



Universiteit
Leiden

The Netherlands

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

Hoogt, C.C. van der

Citation

Hoogt, C. C. van der. (2006, November 28). *The role of ApoCI, LPL and CETP in plasma lipoprotein metabolism - studies in mice*. Retrieved from <https://hdl.handle.net/1887/5414>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/5414>

Note: To cite this publication please use the final published version (if applicable).

1 **General Introduction**

Cardiovascular disease (CVD) is the first cause of death in the Western world and its prevalence is increasing in Eastern Europe and developing countries.¹ Several factors such as diabetes, hypertension, cigarette smoking, obesity, and lipid abnormalities are associated with an increased risk of atherosclerosis development. The research described in this thesis was performed to gain more insight into lipid metabolism. Dietary triglycerides (TG) are absorbed by the intestine to end up in metabolically active tissues (*e.g.* heart or skeletal muscle) to serve as energy source or in adipose tissue for storage. Cholesterol is derived both from the diet and from *de novo* synthesis in the liver and is used for cellular membrane synthesis (most tissues) and as precursor for steroid hormones (produced by endocrine glands such as the adrenal cortex and the gonads) and bile acids (liver). For transport from the site of absorption or synthesis, via the blood circulation towards their destination, cholesterol and TG are packaged into lipoproteins.^{2,3}

1. Lipoproteins and Lipid Metabolism

1.1. Lipoprotein Classes

Lipoproteins are complex, spherical particles that consist of a hydrophobic inner core of neutral lipids, which mainly consist of TG and cholesteryl esters (CE), and a polar outer shell, which is formed by proteins (*e.g.* apolipoproteins), cholesterol, and phospholipids (PL) (Fig. 1).² Since lipoproteins constitute a heterogeneous population of particles, they are traditionally classified and separated according to their densities. The five main categories that are distinguished in human plasma are chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (Table 1). These lipoprotein classes have individual functions in lipid metabolism as will be discussed in the next paragraphs and is schematically represented in figure 2.

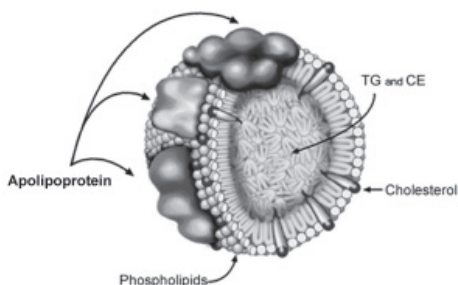


Figure 1. Schematic illustration of a lipoprotein particle. Reproduced with permission from <http://www.peprotech.com>. CE, cholesteryl esters; TG, triglycerides.

1.1.1. Chylomicron Metabolism

Dietary lipids are absorbed in the intestinal lumen, and CE are packaged with TG into large, lipid-rich chylomicron particles, via microsomal triglyceride transfer protein (MTP)-mediated lipidation of apolipoprotein (apo)B-48.⁴ These particles, containing primarily apoB-48, apoAI, apoAII and apoAIV, are secreted into the lymph and subsequently enter the blood circulation. Here, the chylomicrons undergo several modifica-

Table 1. Density, size, and composition of human plasma lipoproteins

	Chylomicrons	VLDL	IDL	LDL	HDL
Density (g/ml)	<0.95	0.95-1.006	1.006-1.019	1.019-1.063	1.063-1.210
Diameter (nm)	75-1200	30-80	25-35	18-25	5-12
Composition (%dry weight)					
proteins	1-2	8	19	22	47
TG	86	55	23	6	4
TC	5	19	38	50	19
PL	7	18	20	22	30
Apolipoproteins					
apoA	AI, AII, AV ¹²	AV	-	-	AI, AII, AIV, AV
apoB	B-48	B-100	B-100	B-100	-
apoC	CI, CII, CIII	CI, CII, CIII	CI, CII, CIII	-	CI, CII, CIII
apoE	E	E	E	-	E
Source	intestine	Liver	VLDL	IDL/VLDL	Liver/Intestine
Main function	Transport of exogenous TG and TC	Transport of endogenous TG	Transport of endogenous TG	Transport of endogenous TC	Reverse cholesterol transport

Apo, apolipoproteins; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; TC, total cholesterol; PL, phospholipids. In this table only the main apolipoproteins are given. Modified from Wasan and Cassidy.²¹⁰

tions. Within the capillary beds of skeletal muscle, heart, and adipose tissue, their TG are hydrolyzed by the lipolytic action of lipoprotein lipase (LPL), thereby generating free fatty acids (FFA) that are used as energy source (muscle) or for storage (adipose tissue), respectively.

Upon entering the circulation, apoAIV rapidly dissociates from the chylomicrons. A small fraction of apoAIV becomes associated with HDL, while more than 90% of the apolipoprotein exists free in the circulation.^{5,6} Similarly, apoAI and apoAII are trans-

ferred to HDL, while the chylomicrons acquire apoCs (*i.e.* apoCI, apoCII, apoCIII) and apoE from circulating lipoproteins. Sequential delipidation steps, which causes depletion of the particles from TG and PL and enrichment in apolipoprotein such as apoE, result in the formation of chylomicron remnants.^{2,3} ApoE is a crucial factor for facilitating the subsequent rapid clearance of the remnants from the circulation by the liver via remnant receptors (*e.g.* low-density lipoprotein receptor [LDLr] and the LDLr-related protein [LRP]).^{7,8} The receptor-mediated internalisation of remnants may be preceded by the “secretion-recapture role” of apoE as was suggested by Ji *et al.*⁹ This process starts with the secretion of apoE by hepatocytes and its accumulation in the space of Disse. There, apoE binds to heparan sulfate proteoglycans (HSPG) that are abundantly present on the surface of hepatocytes. Remnants may initially be sequestered in the space of Disse by apoE-mediated binding to HSPG and subsequently be internalised via lipoprotein receptor-mediated clearance routes (*i.e.* LDLr and LRP). In addition, recent results suggest that, at least in mice, scavenger receptor class B type I (SR-BI) is involved in the hepatic uptake of chylomicron remnants, probably by functioning as an initial recognition site.¹⁰

1.1.2. VLDL Metabolism

The liver, in its turn, generates VLDL particles, consisting of cholesterol (derived from internalized remnants or *de novo* synthesis) and TG (derived from remnant uptake, plasma FFA uptake, or lipogenesis) via MTP-mediated lipitation of apoB, which are subsequently secreted into the circulation.^{2,3} Whereas VLDL in humans is formed by lipitation of apoB-100, mouse VLDL results from lipitation of both apoB-100 and apoB-48.¹¹ Nascent VLDL mainly carries apoB and small amounts of apoCs, apoE and the recently identified apoAV.¹² Similarly to chylomicrons, TG in VLDL are hydrolyzed by LPL, thereby generating FFA, while VLDL is processed into VLDL remnants (*i.e.* IDL). These particles become enriched in apoCs and apoE and will be partly cleared from the plasma via the hepatic remnant receptors (*i.e.* LDLr and LRP). The remainder are processed further, which leads to loss of TG, PL, apoCs and apoE from the particles. This results in the formation of the relatively CE-rich LDL particles, characterized by one apoB-100 molecule as the sole protein constituent.^{2,3}

ApoB-100 is a ligand for the LDLr. About 70% of the LDLr activity is concentrated in the liver, leading to the hepatic uptake of LDL. Part of the remaining LDL is taken up in extrahepatic tissues for maintenance of membrane integrity and for the production of steroids in steroidogenic tissues (*e.g.* adrenal cortex and gonads).¹³ Besides being crucial for cholesterol delivery to peripheral tissues, LDL also has adverse atherogenic properties. LDL can infiltrate the vascular wall, where subsequent modification of LDL in the vascular wall via *e.g.* oxidation causes activation of the endothelium and an inflammatory response. This process leads to recruitment of monocytes from the circulation, which enter the vascular wall, and can differentiate into macrophages. These macrophages scavenge modified LDL via scavenger receptors that include scavenger receptor A (SRA) and CD36, thereby accumulating lipids, mainly cholesterol, and become foam cells.¹⁴ Foam cell formation is considered to be the initial stage in atherosclerosis development.¹⁵

1.1.3. HDL Metabolism - Reverse Cholesterol Transport

Steroidogenic tissues (*i.e.* adrenal cortex and gonads) and skin cells are able to degrade low levels of cholesterol, however other non-hepatic peripheral tissues do not have this ability.¹⁶ To maintain cholesterol homeostasis, excess cholesterol from extrahepatic tissues (*e.g.* lipid-laden macrophages within the arterial wall) can be transported back to the liver to be secreted into the bile as neutral sterols and bile acids. This process is termed reverse cholesterol transport (RCT) and crucially involves apoAI and HDL.¹⁷

Free cholesterol and phospholipids from the macrophage are transported to lipid-poor apoAI via the ATP-binding cassette (ABC) A1 transporter, thereby forming nascent discoidal HDL (nHDL).^{18,19} Subsequently, the enzyme lecithin:cholesterol acyl transferase (LCAT) esterifies the free cholesterol, to generate cholesteryl esters and transform nascent HDL into larger, mature HDL particles (mHDL).²⁰ ABCG1,^{21,22} ABCG4²² and SR-BI^{23,24} then further facilitate the efflux of cholesterol from cells to the mature HDL.

HDL-CE may then be transported to the liver via two pathways. First, cholesteryl ester transfer protein (CETP), which is expressed in species such as humans, rabbits, and hamsters, but not in mice and rats, transfers CE from HDL towards apoB-containing lipoproteins (*e.g.* VLDL, LDL and IDL) that are subsequently cleared from the circulation to the liver. Second, HDL-CE can be selectively taken up via ABC transporters²⁵ or via SR-BI.^{26,27} CETP may also be involved in the direct uptake of HDL-CE into the liver, since administration of the CETP-inhibitor torcetrapib to mice that were treated with an adenovirus expressing CETP did not completely normalize HDL-CE levels. Therefore, cell-associated CETP might be directly involved in the hepatic uptake of HDL-CE.²⁸

Cholesterol that has returned to the liver can be re-used for lipoprotein assembly or can be excreted. The major pathway for elimination of cholesterol, either as neutral sterol or as bile acid, is mediated by the ABC transporters G4, G5 and G8 and results in secretion into the bile.²⁹

HDL particles are anti-atherogenic, partly because of their role in RCT but also due to antioxidative, anti-inflammatory, antithrombotic, and antiapoptotic properties.³⁰⁻³²

1.2. Lipoprotein Remodelling Proteins

The proteins involved in the remodelling of lipoproteins during their residence in the circulation, can be categorized in lipolytic enzymes (*e.g.* endothelial lipase [EL], hepatic lipase [HL] and LPL) and neutral lipid transfer proteins (*e.g.* phospholipid transfer protein [PLTP] and CETP). Two of these proteins, LPL and CETP, will be described in more detail. While LPL is crucially involved in triglyceride metabolism and appears to be a causal factor in obesity, CETP represents a main link between (V)LDL and HDL metabolism by facilitating neutral lipid exchange between these lipoproteins.

