Hyperlipidemia, inflammation and atherosclerosis : roles of apolipoprotein C1 and cholesteryl ester transfer protein

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Summary

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The main cause of cardiovascular disease (CVD) is atherosclerosis. Several genes that affect atherosclerosis development have been identified by the use of genetically modified mice (*i.e.* transgenic and knock-out mouse models). Many of these genes exert their role in atherosclerosis development as a result of effects on lipoprotein metabolism and inflammation. Transgenic mouse models have also been proven to be suitable for evaluating the mechanisms underlying the anti-atherosclerotic action of experimental drugs aimed to reduce atherogenic lipoprotein levels. However, thus far no suitable animal model was present to evaluate the mechanism of action of the anti-atherosclerotic effects of high-density-lipoprotein (HDL)-raising therapeutic strategies. In this thesis, we further explored the role of apolipoprotein CI (apoCI) and cholesteryl ester transfer protein (CETP) in lipoprotein metabolism, inflammation, and atherosclerosis. Furthermore, we developed a mouse model that will be suitable for testing potential HDL raising therapies as a novel strategy to treat CVD.

Data in humans have shown that apoCI levels are associated with hypertriglyceridemia. In line with these findings, mice expressing human apoCI show markedly increased very-low-density-lipoprotein (VLDL)-triglyceride (TG) levels and moderately increased VLDL-cholesterol levels. We recently showed that these effects were independent of the expression of apoE and caused by apoCI-induced lipoprotein lipase (LPL) inhibition. We now show in **chapter 2** that physiological expression of endogenous murine apoCI suffices to markedly increase VLDL-TG levels and moderately increase VLDL-cholesterol levels, independent of apoE expression. Whereas endogenous apoCI expression did not affect the intestinal TG absorption, apoCI increased the hepatic production rate of VLDL particles. In addition, apoCI increased the postprandial TG response to an intragastric olive oil load, suggesting that apoCI interferes with the clearance of VLDL-TG. Furthermore, apoCI decreased the uptake of TG-derived free fatty acids (FFA) from intravenously administered VLDL-like emulsion particles by gonadal and perirenal white adipose tissue (WAT). As LPL is the main enzyme involved in clearance of TG-derived FFA by WAT, and total post-heparin plasma LPL levels were unaffected, these data demonstrate that endogenous apoCI suffices to attenu-

ate the lipolytic activity of LPL. In conclusion, we showed that endogenous plasma apoCI increases VLDL-cholesterol and VLDL-TG in *apoe*$^{-/-}$ mice, resulting from a concurrent stimulation of VLDL-particle production and inhibition of LPL.

Our previous findings that mice overexpressing human apoCI homozygously (*APOC1*$^{+/+}$ mice), show cutaneous abnormalities and resistance to obesity development may, at least partly, be explained by the apoCI-mediated inhibition of LPL, leading to diminished availability of free fatty acids (FFA) for uptake in adipose tissue and the skin. However, FFA levels in the plasma of *APOC1*$^{+/+}$ mice are also elevated and apoCI has been shown to impair the uptake of a FFA analogue into adipose tissue, indicating that apoCI may also directly interfere with FFA uptake. We tested this hypothesis in **chapter 3**. The cutaneous abnormalities of *APOC1*$^{+/+}$ mice were not affected after transplantation to wild-type mice, indicating that locally produced apoCI prevents lipid entry into the skin. Subsequent *in vitro* studies with *apoc1*$^{-/-}$ versus wild-type (*apoc1*$^{+/+}$) macrophages revealed that apoCI reduced the cell association and subsequent esterification of the FA oleic acid. In addition, exogenous human apoCI reduced the association of oleic acid with wild-type macrophages. We thus speculated that apoCI binds FFA extracellularly, thereby preventing cellular uptake of FFA. We showed that elevated plasma levels of FFA as observed in *APOC1*$^{+/+}$ mice were present in VLDL, and that apoCI was indeed able to mediate the binding of oleic acid to otherwise protein-free VLDL-like emulsion particles. This binding depended on the positively charged lysine (*K*) residues in the *KVKEKLK* motif of apoCI, since replacement of these residues with alanines (*A*) residues prevented the binding of apoCI to oleic acid. Since FFAs are negatively charged at neutral pH, the FFA-apoCI binding apparently involves electrostatic interaction. We concluded that apoCI binds to FFA in the circulation, thereby reducing the availability of FFA for uptake by cells. This mechanism can serve as an additional mechanism to explain the resistance to obesity and the cutaneous abnormalities of *APOC1*$^{+/+}$ mice.

