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## **The role of ApoCI, LPL and CETP in plasma lipoprotein metabolism - studies in mice**

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**Summary**

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**Samenvatting**

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**H**yperlipidemia is an important factor in determining risk for cardiovascular disease (CVD). Therefore, reducing hyperlipidemia is a strategy to improve CVD risk. A thorough understanding of lipoprotein metabolism is required to optimize lipid-lowering therapies. In the past decades this knowledge has increased tremendously, especially by the development of mice that have been genetically modified (*i.e.* transgenic and knock-out mice) with respect to genes that are involved in lipoprotein metabolism. In this thesis we focussed on further elucidating the roles of apolipoprotein CI (apoCI), lipoprotein lipase (LPL), and cholesteryl ester transfer protein (CETP) in lipid metabolism. To this end we used several transgenic mouse models, some in combination with adenoviral gene transfer.

Previous studies in humans and mice have shown a positive correlation between plasma levels of apoCI and combined hyperlipidemia, with a more pronounced effect on triglycerides (TG) as compared to cholesterol. Despite several postulated mechanisms via which apoCI might induce hypertriglyceridemia, none of them could satisfactorily explain this effect. In **chapter 2**, we investigated which of the steps in very low-density lipoprotein (VLDL) metabolism were affected in human *APOC1* transgenic mice. The influx of TG into the circulation via intestinal uptake or hepatic VLDL production was not different between *APOC1* and control mice, and could thus be excluded as cause of hypertriglyceridemia. In addition, cross-breeding *APOC1* mice with apoE deficient mice showed that the apoCI-induced hyperlipidemia could not merely be explained by a blockade of apoE-recognizing hepatic lipoprotein receptors. However, we found that the plasma half-life of VLDL-mimicking remnant particles was increased in *APOC1* transgenic mice as compared to controls, indicating that apoCI reduces the lipolytic conversion of VLDL. Although the plasma LPL concentration was not affected in *APOC1* transgenic mice, purified apoCI was able to inhibit LPL-mediated lipolysis *in vitro* and to impair the clearance of VLDL-mimicking emulsion particles *in vivo*. In conclusion, we showed that the hypertriglyceridemic effect of apoCI is primarily the consequence of impaired LPL-mediated TG hydrolysis.

Since the VLDL receptor (VLDLr) and apoCIII are potent modifiers of LPL activity, we subsequently studied whether the inhibition of lipolysis by apoCI depends on interaction with the VLDLr or with apoCIII in **chapter 3**. Hereto, a novel adenoviral vector expressing human *APOC1* (Ad*APOC1*) was used in mice deficient for either apoCIII or the VLDLr, which were also deficient for apoE or double deficient for the low-density lipoprotein receptor (LDLr) and the LDLr-related protein (LRP), respectively. In wild-type mice, Ad*APOC1* expression produced the same phenotype as observed in human *APOC1* transgenic mice. In addition, Ad*APOC1* still increased plasma TG in the absence of the VLDLr or apoCIII. Therefore, we concluded that apoCI is a powerful inhibitor of LPL activity, and can act independent of the presence of the VLDLr and apoCIII.

Recently, our laboratory showed that mice deficient for the three major apoE-recognizing receptors (*i.e.* VLDLr, LDLr and LRP), and thus virtually lack receptor-mediated VLDL clearance mechanisms, have elevated plasma VLDL-cholesterol and TG levels. However, since VLDL particles are continuously produced by the liver, their remnants must still be cleared to attain steady state plasma VLDL levels. In **chapter 4** we stu-

died whether LPL is important in the VLDL clearance in absence of these three main apoE-recognizing receptors. On the one hand, administration of AdAPOC1, thereby inhibiting LPL-mediated lipolysis, resulted in increased TG and cholesterol in VLDL, while the association of particle remnants with the liver was completely abolished. On the other hand, an adenovirus expressing LPL reduced both VLDL TG and cholesterol, concomitant with an increase in hepatic particle remnant association. Thus, in absence of these receptors, the remnant clearance still depends on LPL.

It is known that apoE\*2 can lead to hyperlipidemia in humans, which is mimicked by replacing the endogenous apoE gene by *APOE\*2* in mice (so-called *APOE\*2*-knockin mice). The hyperlipidemia in these mice is caused both by the low binding affinity of apoE\*2 for the LDLr and through apoE-mediated inhibition of LPL, thereby blocking the clearance of VLDL. In **chapter 5**, we addressed the question whether increasing the LPL activity in *APOE2*-knockin mice, either directly (*i.e.* by administration of the LPL activator heparin and by an LPL-expressing adenovirus) or indirectly (*i.e.* by injection of an apoAV-expressing adenovirus and by introducing apoCIII deficiency), could normalize plasma lipid levels. We found that the combined hyperlipidemia in these mice was overcome by direct activation of LPL activity and by indirect activation via apoAV, but not by apoCIII deficiency. Thus, changes in apoAV levels have a dominant effect over changes in apoCIII levels in the improvement of apoE2-associated hyperlipidemia.