1.2.1. Lipoprotein Lipase

LPL is a glycoprotein belonging to the gene family of lipases, which includes HL, EL and pancreatic lipase.³³ It is mainly synthesized in parenchymal cells of tissues that

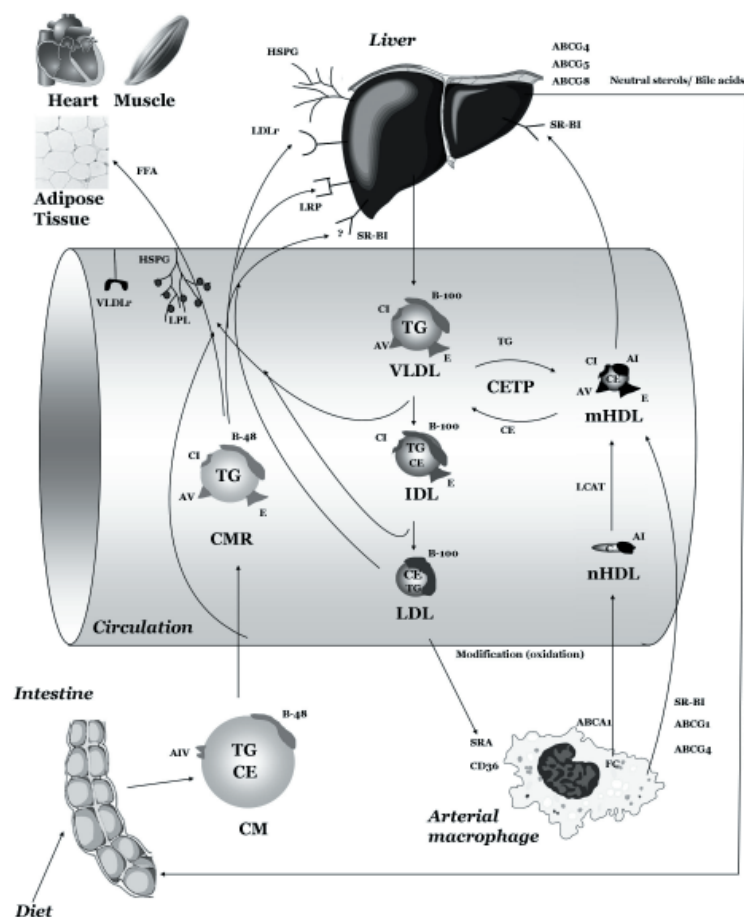


Figure 2. Schematic, simplified representation of human lipoprotein metabolism. See text for explanation. ABC, ATP-binding cassette transporter; AI, AIV, AV, B-48, B-100, CI, E, apolipoproteins AI, AIV, AV, B-48, B-100, CI, E; CETP, cholesteryl ester transfer protein; CM, chylomicron; CMR, chylomicron remnant; FFA, free fatty acids; HSPG, heparan sulphate proteoglycans; IDL, intermediate-density lipoprotein; LCAT, lecithin:cholesterol acyl transferase; LDLr, low-density lipoprotein receptor; LPL, lipoprotein lipase; LRP, LDLr-related protein; mHDL, mature HDL; nHDL, nascent HDL; SR-BI, scavenger receptor class B type I; VLDLr, very low-density lipoprotein receptor.

utilize FFA for energy or storage purposes (*e.g.* cardiac and skeletal muscles, and white adipose tissue). The VLDLr functions as an intracellular chaperone protein that allows LPL to be secreted from the cells to the capillary endothelial surface,^{34,35} where it is anchored by interactions with HSPG.³⁶ Dimeric LPL is activated by its co-factor apoCII, that is present on its substrates (*e.g.* VLDL, chylomicrons), to facilitate lipolysis (*i.e.* hydrolysis of TG within the lipoprotein core to generate glycerol and FFA). The FFA that are generated are subsequently taken up by underlying tissues either via passive diffusional uptake or via a protein-facilitated component (*e.g.* VLDLr, CD36 or FA translocase, FA transport protein).³⁷

The LPL-mediated lipolysis is a prerequisite for the chylomicron and VLDL particles to be removed from the circulation. The LPL-mediated hydrolysis of TG is influenced by several apolipoproteins. Both apoCIII³⁸ and high amounts of apoE³⁹⁻⁴¹ inhibit the lipolysis, whereas recent data indicate that apoAV increases LPL activity either directly or indirectly via still unresolved mechanisms.^{42,43} Lipolytically active dimeric LPL can

dissociate into monomeric LPL, which results in reduced affinity for HSPG and allows LPL to dissociate from the vascular wall and to travel through the circulation bound to lipoproteins. It is proposed that, in addition to apoE, LPL can mediate the lipoprotein binding to HSPG, after which the complex is transferred to lipoprotein receptors. As such, LPL is involved in remnant clearance via both its lipolytic function in the hydrolysis of triglycerides and its non-enzymatic bridging function.⁴⁴⁻⁴⁶

Heterozygous LPL mutations that are associated with a reduction or loss of LPL activity (*i.e.* Asp9Asn, Gly188Glu) are associated with an increased risk for development of combined hyperlipidemia and atherosclerosis.⁴⁷ Also patients with defects in the structure or production of apoCII display hypertriglyceridemia and are indistinguishable from patients with LPL deficiency.⁴⁸ Mice that are deficient for LPL have extremely elevated triglyceride levels and die within the first day after birth, although the exact cause of death is still to be elucidated.⁴⁹ Deficiency for FFA uptake by CD36^{50,51} and overexpression of LPL-inhibitor apoCIII⁵² also lead to hypertriglyceridemia in mice. Interestingly, human *APOC1* transgenic mice also develop hypertriglyceridemia,⁵³ but the effect of apoCI on LPL activity is unresolved as yet.

LPL activity appears a crucial determinant for the development of obesity. Mice in which LPL activity is reduced by deficiency for LPL, CD36, or the LPL-chaperone VLDLr, are protected against diet-induced obesity.^{49,51,54} Similarly, *APOC1* transgenic mice are resistant to the development of diet-induced obesity.⁵⁵ On the other hand, mice with increased LPL activity as caused by apoCIII-deficiency are more prone to develop diet-induced obesity.⁵⁶

1.2.2. Cholesteryl Ester Transfer Protein

CETP is a glycoprotein that is mainly expressed by liver, spleen, and adipose tissue.⁵⁷ Upon secretion into plasma, CETP becomes associated with all lipoprotein classes, but predominantly with HDL.⁵⁸ CETP mediates the transfer of neutral lipids (*i.e.* TG and CE) between lipoproteins. This results in the net flux of CE from HDL towards apoB-containing lipoproteins (*e.g.* VLDL and LDL) in exchange for TG.⁵⁹⁻⁶¹ In mice, which naturally lack CETP, the clearance of HDL-CE occurs almost exclusively via selective delivery to the liver via SR-BI.^{62,63} In rabbits, however, the clearance of HDL-CE from plasma is only partially mediated by the direct uptake of HDL-CE, as 25-70% is cleared after CETP-dependent transfer to apoB-containing lipoproteins.^{64,65} In humans, it has recently been shown that the CE output from plasma is even almost solely facilitated via apoB-containing lipoproteins, whereas selective and holo-particle uptake of HDL-CE by the liver could not be detected.⁶⁶ This suggests that CETP-mediated CE transfer might constitute a major pathway in humans, with only a small contribution of selective HDL-CE uptake,⁶⁶ which is in sheer contrast with the predominant involvement of SR-BI in selective uptake of HDL-CE in mice.

As a consequence of its role in the transfer of lipids, CETP is involved in the remodelling of lipoproteins just like lipases (*e.g.* LPL, HL, EL) and PLTP. The concerted actions of CETP, PLTP, and lipases facilitate the formation of small HDL particles⁶⁷⁻⁶⁹ that are involved in the cholesterol efflux from extrahepatic tissues, thereby increasing the RCT pathway. Subjects deficient for CETP have an increased proportion

of large HDL-particles that are enriched in CE and apoE.⁷⁰⁻⁷³ Thus, CETP may have anti-atherogenic properties by contributing to the remodelling of HDL, thereby facilitating the delivery of HDL-CE to the liver via apoB-containing lipoproteins.

However, by reducing anti-atherogenic HDL-cholesterol levels and increasing the cholesterol content in pro-atherogenic apoB-containing lipoproteins, CETP activity might be considered atherogenic. Indeed, genetic association studies in humans showed an association of the C629A promoter variant of CETP with higher CETP levels, lower HDL-cholesterol levels, and an increased progression of CVD.⁷⁴ Likewise, increased CETP activity was associated with increased risk for CVD in subjects with elevated triglycerides.⁷⁵ However, despite the fact that CETP deficiency is associated with increased levels of HDL cholesterol,^{71-73,76,77} increased CVD risk was found in studies of subjects with partial⁷⁸ or complete^{79,80} loss of CETP. Since mice naturally lack the expression of CETP, transgenic mouse models that express human CETP have been used to gain more insight into the atherogenicity of CETP. Although studies in apoCIII-transgenic mice⁸¹ and LCAT-transgenic mice⁸² showed that human CETP expression reduced atherosclerosis, expression of CETP in mice that are hyperlipidemic because of attenuated hepatic uptake of apoE-containing lipoprotein remnants (*i.e.* apoE-deficient and LDLr-deficient mice) increased atherosclerosis.⁸³

Due to the pro- and anti-atherogenic properties of CETP and conflicting results from human and animal studies, the net effect of CETP on atherosclerosis development has been a subject of debate which has been extensively reviewed,^{61,84,85} but is still not clear.

