identified: inflammation and low levels of high density lipoprotein (HDL)-C. In the first part of this thesis, we focused on cholesterol as an inducer of inflammation, and on the role of inflammation in atherosclerosis. In the second part of this thesis we studied the role of CETP, an important protein in HDL metabolism, in lipid metabolism and atherosclerosis. For these studies we used the APOE*3-Leiden (E3L) and the E3L.CETP transgenic mouse models. These mice respond to a high cholesterol diet with induction of hyperlipidemia and hepatic inflammation and respond to lipid-modifying drugs in a similar way as humans do. Furthermore, these mice develop atherosclerotic lesions that are similar in pathology to atherosclerotic lesions in humans.

The first part of this thesis focused on inflammation. Studies in humans and in mice have shown that cholesterol can induce a hepatic inflammatory response. Since the molecular mechanism underlying the induction of inflammation by cholesterol is not known, we aimed to elucidate this mechanism in **Chapter 2**. To this end, we fed E3L mice a diet without cholesterol (control) or with low cholesterol (LC) or high cholesterol (HC) concentrations, which caused the liver to switch from an adaptive state (control and LC diet) to an inflammatory state (HC diet). Cholesterol feeding dose-dependently increased plasma cholesterol levels and hepatic cholesteryl ester (CE) content and dose-dependently decreased cholesterol synthesis in the liver. In contrast, the hepatic free cholesterol (FC) concentration and plasma levels of the hepatic inflammation marker serum amyloid A (SAA) increased only with HC diet feeding and were found to be significantly correlated. Microarray analysis of livers suggested that HC, but not LC, evokes endoplasmic reticulum (ER) stress. Furthermore, the activity of ER stress-inducible transcription factors and positive regulators of SAA expression, NF-κB and STAT3, were found to be enhanced upon HC, but not LC feeding. We concluded that HC diet feeding induces hepatic inflammation and SAA gene expression in the liver through an increase of hepatic NF-κB and STAT3 activity, putatively as a result of an FC-induced ER stress response.

The stimulatory role of inflammation in atherosclerosis development is now widely established. It is not known if inflammation also plays a role in atherosclerosis regression. In **Chapter 3** we wondered if suppressing inflammation using the NF-κB inhibitor salicylate leads to atherosclerosis regression on top of cholesterol-lowering. To this end, ApoE*3-Leiden (E3L) mice were fed a high cholesterol diet to induce the formation of mild atherosclerotic lesions. Subsequently, one group of mice was

sacrificed to determine lesion area and lesion severity at the start of different regression treatments (reference group). A second group of mice was then fed a high cholesterol diet supplemented with salicylate (HC+SAL) to suppress NF-κB activity. As salicylate not only quenched inflammation, but also reduced plasma cholesterol levels (- ~49%), a third group of mice was fed a low cholesterol (LC) diet to establish similar plasma cholesterol levels as obtained by salicylate treatment. Compared to the reference group, HC+SAL suppressed hepatic NF-κB activity, tended to suppress hepatic STAT3 activity and reduced plasma levels of the hepatic inflammation marker SAA. Compared to HC+SAL, LC diet feeding suppressed hepatic NF-κB activity to a lesser extent, similarly suppressed STAT3 activity, and more strongly reduced plasma SAA levels; HC+SAL and LC feeding similarly suppressed aortic NF-κB activity. At 16 weeks after starting the regression treatments, neither HC+SAL or LC treatment showed an effect on lesion area (compared to the reference group), but HC+SAL had reduced the macrophage area of lesions and increased the plaque stability index (ratio of collagen to macrophage area) more strongly than LC diet feeding. These data led us to conclude that the combined effect of suppressing NF-κB activity and reducing plasma cholesterol with SAL improves lesion severity and promotes lesion regression more efficiently than cholesterol lowering alone.

LXR agonists are a class of compounds that regulate various cellular processes, including inflammation. LXR agonists can both prevent atherosclerosis development and induce regression of pre-existing lesions. However, the mechanisms underlying their atheroprotective effects have not been fully explored. In **Chapter 4** we therefore investigated the mechanisms underlying the anti-atherogenic potency of the LXR agonist T0901317 under both lesion-progressive and lesion-regressive conditions, in a time-dependent manner. Using E3L mice, we showed that T0901317 strongly suppresses lesion evolution and promotes lesion regression. We found that under progressive (high cholesterol diet) as well as regressive (cholesterol-free diet) conditions T0901317 (i) significantly increased plasma triglyceride and total cholesterol levels; (ii) did not affect the systemic inflammation marker SAA; (iii) suppressed endothelial monocyte adhesion; and (iv) induced the expression of the cholesterol efflux-related genes apoE, ABCA1 and ABCG1. Furthermore, under progressive conditions, T0901317 suppressed the vascular inflammatory status, lowered lesional macrophage accumulation, and blocked the transition from lesional stage II to III. Under regressive conditions, T0901317 induced lesional macrophage disappearance and increased the expression of the C-C chemokine receptor CCR7, a factor functionally required for regression. Taken together, we showed that the LXR-agonist T0901317 retards atherosclerotic lesion development and promotes lesion regression by exerting several atheroprotective effects on the vasculature. Furthermore, our findings support that vascular LXR is a potential anti-atherosclerotic target.