Next to expression in the liver, brain, and adipose tissue, apoCI is also expressed in macrophages. *APOC1* mRNA is highly upregulated upon monocyte to macrophage differentiation, and upon treatment of macrophages with an agonist for the lipid sensor the liver X receptor (LXR). These data suggest a role for apoCI in lipid homeostasis in macrophages in addition to its role in

TG and FA metabolism. Since macrophages are crucially involved in atherosclerosis development, we investigated the role of both systemic apoCI expression and local macrophage-specific apoCI expression on atherosclerosis development in **chapter 4**. Systemic endogenous apoCI expression increased the atherosclerotic lesion area in the aortic root of *apoe*$^{-/-}$*apoc1*$^{+/+}$ versus *apoe*$^{-/-}$*apoc1*$^{-/-}$ mice, as related to the apoCI-induced hyperlipidemia as described in chapter 2. However, *in vitro* studies showed that apoCI expression specifically in macrophages protected against foam-cell formation by inducing cholesterol efflux to a similar extent as the established cholesterol acceptors apoAI and apoE, suggesting an anti-atherogenic role of apoCI locally in macrophages. Therefore, we studied the effect of apoCI expression in macrophages on top of apoCI expression in the liver on atherosclerosis by transplantation of bone marrow from *apoe*$^{-/-}$*apoc1*$^{-/-}$ versus *apoe*$^{-/-}$*apoc1*$^{+/+}$ mice into *apoe*$^{-/-}$*apoc1*$^{+/+}$ mice. However, we did not observe any effect of macrophage apoCI expression on atherosclerotic lesion area. We concluded that systemic apoCI expression induces atherosclerosis as a result of increased hyperlipidemia, and that local apoCI production in macrophages of the arterial wall does not contribute to atherosclerosis development *in vivo*, despite enhanced cholesterol efflux from macrophages.

Next to hyperlipidemia, increased inflammation is a risk factor for atherosclerosis. Lipopolysaccharide (LPS) that is released by Gram-negative bacteria upon multiplication or lysis aggravates atherosclerosis in humans and rodents by induction of chronic inflammation via Toll-like receptors. Because apoCI enhances the LPS-induced inflammatory response in macrophages *in vitro* and in mice, we investigated the effect of endogenous apoCI expression on LPS-induced atherosclerosis in **chapter 5**. Hereto, *apoe*$^{-/-}$*apoc1*$^{-/-}$ and *apoe*$^{-/-}$*apoc1*$^{+/+}$ mice were weekly injected intraperitoneally with LPS or vehicle for a period of 10 weeks, and atherosclerosis development was assessed in the aortic root. ApoCI expression increased LPS-induced atherosclerosis, concomitant with an enhancement of the LPS-induced plasma levels of fibrinogen and E-selectin, reflecting a higher inflammatory state, both systemically and at the level of the vessel wall. In contrast, LPS administration did not affect atherosclerotic lesion area in mice that did not express apoCI. We thus concluded that apoCI is crucially involved in the LPS-induced atherosclerosis in *apoe*$^{-/-}$ mice, which relates to an increased inflammatory state. We anticipated that apoCI plasma levels contribute to accelerated atherosclerosis

development in individuals that suffer from chronic infections.

Cholesteryl ester transfer protein (CETP) is a transfer factor that catalyzes the exchange of TG from apoB-containing lipoproteins for CE from HDL, as such being an important determinant for VLDL/LDL and HDL levels in plasma. CETP is expressed in humans yet not in mice. The role of CETP in atherosclerosis is still undergoing debate. Therefore, we studied the effect of CETP on lipoprotein profiles and atherosclerosis in the humanized *APOE*3-Leiden* mouse model in **chapter 6**. These mice show a more human-like lipoprotein profile as compared to wild-type mice, as a consequence of the attenuated clearance of apoB-containing lipoproteins. Expression of human CETP under control of its natural flanking regions increased VLDL-cholesterol in these mice and decreased HDL-cholesterol, resulting in slightly increased total cholesterol. In order to study atherosclerosis, *CETP.APOE*3-Leiden* and *APOE*3-Leiden* mice were fed a cholesterol-rich diet. CETP expression massively increased the size of atherosclerotic lesions in the aortic root, concomitant with more advanced lesions. This can be explained by the CETP-induced increased VLDL and decreased HDL cholesterol levels. Plasma from *CETP.APOE*3-Leiden* mice induced a lower SR-BI-mediated cholesterol efflux from Fu5AH cells as compared to plasma from *APOE*3-Leiden* mice, probably related to the decreased HDL levels. We concluded that CETP expression has a major impact on the cholesterol distribution over lipoproteins and represents a clear pro-atherogenic factor in *APOE*3-Leiden* mice. *APOE*3-Leiden* mice have been shown to exert a human-like response to drugs that affect the level of VLDL/LDL, such as statins and fibrates. We thus anticipated that next to testing drugs that affect VLDL/LDL metabolism, *CETP.APOE*3-Leiden* mice will be suitable for the pre-clinical evaluation of HDL-raising therapies that constitute a novel approach in the treatment of CVD.