1.3. Apolipoproteins

Apolipoproteins are generally composed of a series of class II amphipathic α -helices and play an important role in lipid metabolism by exerting various functions. They stabilize the lipoprotein particles (*e.g.* apoA, apoB, apoC), they serve as co-factors and modulators of enzymatic reactions (*e.g.* apoCs, apoAV), and they direct lipids to target organs by specific receptor interaction (*e.g.* apoA, apoB, apoE). The most abundant apolipoproteins, apoB-100⁸⁶⁻⁸⁹ and apoAI,^{88,90,91} have been extensively studied. The role of these apolipoproteins in lipoprotein metabolism are quite well-defined: apoB-100 regulates LDL metabolism by mediating the endocytosis of LDL by the LDL receptor, and apoAI is involved in HDL metabolism by mediating both the influx of cholesterol from peripheral cells into HDL and the efflux of cholesterol from HDL to the liver. However, the less abundant apolipoproteins apoCI, apoCIII, apoE, and apoAV seem to have overlapping functions in (V)LDL and HDL metabolism, as will be outlined below.

1.3.1. Apolipoprotein CI

The gene encoding for the smallest of the known apolipoproteins, *APOC1*, is part of the *APOE/APOC1/APOC2/APOC4* gene-cluster,⁹² located on chromosome 19 in humans.⁹³⁻⁹⁵ The gene is 4.7 kb in size and consists of 4 exons. *APOC1* is primarily expressed by the liver, but is also found at low levels in lung, skin, spleen, adipose tissue, and brain.⁹⁶ ApoCI is synthesized with a 26-residue signal peptide, which is cleaved co-translationally in the rough endoplasmic reticulum, generating a mature protein of 57

amino acids.⁹⁷ ApoCI consists of only two helices separated by a flexible hinge. The protein is subsequently secreted into the circulation where it is present at a high concentration of approximately 10 mg/dl,⁹⁸ associated with chylomicrons, VLDL and HDL.⁹⁹

Up to now, no apoCI polymorphisms have been identified in humans that result in functional apoCI variants. However, an *HpaI* polymorphism in the promoter region has been described, which results in the increased expression of the *APOC1* gene (+57%),¹⁰⁰ and is associated with elevated TG.¹⁰¹ To study the function of apoCI in lipid metabolism, mice were generated either lacking endogenous apoCI^{102,103} or overexpressing the human *APOC1* gene.^{98,104} Whereas apoCI deficient mice did not have an obvious plasma lipid phenotype,¹⁰² *APOC1* overexpression markedly increased the levels of plasma TC and TG.^{104,105} The observation in humans that apoCI levels are positively associated with plasma TG levels may thus be explained by a causal effect of apoCI. The apoCI-induced hyperlipidemia in *APOC1* mice has initially been explained by interference of apoCI with the apoE-dependent hepatic uptake of remnants by the LDLr¹⁰⁶ and LRP,¹⁰⁷ and by the inhibition of binding of apoE-containing lipoproteins to the VLDLr as present on peripheral cells,¹⁰⁸ thereby impeding remnant clearance. However, *APOC1* mice show predominantly hypertriglyceridemia rather than hypercholesterolemia, which is not consistent with either of these proposed effects of apoCI. In addition, *in vitro* studies showed that apoCI is involved in the partial activation of LCAT,^{109,110} the inhibition of LPL,^{111,112} and the inhibition of HL¹¹³. Most likely, apoCI interferes with the lipolytic function of LPL, thereby inducing hypertriglyceridemia and protecting against diet-induced obesity, but such an effect has not been shown yet *in vivo*.

Furthermore, human apoCI¹¹⁴ and the N-terminal fragment of baboon apoCI¹¹⁵ have been reported to inhibit CETP *in vitro*. Indeed, deficiency of apoCI in *CETP* transgenic mice resulted in increased CETP activity as compared to control mice.¹¹⁶ Similarly, human *APOA1* overexpression in *CETP* transgenic rats leads to an increase in CETP activity possibly related to a reduction of the amount of apoCI associated with HDL.¹¹⁷

1.3.2. Apolipoprotein CIII

The *APOC3* gene is located in the *APOA1/APOC3/APOA4/APOA5* gene-cluster¹¹⁸ located on chromosome 11 in humans and is primarily expressed in liver and intestine.^{93,95} After removal of the 20-amino acid signal peptide, the mature apoCIII protein (79 amino acids; 8.8 kDa) is secreted into the circulation where it is associated with chylomicrons, VLDL and HDL, and has a concentration of approximately 12 mg/dl.¹¹⁹

ApoCIII plasma levels are positively correlated with plasma TG concentration.¹²⁰⁻¹²² Indeed, human *APOC3* transgenic mice display severely increased TG levels, whereas a reduction was observed in *apoc3* deficient mice. Although a direct function on receptors has been postulated,¹²³ the hypertriglyceridemia is most likely explained by the strong LPL inhibitory action of apoCIII that was demonstrated both *in vitro*^{38,124,125} and *in vivo*.^{93,95,126-128}

1.3.3. Apolipoprotein AV

In 2001, a novel apolipoprotein has been discovered independently in two research groups.^{118,129} Comparative sequence analysis of the mammalian *APOA1/APOC3/APOC4*

gene cluster between humans and mice¹³⁰ identified a highly homologous sequence encoding an additional apolipoprotein, which was named apoAV.¹¹⁸ At the same time, apoAV was discovered as a protein that is highly upregulated in rats after a 70% hepatectomy.¹²⁹ ApoAV is synthesized exclusively in the liver. After cleavage of its signal peptide, the 39 kDa mature protein (343 amino acids) is secreted into the circulation where it is present at low concentrations (approx. 125-180 ng/ml),^{12,131} mainly bound to chylomicrons, VLDL and HDL.^{12,132,133}

Apoa5 deficient mice displayed 4-fold increased plasma TG levels, whereas overexpression of human *APOA5* in mice reduced TG by 65%.¹³² In addition, adenoviral expression of murine *apoa5* dose-dependently decreased plasma TG up to 70%.^{43,134} *In vitro* studies suggested that the hepatic VLDL production may be decreased,¹³⁵ which was confirmed *in vivo*.⁴³ Strong evidence was found that apoAV facilitates the clearance of TG from the plasma^{42,43,136} by increasing the LPL-mediated TG hydrolysis,^{42,43,136,137} although it is not sure whether apoAV acts via direct activation of LPL or via an indirect pathway. Several mechanisms by which apoAV might enhance lipolysis have been postulated.^{43,136} 1) Since apoAV was shown to increase the binding of lipoprotein particles to HSPG,^{136,138} apoAV may bring the particles in close proximity of LPL thereby enhancing lipolysis. 2) Since apoAV is very hydrophobic,¹³⁹ it penetrates more deeply into the lipoprotein particle than other lipoproteins, thereby potentially facilitating the access of LPL to TG, which are present in the core of the lipoprotein. Indeed, apoAV was shown to increase the LPL-mediated lipolysis in the absence of HSPG *in vitro*,^{42,43} although it should be noted that these effects were only obtained at high apoAV concentrations. 3) ApoAV might interact directly with LPL, thereby enhancing enzymatic activity, for example by stabilizing the LPL dimer. This is supported by the findings that both *apoa5* deficient mice¹⁴⁰ and humans that lack apoAV due to a mutation in apoAV that generates a truncated protein devoid of key functional domains¹⁴¹ have reduced levels of post-heparin LPL levels. So far, no experiments have been performed to address this possibility.

Apart from its role in TG metabolism, apoAV may also play a role in HDL metabolism,¹⁴² because *apoa5* deficient mice displayed increased HDL-cholesterol,¹⁴⁰ whereas adenoviral *APOA5* overexpression in mice dose-dependently reduced HDL-cholesterol levels.⁴³ Further studies will be needed to resolve the mechanism(s) underlying the effect on HDL.

1.3.4. Apolipoprotein E

Just like apoCI, human *APOE* is part of the *APOE/APOC1/APOC2/APOC4* gene-cluster,⁹² located on chromosome 19,⁹⁴ and is mainly expressed in liver, macrophages and brain.⁷ After co-translational cleavage of an 18-amino acid signal peptide, the 299-amino acid mature protein circulates at plasma levels of approximately 2.5-5 mg/dl as a constituent of chylomicrons, VLDL, IDL and HDL.¹⁴³

High amounts of apoE have been shown to inhibit LPL-mediated lipolysis, thus resulting in increased plasma levels of triglycerides.³⁹⁻⁴¹ However, physiological expression of apoE is crucial for mediating the binding and uptake of remnant particles via the LDLr, LRP and the VLDLr,^{7,144,145} thereby playing an important role in lipoprotein

metabolism. In addition, apoE has been suggested to be involved in the secretion-recapture process as was discussed in 1.1.1.

The human *APOE* gene is polymorphic, with three common alleles (*E**2, *E**3 and *E**4) of which *E**3 is the most frequent allele (70-85%).^{7,143,146} These isoforms differ with respect to the association with lipoproteins,^{147,148} binding affinity for the LDLr⁷ and interaction with HSPG.¹⁴⁹

ApoE*2 displays only about 1% of the binding affinity of apoE*3 to the LDLr.¹⁵⁰ As a consequence, *APOE**2 knockin mice display elevated levels of plasma TG and cholesterol.¹⁵¹ Simultaneously, apoE*2 accumulates in plasma, resulting in an increase in the apoE-mediated inhibition of LPL-mediated lipolysis,¹⁵² via reducing the interaction of particles with HSPG-bound LPL.¹⁵³ Whereas in humans most of the homozygous *APOE**2 carriers are normolipidemic, only about 10% develops type III hyperlipidemia. On the other hand, most of the subjects with type III hyperlipidemia are *APOE**2 homozygotes. This indicates that certain additional factors are needed to develop the hyperlipidemia. The apoAV S19W polymorphism may constitute one of these factors. In this polymorphism, apoAV has a substitution at residue 19 in its predicted signal sequence, which was by *in vitro* experiments suggested to be required for the translocation across the endoplasmic reticulum membrane and the subsequent secretion into the circulation.¹⁵⁴ In initial studies, the S19W rare allele has been associated with higher plasma TG levels.¹⁵⁵ Strikingly, in a pilot study 6 out of 7 hypertriglyceridemic *APOE**2/*E**2 carriers the S19W polymorphism was found,¹⁵⁶ which was later confirmed in a larger population, albeit that the effects were less dramatic (*i.e.* 53% in the *APOE**2/*E**2 carriers vs. 20% in the controls).¹⁵⁷ These findings suggest that apoAV may be used to reverse type III hyperlipidemia in *APOE**2/*E**2 subjects, which will require further investigation.