The second part of this thesis addressed the role of CETP in lipid metabolism and atherosclerosis. As CETP decreases HDL-C levels, CETP inhibition is being regarded a

promising strategy to increase HDL-C levels and thereby reduce CVD. The first CETP inhibitor tested in large clinical trials was torcetrapib. Although torcetrapib increased HDL-C levels with about 60% in humans on a background of atorvastatin, torcetrapib did not reduce atherosclerosis progression, and even increased cardiovascular death rate. Importantly, the effect of torcetrapib on atherosclerosis progression and cardiovascular endpoints in humans was only studied in combination with atorvastatin. Therefore, we evaluated the anti-atherogenic potential of torcetrapib with and without atorvastatin in E3L.CETP mice in **Chapter 5**. Furthermore, we aimed to gain insight in the adverse effects underlying the increase in cardiovascular death rate in humans. To this end, E3L.CETP mice were fed a cholesterol-rich without drugs or with torcetrapib, atorvastatin or both. Torcetrapib decreased plasma cholesterol, albeit to a lesser extent than atorvastatin or the combination of torcetrapib and atorvastatin. Torcetrapib similarly increased HDL-C in the absence and in the presence of atorvastatin. Torcetrapib and atorvastatin alone reduced atherosclerotic lesion size to a similar extent, and combination therapy did not further reduce atherosclerosis. Remarkably, as compared to atorvastatin, torcetrapib induced enhanced monocyte recruitment and expression of monocyte chemoattractant protein-1 and resulted in lesions of a more inflammatory phenotype, as reflected by an increased macrophage content and a reduced collagen content. In conclusion, we showed that CETP inhibition by torcetrapib *per se* reduces atherosclerotic lesion size but does not enhance the anti-atherogenic potential of atorvastatin. However, as compared to atorvastatin, torcetrapib introduces lesions of a less stable phenotype.

CETP adversely affects the plasma lipoprotein profile by increasing VLDL-C and decreasing HDL-C. However, the relative contribution of either of these changes to atherosclerosis development is not known. Therefore, we investigated in **Chapter 6** to what extent the increase in VLDL-C can explain the atherogenic action of human CETP expression in E3L mice. For this purpose, E3L and E3L.CETP mice were fed a low cholesterol (LC) diet, resulting in an increased VLDL-C level as well as an increased atherosclerotic lesion area in the aortic root in E3L.CETP mice compared to E3L-LC mice. E3L mice fed a high cholesterol (HC) diet to match for the increased VLDL-C levels in E3L.CETP mice, displayed a similar atherosclerotic lesion area as observed in E3L.CETP mice. Despite similar atherosclerosis development, E3L.CETP mice had lower HDL-C as compared to E3L-HC mice. Remarkably, atherosclerotic lesions in CETP-expressing mice were enriched in collagen, suggesting a role of CETP or the diet in modifying the collagen content of lesions. These data led us to conclude that, in this experimental setting, the pro-atherogenic effect of CETP is largely explained by increased VLDL-C.

The drug bexarotene, an RXR agonist, is being used as a strategy to treat patients with different types of cancer. A common dose-limiting side effect of treatment with bexarotene is dyslipidemia. In **Chapter 7**, we evaluated the effects of bexarotene on plasma lipid metabolism in patients with metastatic differentiated thyroid carcinoma (DTC). To this end, ten patients with metastatic DTC were treated with bexarotene. Bexarotene increased plasma TG, primarily in VLDL, and raised plasma total cholesterol (TC). However, while bexarotene increased VLDL-C and LDL-C, it decreased HDL-C and

tended to decrease apoAI concomitant with an increase in endogenous CETP activity. To evaluate the cause of the bexarotene-induced hypertriglyceridemia and the role of CETP in the bexarotene-induced shift in cholesterol distribution, E3L and E3L.CETP mice were treated with bexarotene. Bexarotene increased VLDL-TG in both E3L and E3L.CETP mice, by increasing VLDL-TG production. Bexarotene did not affect the TC levels or distribution in E3L mice, but increased VLDL-C and decreased HDL-C as well as apoAI in E3L.CETP mice, concomitant with increased endogenous CETP activity. This increased CETP activity by bexarotene treatment is likely due to the increase in VLDL-TG, a CETP substrate that drives CETP activity. We concluded that bexarotene causes combined dyslipidemia as reflected by increased TG, VLDL-C and LDL-C and decreased HDL-C, which is the result of an increased VLDL-TG production that causes an increase of the endogenous CETP activity.

Taken together, the studies described in this thesis show that inflammation and CETP are both important factors in lipid metabolism and atherosclerosis. In the first part of this thesis we showed that high dietary cholesterol can induce hepatic inflammation via disturbed cholesterol homeostasis and ER stress, revealing new targets for the treatment of metabolic inflammation. Next, we demonstrated that intervention in both systemic and vascular inflammation can reduce atherosclerosis progression and/or induce regression, highlighting the importance of developing drugs targeting the inflammatory component of atherosclerotic disease. In the second part of this thesis we showed that CETP inhibition per se may be anti-atherogenic, but that combination therapy of the CETP inhibitor torcetrapib with atorvastatin may have obscured its atheroprotective effect. Furthermore, we showed that the VLDL-increasing effect of CETP largely explains its atherogenic effect, at least in APOE*3-Leiden.CETP mice, and that CETP inhibition may negatively affect lesion stability. Our data suggest that CETP inhibition may not be the most optimal strategy to increase HDL-C levels and thereby reduce atherosclerosis. We anticipate that strategies improving HDL functionality, rather than raising the HDL level, are more likely to effectively reduce CVD.