CETP is a crucial factor for the cross-talk between the metabolism of apoB-containing lipoproteins and HDL, and is expressed in men but not in mice. Since the atherogenicity of CETP is still under debate, we studied in **chapter 6** the effect of human CETP expression on plasma lipoprotein metabolism and atherosclerosis development in *APOE\*3-Leiden* mice. These mice show a human-like lipoprotein profile due to attenuated clearance of VLDL as compared to wild-type mice. In addition to a slight increase in cholesterol levels, CETP expression increased VLDL-cholesterol at the expense of high-density lipoprotein (HDL)-cholesterol. To evaluate the effects of CETP on the development of atherosclerosis, mice were fed a cholesterol-containing diet, leading to elevated VLDL-cholesterol levels both in CETP expressing mice and in controls. The mean lesion area was severely increased in sequential cross-sections in the aortic root of mice that express CETP, concomitant with more advanced lesions. This was accompanied by increased levels of VLDL in plasma of the *CETP.APOE\*3-Leiden* mice as compared to *APOE\*3-Leiden* littermates. Moreover, plasma of *CETP.APOE\*3-Leiden* mice had reduced capacity to induce SR-BI-mediated cholesterol efflux from Fu5AH cells as compared to control plasma. We concluded that CETP expression has a major impact on the cholesterol distribution between lipoproteins and represents a clear pro-atherogenic factor in *APOE\*3-Leiden* mice.

Two groups of drugs that are often prescribed to subjects with dyslipidemia are the fibrates and the statins. Although fibrates primarily lower VLDL-TG and statins mainly reduce LDL-cholesterol, both groups of drugs appear to modestly increase HDL-cholesterol levels in humans. However, this effect on HDL-cholesterol is not observed in mice, a species that naturally lacks CETP expression. In **chapter 7**, we addressed the

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question whether the fenofibrate-induced increase in HDL depends on CETP expression, by using *APOE\*3-Leiden* mice and *CETP.APOE\*3-Leiden* littermates. Whereas administration of fenofibrate to *APOE\*3-Leiden* mice did not affect HDL-cholesterol, it increased HDL-cholesterol when CETP was present in these mice. Fenofibrate did not affect the clearance of HDL-cholesteryl esters from serum, indicating that fenofibrate causes a higher steady-state HDL-cholesterol level without altering the HDL-cholesterol flux through plasma. Since apoAI, adenosine binding cassette transporter A1 (ABCA1), phospholipid transfer protein (PLTP) and scavenger receptor class B type I (SR-BI) are involved in determining plasma HDL-cholesterol levels, we tested the possibility that fenofibrate affected these genes differently in *APOE\*3-Leiden* and *CETP.APOE\*3-Leiden* mice. However, hepatic mRNA expression levels of these genes were similarly affected in both types of mice, thus excluding that possibility. Strikingly, fenofibrate resulted in a dramatic reduction of hepatic CETP mRNA levels, which was accompanied by reductions in plasma CETP mass and activity levels. It has been previously reported that apoAI, ABCA1, PLTP, and possibly CD36- and LIMPII-analogous 1 (CLA1), the human homologue of SR-BI, are involved in determining the HDL-increasing effects of fibrates. Based on our present results we conclude that decreased CETP expression is a crucial additional causal factor for the HDL-raising effect of fenofibrate.

In **chapter 8**, a similar approach was used to test whether the statin-induced increase in HDL also depends on CETP expression. Indeed, whereas administration of atorvastatin to *APOE\*3-Leiden* mice did not affect HDL-cholesterol, it increased HDL-cholesterol in *CETP*-expressing mice. In addition, atorvastatin treatment caused an increase in HDL-cholesteryl ester turnover in both groups of mice by approximately 30%, indicating that atorvastatin does not increase steady-state HDL-cholesterol levels by decreasing HDL-turnover. In addition, atorvastatin did not differently affect hepatic expression of genes involved in determining HDL-cholesterol levels (*i.e.* apoAI, ABCA1, PLTP, and SR-BI) in *CETP.APOE\*3-Leiden* as compared to *APOE\*3-Leiden* mice. However, atorvastatin treatment reduced the hepatic mRNA expression of CETP in *CETP.APOE\*3-Leiden* mice. This was accompanied by reductions in plasma CETP mass and activity levels. Therefore, we concluded that atorvastatin increased HDL-cholesterol in *CETP.APOE\*3-Leiden* mice by reducing the CETP-dependent HDL-clearance.

Taken together, this thesis contributes to a better understanding of the roles of apoCI, LPL, and CETP in lipoprotein metabolism. Our data illustrate that the activity of LPL, and thereby the level of plasma TG, is crucially determined by the relative abundance of apolipoproteins. In addition, we showed that LPL is an important determinant in remnant-particle clearance in the absence of the three main apoE-recognizing receptors. Finally, we demonstrated that CETP presents a pro-atherogenic factor in mice resembling a human lipid distribution over lipoproteins and that atorvastatin and fenofibrate treatment influence HDL-metabolism via inhibition of CETP, which may thus add to their therapeutic benefit. Since there were initial concerns that inhibition of CETP would reduce the flux of cholesteryl esters from the periphery back to the liver, thereby possibly increasing the risk for atherosclerosis, it is of interest that we found that fenofibrate-mediated inhibition of CETP did not hamper the total flux of HDL-cholesteryl esters. This holds promise for therapies based on CETP inhibition.