Apart from the three common isoforms of apoE, several other rare variants are known, which are often associated with lipid disorders. For example, the *APOE**3-*Leiden* mutation has a 7-amino acid tandem repeat outside the binding domain.^{158,159} However, the conformation of the receptor-binding domain is dramatically changed by the large insertion, leading to a binding defect of the protein to the LDLr (20-40% of apoE*3)¹⁶⁰ and HSPG.^{161,162} *APOE**3-*Leiden* has been implicated in Familial Dysbetalipoproteinemia (FD), which is characterized by accumulation of chylomicron and VLDL remnants.¹⁶³

To study the direct effect of the *APOE**3-*Leiden* mutation on lipoprotein metabolism, mice have been generated that express the *APOE**3-*Leiden* gene as well as the adjacent *APOC1* gene.¹⁶⁴ It appeared that the mice developed a moderate dyslipidemia, as explained by a reduced VLDL turnover. Because VLDL turnover was reduced, leading to the accumulation of apoB-containing lipoproteins, the lipoprotein profile of *APOE**3-*Leiden* mice appeared to resemble that of humans.^{164,165} In addition, plasma cholesterol in these mice can be titrated by adjustment of dietary cholesterol intake and there is a clear relationship between plasma cholesterol and atherosclerosis severity.¹⁶⁶ *APOE**3-*Leiden* mice respond to lipid lowering therapies such as statins and fibrates.¹⁶⁷ Despite these properties, the major difference in lipoprotein metabolism between mice and humans is the absence of CETP in mice. Since CETP is important in the cross-talk

between HDL and (V)LDL metabolism, expression of CETP in *APOE*3-Leiden* mice might enable us to study HDL metabolism in a model that resembles the human situation even more.

2. Lipid Modulating Therapies

To improve atherogenic lipoprotein profiles, thereby aiming to reduce cardiovascular risk, several classes of lipid-modifying drugs are available. These include bile acid-binding resins (*e.g.* cholestyramine, colestipol, colesevalam), which bind bile acids in the gastrointestinal tract to prevent re-uptake, cholesterol-absorption inhibitors (*e.g.* ezetimibe), fibrates (*e.g.* fenofibrate, clofibrate, gemfibrozil, bezafibrate) and 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (*e.g.* atorvastatin, rosuvastatin, simvastatin). The most widely prescribed drugs are the statins and fibrates, which will be discussed in more detail in the following paragraphs.

2.1. Statins

Statins are inhibitors of HMG-CoA reductase, the rate-determining enzyme in hepatic cholesterol biosynthesis, which converts HMG-CoA into the cholesterol-precursor mevalonate.¹⁶⁸ Initially, semisynthetic fungal derivatives were used as HMG-CoA reductase inhibitors (*e.g.* simvastatin, pravastatin). Later, synthetic statins were designed (*e.g.* rosuvastatin, atorvastatin).¹⁶⁹ The members in this class of drugs share a similar structure resembling the HMG-moiety that plays an important role in the inhibition of the enzyme.¹⁷⁰ In the liver, the lowering of cholesterol biosynthesis results in depletion of hepatic intracellular cholesterol. To compensate for the depletion in intracellular cholesterol, LDLr receptor expression levels are increased to facilitate the uptake of plasma LDL and its precursors VLDL and IDL.¹⁷¹ At the same time, hepatic apoB-100 production is decreased, thereby reducing the synthesis and secretion of VLDL.¹⁷²⁻¹⁷⁵ These statin-mediated actions result in a reduction in plasma LDL-cholesterol,¹⁷⁶⁻¹⁷⁸ which in hypertriglyceridemic subjects often are accompanied with reductions in TG levels.^{175,179}

Indeed, a meta-analysis of 25 studies enrolling nearly 70,000 subjects with CVD indicated that statins reduce LDL-cholesterol levels up to 40%, which was associated with a reduction of CVD mortality by 23%.¹⁸⁰ Apart from the effects on LDL-cholesterol, statins also decreased plasma TG and increased plasma HDL-cholesterol by about 5-15%.¹⁸¹⁻¹⁸³ Although clinical studies indicate that a reduction in CETP activity may be involved in the statin-induced increase in HDL,^{184,185} this has not been firmly established as a causal factor yet. In addition, statins have been reported to exert a wide range of pleiotropic effects that may contribute to their beneficial actions. These include vasodilation, plaque stabilization, and antithrombotic, antioxidant, anti-proliferative and anti-inflammatory actions.^{169,186,187}

2.2. Fibrates

By mimicking the structure and function of FFA, fibrates are peroxisome proliferator-activated receptor α (PPAR α) agonists.¹⁸⁸⁻¹⁹⁰ PPAR α is mainly expressed in the

liver,¹⁹¹ and upon activation it translocates from the cytoplasm to the nucleus, where it heterodimerizes with retinoid X receptor (RXR). This complex subsequently binds to specific peroxisome proliferator response elements (PPREs) in a range of target genes, thereby either activating or repressing their transcription.¹⁹⁰

The hypotriglyceridemic effect of fibrates is based on increased lipolysis of TG-rich lipoproteins in plasma via a PPAR α -mediated increase in hepatic LPL expression¹⁹² and decrease in hepatic apoCIII expression.^{193,194} Indeed, fibrate administration is associated with increased plasma LPL activity levels¹⁹⁵ and a reduction in apoCIII synthesis¹⁹³ in humans. In addition, fibrates reduce VLDL-TG production through enhanced FFA catabolism (via an increase in genes of the β -oxidation pathway) and reduced FFA production (via downregulation of acetyl CoA carboxylase).¹⁹⁶⁻¹⁹⁸

Although the role of plasma TG as CVD risk factor has long been controversial, strong evidence has been provided that elevated TG levels indeed are correlated with increased risk.¹⁹⁹ This is illustrated by a meta-analysis of 17 population-based, prospective studies, enrolling 46,413 men and 10,864 women, where TG were found to be an independent risk factor for CVD.²⁰⁰ In subsequent years this has been confirmed in the Prospective Cardiovascular Münster (PRO-CAM) study²⁰¹ and the Copenhagen Male study,²⁰² both prospective studies that only included men without evidence of myocardial infarction at entry.

Fibrates are widely used to reduce hypertriglyceridemia, thereby reaching TG reductions, indicating lower levels of VLDL that exceed 50% in subjects with hypertriglyceridemia. In hypercholesterolemic patients TG reductions are generally less than 30%.¹⁹⁸ Although it appears that the extent of TG-lowering by fibrates is dependent on the lipid phenotype and the fibrate used, a meta-analysis with data from 53 trials (16,802 subjects), including subjects with hypercholesterolemia, hypertriglyceridemia, type II diabetes mellitus and combined hyperlipidemia, clearly demonstrated a 36% reduction in TG.²⁰³ In addition to TG-reduction, fibrates exert a spectrum of lipid modulating actions, involving reduction of cholesterol in VLDL, VLDL remnants, IDL and LDL, and elevation of HDL-cholesterol by approximately 10%.^{198,203} Except for their lipid-modulating effects, fibrates also exert pleiotropic anti-inflammatory effects by downregulating the expression of genes encoding inflammatory cytokines and acute phase response proteins, as was recently reviewed.²⁰⁴ Altogether, the previously mentioned meta-analysis showed that fibrate administration is associated with a CVD risk reduction of 25%.²⁰³

The mechanism underlying the fibrate-induced increase in HDL-cholesterol has been intensively studied. In contrast to humans, fibrates do not affect or even decrease HDL-cholesterol levels in mice.²⁰⁵⁻²⁰⁸ This effect may be attributed to the fact that, in contrast to the human *APOA1* promoter, which contains a functional positive PPRE leading to increase *APOA1* transcription, the murine *apoa1* promoter contains a non-functional PPRE.²⁰⁵ However, since not all clinical studies show an increase in apoAI plasma levels,²⁰⁹ it is likely that fibrates may increase HDL-cholesterol via apoAI-independent mechanisms. As was mentioned previously, a major difference between humans and mice is that mice do not express CETP. Whether the fibrate-induced increase in HDL-cholesterol depends on CETP expression has not yet been investigated.

3. Outline of this Thesis

Since lipid homeostasis presents an important factor in determining CVD risk, a thorough understanding of lipid metabolism is required to optimize lipid-lowering therapies. In the past decades this knowledge has increased tremendously, especially by the development of mice deficient or transgenic with respect to proteins in plasma lipid metabolism. In this thesis we aimed to expand our knowledge of the roles of apoCI, LPL and CETP in lipid metabolism, by performing studies using a combination of genetically modified (*i.e.* transgenic and knockout) mice, adenovirus-mediated gene expression, and *in vitro* studies.

Data obtained from studies in humans and mice have shown that plasma apoCI protein levels are positively correlated with combined hyperlipidemia, displaying the most pronounced effect on TG levels. Thus far, the apoCI-induced hyperlipidemia in *APOC1* mice has been explained by mainly inhibition of receptor-mediated remnant clearance, but this does not satisfactorily explain the predominant hypertriglyceridemia. In **chapter 2**, human *APOC1* expressing mice were used to elucidate the main underlying cause. We showed that the hypertriglyceridemia was predominantly caused by impaired LPL-mediated lipolytic conversion of VLDL-TG. Therefore, we further studied whether apoCI inhibits the LPL-mediated lipolysis via interaction with the VLDLr or with apoCIII in **chapter 3**. Our results showed that apoCI is a powerful inhibitor of LPL activity *in vivo*, independent of the VLDLr and apoCIII.

Chapter 4 focuses on the role of LPL in the apoE-receptor independent VLDL remnant clearance. This was studied by adenoviral-mediated gene transfer of human *APOC1* and human *LPL* into mice that lack the three main apoE-recognizing receptors, *i.e.* LDLr, the VLDLr and hepatic LRP. We found that also in the absence of these receptors the remnant clearance depends on LPL. In **chapter 5**, we addressed whether increasing LPL activity can lead to reduction of the combined hyperlipidemia in *APOE*2*-knockin mice. These studies revealed that the hyperlipidemia in these mice can be ameliorated by a direct increase in LPL activity and by overexpression of apoAV, but not by deletion of apoCIII.

Since a major difference between mice and men regarding lipid metabolism is the expression of CETP and the atherogenicity of CETP is still under debate, we studied in **chapter 6** the effect of human CETP expression in *APOE*3-Leiden* mice with a human-like distribution of cholesterol over lipoprotein particles. These results showed that CETP expression has a major impact on the cholesterol distribution between lipoproteins and represents a clear pro-atherogenic factor in these mice. Since both statins and fenofibrate increase HDL-cholesterol levels in humans, whereas this is not observed in mice, we addressed the hypothesis that this increase in HDL-cholesterol could depend on CETP in **chapter 7** and **chapter 8**. Therefore, mice on an *APOE*3-Leiden* background with or without CETP expression received a cholesterol-rich diet with or without fenofibrate or atorvastatin. Indeed, the mice with CETP displayed an increase in HDL-cholesterol, that was absent in the control mice, and could not be explained by differences in other genes involved in HDL-metabolism.

Chapter 9 discusses the results of these studies, together with the future perspectives.

References

1. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med.* 2005;352:1685-1695.
2. Davis RA. Chapter 14: Lipoprotein structure and secretion; 403-426. In: *Biochemistry of lipids, lipoproteins and membranes.* (1991) Edited by: Vance DE and Vance JE. Elsevier;New York.
3. Shepherd J and Packard ChJ. Chapter 3: Lipoprotein metabolism; 55-78. In: *Human plasma lipoproteins.* (1989) Edited by: Fruchart JC and Shepherd J. Walter de Gruyter;Berlin.
4. Hussain MM, Kancha RK, Zhou Z, Luchoomun J, Zu H, Bakillah A. Chylomicron assembly and catabolism: role of apolipoproteins and receptors. *Biochim Biophys Acta.* 1996;1300:151-170.
5. Lai CQ, Parnell LD, Ordovas JM. The APOA1/C3/A4/A5 gene cluster, lipid metabolism and cardiovascular disease risk. *Curr Opin Lipidol.* 2005;16:153-166.
6. Utermann G and Beisiegel U. Apolipoprotein A-IV: a protein occurring in human mesenteric lymph chylomicrons and free in plasma. Isolation and quantification. *Eur J Biochem.* 1979;99:333-343.
7. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* 1988;240:622-630.
8. Mahley RW, Hui DY, Innerarity TL, Beisiegel U. Chylomicron remnant metabolism. Role of hepatic lipoprotein receptors in mediating uptake. *Arteriosclerosis.* 1989;9:I14-I18.
9. Ji ZS, Fazio S, Lee YL, Mahley RW. Secretion-capture role for apolipoprotein E in remnant lipoprotein metabolism involving cell surface heparan sulfate proteoglycans. *J Biol Chem.* 1994;269:2764-2772.
10. Out R, Kruijt JK, Rensen PC, Hildebrand RB, de Vos P, Van Eck M, Van Berkel TJ. Scavenger receptor BI plays a role in facilitating chylomicron metabolism. *J Biol Chem.* 2004;279:18401-18406.
11. Greeve J, Altkemper I, Dieterich JH, Greten H, Windler E. Apolipoprotein B mRNA editing in 12 different mammalian species: hepatic expression is reflected in low concentrations of apoB-containing plasma lipoproteins. *J Lipid Res.* 1993;34:1367-1383.
12. O'Brien PJ, Alborn WE, Sloan JH, Ulmer M, Boodhoo A, Knierman MD, Schultze AE, Konrad RJ. The novel apolipoprotein A5 is present in human serum, is associated with VLDL, HDL, and chylomicrons, and circulates at very low concentrations compared with other apolipoproteins. *Clin Chem.* 2005;51:351-359.
13. Rigotti A, Miettinen HE, Krieger M. The role of the high-density lipoprotein receptor SR-BI in the lipid metabolism of endocrine and other tissues. *Endocr Rev.* 2003;24:357-387.
14. Freeman MW. Macrophage scavenger receptors. *Curr Opin Lipidol.* 1994;5:143-148.
15. Brown MS and Goldstein JL. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu Rev Biochem.* 1983;52:223-261.
16. Stein Y, Dabach Y, Hollander G, Halperin G, Stein O. Metabolism of HDL-cholesteryl ester in the rat, studied with a nonhydrolyzable analog, cholesteryl linoleyl ether. *Biochim Biophys Acta.* 1983;752:98-105.
17. Lewis GF and Rader DJ. New insights into the regulation of HDL metabolism and reverse cholesterol transport. *Circ Res.* 2005;96:1221-1232.
18. Linsel-Nitschke P and Tall AR. HDL as a target in the treatment of atherosclerotic cardiovascular disease. *Nat Rev Drug Discov.* 2005;4:193-205.
19. Oram JF and Heinecke JW. ATP-binding cassette transporter A1: a cell cholesterol exporter that protects against cardiovascular disease. *Physiol Rev.* 2005;85:1343-1372.
20. Jonas A. Lecithin cholesterol acyltransferase. *Biochim Biophys Acta.* 2000;1529:245-256.

21. Nakamura K, Kennedy MA, Baldan A, Bojanic DD, Lyons K, Edwards PA. Expression and regulation of multiple murine ATP-binding cassette transporter G1 mRNAs/isoforms that stimulate cellular cholesterol efflux to high density lipoprotein. *J Biol Chem.* 2004;279:45980-45989.
22. Wang N, Lan D, Chen W, Matsuura F, Tall AR. ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. *Proc Natl Acad Sci U S A.* 2004;101:9774-9779.
23. Asztalos BF, Llera-Moya M, Dallal GE, Horvath KV, Schaefer EJ, Rothblat GH. Differential effects of HDL subpopulations on cellular A. *J Lipid Res.* 2005;46:2246-2253.
24. Jian B, Llera-Moya M, Ji Y, Wang N, Phillips MC, Swaney JB, Tall AR, Rothblat GH. Scavenger receptor class B type I as a mediator of cellular cholesterol efflux to lipoproteins and phospholipid acceptors. *J Biol Chem.* 1998;273:5599-5606.
25. Stefkova J, Poledne R, Hubacek JA. ATP-binding cassette (ABC) transporters in human metabolism and diseases. *Physiol Res.* 2004;53:235-243.
26. Collet X, Tall AR, Serajuddin H, Guendouzi K, Royer L, Oliveira H, Barbaras R, Jiang XC, Francone OL. Remodeling of HDL by CETP in vivo and by CETP and hepatic lipase in vitro results in enhanced uptake of HDL CE by cells expressing scavenger receptor B-I. *J Lipid Res.* 1999;40:1185-1193.
27. Van Eck M, Pennings M, Hoekstra M, Out R, Van Berkel TJ. Scavenger receptor BI and ATP-binding cassette transporter A1 in reverse cholesterol transport and atherosclerosis. *Curr Opin Lipidol.* 2005;16:307-315.
28. Gauthier A, Lau P, Zha X, Milne R, McPherson R. Cholesteryl ester transfer protein directly mediates selective uptake of high density lipoprotein cholesteryl esters by the liver. *Arterioscler Thromb Vasc Biol.* 2005;25:2177-2184.
29. Yu L, Gupta S, Xu F, Liverman AD, Moschetta A, Mangelsdorf DJ, Repa JJ, Hobbs HH, Cohen JC. Expression of ABCG5 and ABCG8 is required for regulation of biliary cholesterol secretion. *J Biol Chem.* 2005;280:8742-8747.
30. Assmann G and Gotto AM, Jr. HDL cholesterol and protective factors in atherosclerosis. *Circulation.* 2004;109:III8-III14.
31. Calabresi L, Gomaschi M, Franceschini G. Endothelial protection by high-density lipoproteins: from bench to bedside. *Arterioscler Thromb Vasc Biol.* 2003;23:1724-1731.
32. Chapman MJ, Assmann G, Fruchart JC, Shepherd J, Sirtori C. Raising high-density lipoprotein cholesterol with reduction of cardiovascular risk: the role of nicotinic acid--a position paper developed by the European Consensus Panel on HDL-C. *Curr Med Res Opin.* 2004;20:1253-1268.
33. Hide WA, Chan L, Li WH. Structure and evolution of the lipase superfamily. *J Lipid Res.* 1992;33:167-178.
34. Fielding PE and Fielding CJ. Chapter 15: Dynamics of lipoprotein transport in the circulatory system; 427-459. In: *Biochemistry of lipids, lipoproteins and membranes.* (1991) Edited by: Vance DE and Vance JE. Elsevier;New York.
35. Tikkanen MJ. Chapter 6: Intravascular lipoprotein remodelling: lipolytic enzymes; 143-151. In: *Human plasma lipoproteins.* (1989) Edited by: Fruchart JC and Shepherd J. Walter de Gruyter;Berlin.
36. Goldberg IJ. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. *J Lipid Res.* 1996;37:693-707.
37. Teusink B, Voshol PJ, Dahlmans VE, Rensen PC, Pijl H, Romijn JA, Havekes LM. Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. *Diabetes.* 2003;52:614-620.
38. Wang CS, McConathy WJ, Kloer HU, Alaupovic P. Modulation of lipoprotein lipase activity by apolipoproteins. Effect of apolipoprotein C-III. *J Clin Invest.* 1985;75:384-390.
39. Rensen PC and Van Berkel TJ. Apolipoprotein E effectively inhibits lipoprotein lipase-mediated lipolysis of chy-

- lomicron-like triglyceride-rich lipid emulsions in vitro and in vivo. *J Biol Chem.* 1996;271:14791-14799.
40. Jong MC, Dahlmans VE, Hofker MH, Havekes LM. Nascent very-low-density lipoprotein triacylglycerol hydrolysis by lipoprotein lipase is inhibited by apolipoprotein E in a dose-dependent manner. *Biochem J.* 1997;328 (Pt 3):745-750.
 41. van Dijk KW, van Vlijmen BJ, van't Hof HB, van der ZA, Santamarina-Fojo S, Van Berkel TJ, Havekes LM, Hofker MH. In LDL receptor-deficient mice, catabolism of remnant lipoproteins requires a high level of apoE but is inhibited by excess apoE. *J Lipid Res.* 1999;40:336-344.
 42. Fruchart-Najib J, Bauge E, Niculescu LS, Pham T, Thomas B, Rommens C, Majd Z, Brewer B, Pennacchio LA, Fruchart JC. Mechanism of triglyceride lowering in mice expressing human apolipoprotein A5. *Biochem Biophys Res Commun.* 2004;319:397-404.
 43. Schaap FG, Rensen PC, Voshol PJ, Vriens C, van der Vliet HN, Chamuleau RA, Havekes LM, Groen AK, van Dijk KW. ApoAV reduces plasma triglycerides by inhibiting very low density lipoprotein-triglyceride (VLDL-TG) production and stimulating lipoprotein lipase-mediated VLDL-TG hydrolysis. *J Biol Chem.* 2004;279:27941-27947.
 44. Mulder M, Lombardi P, Jansen H, Van Berkel TJ, Frants RR, Havekes LM. Low density lipoprotein receptor internalizes low density and very low density lipoproteins that are bound to heparan sulfate proteoglycans via lipoprotein lipase. *J Biol Chem.* 1993;268:9369-9375.
 45. Niemeier A, Gafvels M, Heeren J, Meyer N, Angelin B, Beisiegel U. VLDL receptor mediates the uptake of human chylomicron remnants in vitro. *J Lipid Res.* 1996;37:1733-1742.
 46. Takahashi S, Sakai J, Fujino T, Hattori H, Zenimaru Y, Suzuki J, Miyamori I, Yamamoto TT. The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. *J Atheroscler Thromb.* 2004;11:200-208.
 47. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. *J Lipid Res.* 2002;43:1997-2006.
 48. Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. *Arterioscler Thromb Vasc Biol.* 1999;19:472-484.
 49. Strauss JG, Frank S, Kratky D, Hammerle G, Hrzenjak A, Knipping G, von Eckardstein A, Kostner GM, Zechner R. Adenovirus-mediated rescue of lipoprotein lipase-deficient mice. Lipolysis of triglyceride-rich lipoproteins is essential for high density lipoprotein maturation in mice. *J Biol Chem.* 2001;276:36083-36090.
 50. Febbraio M, Guy E, Coburn C, Knapp FF, Jr., Beets AL, Abumrad NA, Silverstein RL. The impact of overexpression and deficiency of fatty acid translocase (FAT)/CD36. *Mol Cell Biochem.* 2002;239:193-197.
 51. Goudriaan JR, den Boer MA, Rensen PC, Febbraio M, Kuipers F, Romijn JA, Havekes LM, Voshol PJ. CD36 deficiency in mice impairs lipoprotein lipase-mediated triglyceride clearance. *J Lipid Res.* 2005;46:2175-2181.
 52. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science.* 1990;249:790-793.
 53. Jong MC, Gijbels MJ, Dahlmans VE, Gorp PJ, Koopman SJ, Ponc M, Hofker MH, Havekes LM. Hyperlipidemia and cutaneous abnormalities in transgenic mice overexpressing human apolipoprotein C1. *J Clin Invest.* 1998;101:145-152.
 54. Goudriaan JR, Tacke PJ, Dahlmans VE, Gijbels MJ, van Dijk KW, Havekes LM, Jong MC. Protection from obesity in mice lacking the VLDL receptor. *Arterioscler Thromb Vasc Biol.* 2001;21:1488-1493.
 55. Jong MC, Voshol PJ, Muurling M, Dahlmans VE, Romijn JA, Pijl H, Havekes LM. Protection from obesity and insulin resistance in mice overexpressing human apolipoprotein C1. *Diabetes.* 2001;50:2779-2785.
 56. Duivenvoorden I, Teusink B, Rensen PC, Romijn JA, Havekes LM, Voshol PJ. Apolipoprotein C3 deficiency results in diet-induced obesity and aggravated insulin resistance in mice. *Diabetes.* 2005;54:664-671.
 57. Tall AR. Plasma cholesteryl ester transfer protein. *J Lipid Res.* 1993;34:1255-1274.

58. Nishida HI, Arai H, Nishida T. Cholesterol ester transfer mediated by lipid transfer protein as influenced by changes in the charge characteristics of plasma lipoproteins. *J Biol Chem.* 1993;268:16352-16360.
59. Barter PJ and Lally JL. In vitro exchanges of esterified cholesterol between serum lipoprotein fractions: studies of humans and rabbits. *Metabolism.* 1979;28:230-236.
60. Hesler CB, Swenson TL, Tall AR. Purification and characterization of a human plasma cholesteryl ester transfer protein. *J Biol Chem.* 1987;262:2275-2282.
61. Le Goff W, Guerin M, Chapman MJ. Pharmacological modulation of cholesteryl ester transfer protein, a new therapeutic target in atherogenic dyslipidemia. *Pharmacol Ther.* 2004;101:17-38.
62. Brundert M, Ewert A, Heeren J, Behrendt B, Ramakrishnan R, Greten H, Merkel M, Rinninger F. Scavenger receptor class B type I mediates the selective uptake of high-density lipoprotein-associated cholesteryl ester by the liver in mice. *Arterioscler Thromb Vasc Biol.* 2005;25:143-148.
63. Out R, Hoekstra M, Spijkers JA, Kruijt JK, Van Eck M, Bos IS, Twisk J, Van Berkel TJ. Scavenger receptor class B type I is solely responsible for the selective uptake of cholesteryl esters from HDL by the liver and the adrenals in mice. *J Lipid Res.* 2004;45:2088-2095.
64. Goldberg DI, Beltz WF, Pittman RC. Evaluation of pathways for the cellular uptake of high density lipoprotein cholesterol esters in rabbits. *J Clin Invest.* 1991;87:331-346.
65. Kee P, Caiazza D, Rye KA, Barrett PH, Morehouse LA, Barter PJ. Effect of inhibiting cholesteryl ester transfer protein on the kinetics of high-density lipoprotein cholesteryl ester transport in plasma: in vivo studies in rabbits. *Arterioscler Thromb Vasc Biol.* 2006;26:884-890.
66. Schwartz CC, VandenBroek JM, Cooper PS. Lipoprotein cholesteryl ester production, transfer, and output in vivo in humans. *J Lipid Res.* 2004;45:1594-1607.
67. Clay MA, Newnham HH, Forte TM, Barter PJ. Cholesteryl ester transfer protein and hepatic lipase activity promote shedding of apo A-I from HDL and subsequent formation of discoidal HDL. *Biochim Biophys Acta.* 1992;1124:52-58.
68. Newnham HH and Barter PJ. Synergistic effects of lipid transfers and hepatic lipase in the formation of very small high-density lipoproteins during incubation of human plasma. *Biochim Biophys Acta.* 1990;1044:57-64.
69. Newnham HH and Barter PJ. Changes in particle size of high density lipoproteins during incubation with very low density lipoproteins, cholesteryl ester transfer protein and lipoprotein lipase. *Biochim Biophys Acta.* 1992;1125:297-304.
70. Arai T, Tsukada T, Murase T, Matsumoto K. Particle size analysis of high density lipoproteins in patients with genetic cholesteryl ester transfer protein deficiency. *Clin Chim Acta.* 2000;301:103-117.
71. Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Maruhama Y, Mabuchi H, Tall AR. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med.* 1990;323:1234-1238.
72. Yamashita S, Sprecher DL, Sakai N, Matsuzawa Y, Tarui S, Hui DY. Accumulation of apolipoprotein E-rich high density lipoproteins in hyperalphalipoproteinemic human subjects with plasma cholesteryl ester transfer protein deficiency. *J Clin Invest.* 1990;86:688-695.
73. Yamashita S, Hui DY, Wetterau JR, Sprecher DL, Harmony JA, Sakai N, Matsuzawa Y, Tarui S. Characterization of plasma lipoproteins in patients heterozygous for human plasma cholesteryl ester transfer protein (CETP) deficiency: plasma CETP regulates high-density lipoprotein concentration and composition. *Metabolism.* 1991;40:756-763.
74. Kuivenhoven JA, Jukema JW, Zwinderman AH, de Knijff P, McPherson R, Bruschke AV, Lie KI, Kastelein JJ. The role of a common variant of the cholesteryl ester transfer protein gene in the progression of coronary atherosclerosis. The Regression Growth Evaluation Statin Study Group. *N Engl J Med.* 1998;338:86-93.

75. Boekholdt SM, Kuivenhoven JA, Wareham NJ, Peters RJ, Jukema JW, Luben R, Bingham SA, Day NE, Kastelein JJ, Khaw KT. Plasma levels of cholesteryl ester transfer protein and the risk of future coronary artery disease in apparently healthy men and women: the prospective EPIC (European Prospective Investigation into Cancer and nutrition)-Norfolk population study. *Circulation*. 2004;110:1418-1423.
76. Brown ML, Inazu A, Hesler CB, Agellon LB, Mann C, Whitlock ME, Marcel YL, Milne RW, Koizumi J, Mabuchi H. Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. *Nature*. 1989;342:448-451.
77. Yamashita S, Matsuzawa Y, Okazaki M, Kako H, Yasugi T, Akioka H, Hirano K, Tarui S. Small polydisperse low density lipoproteins in familial hyperalphalipoproteinemia with complete deficiency of cholesteryl ester transfer activity. *Atherosclerosis*. 1988;70:7-12.
78. Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest*. 1996;97:2917-2923.
79. Hirano K, Yamashita S, Kuga Y, Sakai N, Nozaki S, Kihara S, Arai T, Yanagi K, Takami S, Menju M. Atherosclerotic disease in marked hyperalphalipoproteinemia. Combined hyperreduction of cholesteryl ester transfer protein and hepatic triglyceride lipase. *Arterioscler Thromb Vasc Biol*. 1995;15:1849-1856.
80. Hirano K, Yamashita S, Nakajima N, Arai T, Maruyama T, Yoshida Y, Ishigami M, Sakai N, Kameda-Takemura K, Matsuzawa Y. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. *Arterioscler Thromb Vasc Biol*. 1997;17:1053-1059.
81. Hayek T, Masucci-Magoulas L, Jiang X, Walsh A, Rubin E, Breslow JL, Tall AR. Decreased early atherosclerotic lesions in hypertriglyceridemic mice expressing cholesteryl ester transfer protein transgene. *J Clin Invest*. 1995;96:2071-2074.
82. Foger B, Chase M, Amar MJ, Vaisman BL, Shamburek RD, Paigen B, Fruchart-Najib J, Paiz JA, Koch CA, Hoyt RF, Brewer HB, Jr., Santamarina-Fojo S. Cholesteryl ester transfer protein corrects dysfunctional high density lipoproteins and reduces aortic atherosclerosis in lecithin cholesterol acyltransferase transgenic mice. *J Biol Chem*. 1999;274:36912-36920.
83. Plump AS, Masucci-Magoulas L, Bruce C, Bisgaier CL, Breslow JL, Tall AR. Increased atherosclerosis in ApoE and LDL receptor gene knock-out mice as a result of human cholesteryl ester transfer protein transgene expression. *Arterioscler Thromb Vasc Biol*. 1999;19:1105-1110.
84. Barter PJ, Brewer HB, Jr., Chapman MJ, Hennekens CH, Rader DJ, Tall AR. Cholesteryl ester transfer protein: a novel target for raising HDL and inhibiting atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2003;23:160-167.
85. de Grooth GJ, Klerkx AH, Stroes ES, Stalenhoef AF, Kastelein JJ, Kuivenhoven JA. A review of CETP and its relation to atherosclerosis. *J Lipid Res*. 2004;45:1967-1974.
86. Avramoglu RK and Adeli K. Hepatic regulation of apolipoprotein B. *Rev Endocr Metab Disord*. 2004;5:293-301.
87. Olofsson SO and Boren J. Apolipoprotein B: a clinically important apolipoprotein which assembles atherogenic lipoproteins and promotes the development of atherosclerosis. *J Intern Med*. 2005;258:395-410.
88. Walldius G and Jungner I. Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. *J Intern Med*. 2004;255:188-205.
89. Shelness GS and Ledford AS. Evolution and mechanism of apolipoprotein B-containing lipoprotein assembly. *Curr Opin Lipidol*. 2005;16:325-332.
90. Marcel YL and Kiss RS. Structure-function relationships of apolipoprotein A-I: a flexible protein with dynamic lipid associations. *Curr Opin Lipidol*. 2003;14:151-157.
91. Rye KA and Barter PJ. Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. *Arterio-*

- scler Thromb Vasc Biol. 2004;24:421-428.
92. Allan CM, Walker D, Segrest JP, Taylor JM. Identification and characterization of a new human gene (APOC4) in the apolipoprotein E, C-I, and C-II gene locus. *Genomics*. 1995;28:291-300.
 93. Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. *Arterioscler Thromb Vasc Biol*. 1999;19:472-484.
 94. Scott J, Knott TJ, Shaw DJ, Brook JD. Localization of genes encoding apolipoproteins CI, CII, and E to the p13---cen region of human chromosome 19. *Hum Genet*. 1985;71:144-146.
 95. Shachter NS. Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. *Curr Opin Lipidol*. 2001;12:297-304.
 96. Lauer SJ, Walker D, Elshourbagy NA, Reardon CA, Levy-Wilson B, Taylor JM. Two copies of the human apolipoprotein C-I gene are linked closely to the apolipoprotein E gene. *J Biol Chem*. 1988;263:7277-7286.
 97. Knott TJ, Robertson ME, Priestley LM, Urdea M, Wallis S, Scott J. Characterisation of mRNAs encoding the precursor for human apolipoprotein CI. *Nucleic Acids Res*. 1984;12:3909-3915.
 98. Shachter NS, Ebara T, Ramakrishnan R, Steiner G, Breslow JL, Ginsberg HN, Smith JD. Combined hyperlipidemia in transgenic mice overexpressing human apolipoprotein CI. *J Clin Invest*. 1996;98:846-855.
 99. Shulman RS, Herbert PN, Wehrly K, Fredrickson DS. The complete amino acid sequence of C-I (apoLP-Ser), an apolipoprotein from human very low density lipoproteins. *J Biol Chem*. 1975;250:182-190.
 100. Xu Y, Berglund L, Ramakrishnan R, Mayeux R, Ngai C, Holleran S, Tycko B, Leff T, Shachter NS. A common Hpa I RFLP of apolipoprotein C-I increases gene transcription and exhibits an ethnically distinct pattern of linkage disequilibrium with the alleles of apolipoprotein E. *J Lipid Res*. 1999;40:50-58.
 101. Hubacek JA, Pitha J, Adamkova V, Skodova Z, Lanska V, Poledne R. Apolipoprotein E and apolipoprotein CI polymorphisms in the Czech population: almost complete linkage disequilibrium of the less frequent alleles of both polymorphisms. *Physiol Res*. 2003;52:195-200.
 102. Jong MC, van Ree JH, Dahlmans VE, Frants RR, Hofker MH, Havekes LM. Reduced very-low-density lipoprotein fractional catabolic rate in apolipoprotein C1-deficient mice. *Biochem J*. 1997;321 (Pt 2):445-450.
 103. van Ree JH, Hofker MH, van den Broek WJ, van Deursen JM, van der BH, Frants RR, Wieringa B, Havekes LM. Increased response to cholesterol feeding in apolipoprotein C1-deficient mice. *Biochem J*. 1995;305 (Pt 3):905-911.
 104. Jong MC, Dahlmans VE, van Gorp PJ, van Dijk KW, Breuer ML, Hofker MH, Havekes LM. In the absence of the low density lipoprotein receptor, human apolipoprotein C1 overexpression in transgenic mice inhibits the hepatic uptake of very low density lipoproteins via a receptor-associated protein-sensitive pathway. *J Clin Invest*. 1996;98:2259-2267.
 105. Jong MC, Gijbels MJ, Dahlmans VE, Gorp PJ, Koopman SJ, Ponc M, Hofker MH, Havekes LM. Hyperlipidemia and cutaneous abnormalities in transgenic mice overexpressing human apolipoprotein C1. *J Clin Invest*. 1998;101:145-152.
 106. Sehayek E and Eisenberg S. Mechanisms of inhibition by apolipoprotein C of apolipoprotein E-dependent cellular metabolism of human triglyceride-rich lipoproteins through the low density lipoprotein receptor pathway. *J Biol Chem*. 1991;266:18259-18267.
 107. Weisgraber KH, Mahley RW, Kowal RC, Herz J, Goldstein JL, Brown MS. Apolipoprotein C-I modulates the interaction of apolipoprotein E with beta-migrating very low density lipoproteins (beta-VLDL) and inhibits binding of beta-VLDL to low density lipoprotein receptor-related protein. *J Biol Chem*. 1990;265:22453-22459.
 108. Jong MC, van Dijk KW, Dahlmans VE, van der BH, Kobayashi K, Oka K, Siest G, Chan L, Hofker MH, Havekes LM. Reversal of hyperlipidaemia in apolipoprotein C1 transgenic mice by adenovirus-mediated gene delivery of the low-density-lipoprotein receptor, but not by the very-low-density-lipoprotein receptor. *Biochem J*. 1999;338

- (Pt 2):281-287.
109. Soutar AK, Garner CW, Baker HN, Sparrow JT, Jackson RL, Gotto AM, Smith LC. Effect of the human plasma apolipoproteins and phosphatidylcholine acyl donor on the activity of lecithin: cholesterol acyltransferase. *Biochemistry*. 1975;14:3057-3064.
 110. Steyrer E and Kostner GM. Activation of lecithin-cholesterol acyltransferase by apolipoprotein D: comparison of proteoliposomes containing apolipoprotein D, A-I or C-I. *Biochim Biophys Acta*. 1988;958:484-491.
 111. Ekman R and Nilsson-Ehle P. Effects of apolipoproteins on lipoprotein lipase activity of human adipose tissue. *Clin Chim Acta*. 1975;63:29-35.
 112. Havel RJ, Fielding CJ, Olivecrona T, Shore VG, Fielding PE, Egelrud T. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoproteins lipase from different sources. *Biochemistry*. 1973;12:1828-1833.
 113. Kinnunen PK and Ehnolm C. Effect of serum and C-apoproteins from very low density lipoproteins on human postheparin plasma hepatic lipase. *FEBS Lett*. 1976;65:354-357.
 114. Gautier T, Masson D, de Barros JP, Athias A, Gambert P, Aunis D, Metz-Boutigue MH, Lagrost L. Human apolipoprotein C-I accounts for the ability of plasma high density lipoproteins to inhibit the cholesteryl ester transfer protein activity. *J Biol Chem*. 2000;275:37504-37509.
 115. Kushwaha RS, Hasan SQ, McGill HC, Jr., Getz GS, Dunham RG, Kanda P. Characterization of cholesteryl ester transfer protein inhibitor from plasma of baboons (*Papio sp.*). *J Lipid Res*. 1993;34:1285-1297.
 116. Gautier T, Masson D, Jong MC, Duverneuil L, Le Guern N, Deckert V, Pais de Barros JP, Dumont L, Bataille A, Zak Z, Jiang XC, Tall AR, Havekes LM, Lagrost L. Apolipoprotein CI deficiency markedly augments plasma lipoprotein changes mediated by human cholesteryl ester transfer protein (CETP) in CETP transgenic/ApoCI-knocked out mice. *J Biol Chem*. 2002;277:31354-31363.
 117. Masson D, Pais de Barros JP, Zak Z, Gautier T, Le Guern N, Assem M, Chisholm JW, Paterniti JR, Jr., Lagrost L. Human apoA-I expression in CETP transgenic rats leads to lower levels of apoC-I in HDL and to magnification of CETP-mediated lipoprotein changes. *J Lipid Res*. 2006;47:356-365.
 118. Pennacchio LA and Rubin EM. Genomic strategies to identify mammalian regulatory sequences. *Nat Rev Genet*. 2001;2:100-109.
 119. Nestel PJ and Fidge NH. Apoprotein C metabolism in man. *Adv Lipid Res*. 1982;19:55-83.
 120. Le NA, Gibson JC, Ginsberg HN. Independent regulation of plasma apolipoprotein C-II and C-III concentrations in very low density and high density lipoproteins: implications for the regulation of the catabolism of these lipoproteins. *J Lipid Res*. 1988;29:669-677.
 121. Schonfeld G, George PK, Miller J, Reilly P, Witztum J. Apolipoprotein C-II and C-III levels in hyperlipoproteinaemia. *Metabolism*. 1979;28:1001-1010.
 122. Shoulders CC, Harry PJ, Lagrost L, White SE, Shah NF, North JD, Gilligan M, Gambert P, Ball MJ. Variation at the apo AI/CIII/AIV gene complex is associated with elevated plasma levels of apo CIII. *Atherosclerosis*. 1991;87:239-247.
 123. Aalto-Setälä K, Weinstock PH, Bisgaier CL, Wu L, Smith JD, Breslow JL. Further characterization of the metabolic properties of triglyceride-rich lipoproteins from human and mouse apoC-III transgenic mice. *J Lipid Res*. 1996;37:1802-1811.
 124. Jackson RL, Tajima S, Yamamura T, Yokoyama S, Yamamoto A. Comparison of apolipoprotein C-II-deficient triacylglycerol-rich lipoproteins and trioleoylglycerol/phosphatidylcholine-stabilized particles as substrates for lipoprotein lipase. *Biochim Biophys Acta*. 1986;875:211-219.
 125. McConathy WJ, Gesquiere JC, Bass H, Tartar A, Fruchart JC, Wang CS. Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III. *J Lipid Res*. 1992;33:995-1003.

126. de Silva HV, Lauer SJ, Wang J, Simonet WS, Weisgraber KH, Mahley RW, Taylor JM. Overexpression of human apolipoprotein C-III in transgenic mice results in an accumulation of apolipoprotein B48 remnants that is corrected by excess apolipoprotein E. *J Biol Chem.* 1994;269:2324-2335.
127. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. *Science.* 1990;249:790-793.
128. Maeda N, Li H, Lee D, Oliver P, Quarfordt SH, Osada J. Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J Biol Chem.* 1994;269:23610-23616.
129. van der Vliet HN, Sammels MG, Leegwater AC, Levels JH, Reitsma PH, Boers W, Chamuleau RA. Apolipoprotein A-V: a novel apolipoprotein associated with an early phase of liver regeneration. *J Biol Chem.* 2001;276:44512-44520.
130. Karathanasis SK. Apolipoprotein multigene family: tandem organization of human apolipoprotein AI, CIII, and AIV genes. *Proc Natl Acad Sci U S A.* 1985;82:6374-6378.
131. Ishihara M, Kujiraoka T, Iwasaki T, Nagano M, Takano M, Ishii J, Tsuji M, Ide H, Miller IP, Miller NE, Hattori H. A sandwich enzyme-linked immunosorbent assay for human plasma apolipoprotein A-V concentration. *J Lipid Res.* 2005;46:2015-2022.
132. Pennacchio LA, Olivier M, Hubacek JA, Cohen JC, Cox DR, Fruchart JC, Krauss RM, Rubin EM. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science.* 2001;294:169-173.
133. van Dijk KW, Rensen PC, Voshol PJ, Havekes LM. The role and mode of action of apolipoproteins CIII and AV: synergistic actors in triglyceride metabolism? *Curr Opin Lipidol.* 2004;15:239-246.
134. van der Vliet HN, Schaap FG, Levels JH, Ottenhoff R, Looije N, Wesseling JG, Groen AK, Chamuleau RA. Adenoviral overexpression of apolipoprotein A-V reduces serum levels of triglycerides and cholesterol in mice. *Biochem Biophys Res Commun.* 2002;295:1156-1159.
135. Weinberg RB, Cook VR, Beckstead JA, Martin DD, Gallagher JW, Shelness GS, Ryan RO. Structure and interfacial properties of human apolipoprotein A-V. *J Biol Chem.* 2003;278:34438-34444.
136. Merkel M, Loeffler B, Kluger M, Fabig N, Geppert G, Pennacchio LA, Laatsch A, Heeren J. Apolipoprotein AV accelerates plasma hydrolysis of triglyceride-rich lipoproteins by interaction with proteoglycan-bound lipoprotein lipase. *J Biol Chem.* 2005;280:21553-21560.
137. Lookene A, Beckstead JA, Nilsson S, Olivecrona G, Ryan RO. Apolipoprotein A-V-heparin interactions: implications for plasma lipoprotein metabolism. *J Biol Chem.* 2005;280:25383-25387.
138. Lookene A, Beckstead JA, Nilsson S, Olivecrona G, Ryan RO. Apolipoprotein A-V-heparin interactions: implications for plasma lipoprotein metabolism. *J Biol Chem.* 2005;280:25383-25387.
139. Weinberg RB, Cook VR, Beckstead JA, Martin DD, Gallagher JW, Shelness GS, Ryan RO. Structure and interfacial properties of human apolipoprotein A-V. *J Biol Chem.* 2003;278:34438-34444.
140. Grosskopf I, Baroukh N, Lee SJ, Kamari Y, Harats D, Rubin EM, Pennacchio LA, Cooper AD. Apolipoprotein A-V deficiency results in marked hypertriglyceridemia attributable to decreased lipolysis of triglyceride-rich lipoproteins and removal of their remnants. *Arterioscler Thromb Vasc Biol.* 2005;25:2573-2579.
141. Oliva CP, Pisciotto L, Li VG, Sambataro MP, Cantafora A, Bellocchio A, Catapano A, Tarugi P, Bertolini S, Calandra S. Inherited apolipoprotein A-V deficiency in severe hypertriglyceridemia. *Arterioscler Thromb Vasc Biol.* 2005;25:411-417.
142. Rensen PC, van Dijk KW, Havekes LM. Apolipoprotein AV: low concentration, high impact. *Arterioscler Thromb Vasc Biol.* 2005;25:2445-2447.
143. Rall SC, Jr., Weisgraber KH, Mahley RW. Human apolipoprotein E. The complete amino acid sequence. *J Biol*

- Chem. 1982;257:4171-4178.
144. Kowal RC, Herz J, Goldstein JL, Esser V, Brown MS. Low density lipoprotein receptor-related protein mediates uptake of cholesterol esters derived from apoprotein E-enriched lipoproteins. *Proc Natl Acad Sci U S A*. 1989;86:5810-5814.
 145. Takahashi S, Suzuki J, Kohno M, Oida K, Tamai T, Miyabo S, Yamamoto T, Nakai T. Enhancement of the binding of triglyceride-rich lipoproteins to the very low density lipoprotein receptor by apolipoprotein E and lipoprotein lipase. *J Biol Chem*. 1995;270:15747-15754.
 146. Weisgraber KH, Rall SC, Jr., Mahley RW. Human E apoprotein heterogeneity. Cysteine-arginine interchanges in the amino acid sequence of the apo-E isoforms. *J Biol Chem*. 1981;256:9077-9083.
 147. Dong LM, Wilson C, Wardell MR, Simmons T, Mahley RW, Weisgraber KH, Agard DA. Human apolipoprotein E. Role of arginine 61 in mediating the lipoprotein preferences of the E3 and E4 isoforms. *J Biol Chem*. 1994;269:22358-22365.
 148. Dong LM and Weisgraber KH. Human apolipoprotein E4 domain interaction. Arginine 61 and glutamic acid 255 interact to direct the preference for very low density lipoproteins. *J Biol Chem*. 1996;271:19053-19057.
 149. Mann WA, Meyer N, Weber W, Meyer S, Greten H, Beisiegel U. Apolipoprotein E isoforms and rare mutations: parallel reduction in binding to cells and to heparin reflects severity of associated type III hyperlipoproteinemia. *J Lipid Res*. 1995;36:517-525.
 150. Weisgraber KH, Innerarity TL, Mahley RW. Role of lysine residues of plasma lipoproteins in high affinity binding to cell surface receptors on human fibroblasts. *J Biol Chem*. 1978;253:9053-9062.
 151. Sullivan PM, Mezdour H, Quarfordt SH, Maeda N. Type III hyperlipoproteinemia and spontaneous atherosclerosis in mice resulting from gene replacement of mouse Apoe with human Apoe*2. *J Clin Invest*. 1998;102:130-135.
 152. Huang Y, Liu XQ, Rall SC, Jr., Mahley RW. Apolipoprotein E2 reduces the low density lipoprotein level in transgenic mice by impairing lipoprotein lipase-mediated lipolysis of triglyceride-rich lipoproteins. *J Biol Chem*. 1998;273:17483-17490.
 153. de Man FH, de Beer F, van de LA, Smelt AH, Leuven JA, Havekes LM. Effect of apolipoprotein E variants on lipolysis of very low density lipoproteins by heparan sulphate proteoglycan-bound lipoprotein lipase. *Atherosclerosis*. 1998;136:255-262.
 154. Talmud PJ, Palmen J, Putt W, Lins L, Humphries SE. Determination of the functionality of common APOA5 polymorphisms. *J Biol Chem*. 2005;280:28215-28220.
 155. Pennacchio LA, Olivier M, Hubacek JA, Krauss RM, Rubin EM, Cohen JC. Two independent apolipoprotein A5 haplotypes influence human plasma triglyceride levels. *Hum Mol Genet*. 2002;11:3031-3038.
 156. Schaefer JR, Sattler AM, Hackler B, Kurt B, Hackler R, Maisch B, Soufi M. Hyperlipidemia in patients with apolipoprotein E 2/2 phenotype: apolipoprotein A5 S19W mutation as a cofactor. *Clin Chem*. 2004;50:2214-2215.
 157. Evans D, Seedorf U, Beil FU. Polymorphisms in the apolipoprotein A5 (APOA5) gene and type III hyperlipidemia. *Clin Genet*. 2005;68:369-372.
 158. van den Maagdenberg AM, de Knijff P, Stalenhoef AF, Gevers Leuven JA, Havekes LM, Frants RR. Apolipoprotein E*3-Leiden allele results from a partial gene duplication in exon 4. *Biochem Biophys Res Commun*. 1989;165:851-857.
 159. Wardell MR, Weisgraber KH, Havekes LM, Rall SC, Jr. Apolipoprotein E3-Leiden contains a seven-amino acid insertion that is a tandem repeat of residues 121-127. *J Biol Chem*. 1989;264:21205-21210.
 160. Chappell DA. High receptor binding affinity of lipoproteins in atypical dysbetalipoproteinemia (type III hyperlipoproteinemia). *J Clin Invest*. 1989;84:1906-1915.
 161. Ji ZS, Fazio S, Mahley RW. Variable heparan sulfate proteoglycan binding of apolipoprotein E variants may

- modulate the expression of type III hyperlipoproteinemia. *J Biol Chem.* 1994;269:13421-13428.
162. Mann WA, Meyer N, Weber W, Meyer S, Greten H, Beisiegel U. Apolipoprotein E isoforms and rare mutations: parallel reduction in binding to cells and to heparin reflects severity of associated type III hyperlipoproteinemia. *J Lipid Res.* 1995;36:517-525.
 163. Havekes L, de Wit E, Leuven JG, Klasen E, Utermann G, Weber W, Beisiegel U. Apolipoprotein E3-Leiden. A new variant of human apolipoprotein E associated with familial type III hyperlipoproteinemia. *Hum Genet.* 1986;73:157-163.
 164. van den Maagdenberg AM, Hofker MH, Krimpenfort PJ, de B, I, van Vlijmen B, van der BH, Havekes LM, Frants RR. Transgenic mice carrying the apolipoprotein E3-Leiden gene exhibit hyperlipoproteinemia. *J Biol Chem.* 1993;268:10540-10545.
 165. van Vlijmen BJ, van den Maagdenberg AM, Gijbels MJ, van der BH, HogenEsch H, Frants RR, Hofker MH, Havekes LM. Diet-induced hyperlipoproteinemia and atherosclerosis in apolipoprotein E3-Leiden transgenic mice. *J Clin Invest.* 1994;93:1403-1410.
 166. Groot PH, van Vlijmen BJ, Benson GM, Hofker MH, Schiffelers R, Vidgeon-Hart M, Havekes LM. Quantitative assessment of aortic atherosclerosis in APOE*3 Leiden transgenic mice and its relationship to serum cholesterol exposure. *Arterioscler Thromb Vasc Biol.* 1996;16:926-933.
 167. van Vlijmen BJ, Pearce NJ, Bergo M, Staels B, Yates JW, Gribble AD, Bond BC, Hofker MH, Havekes LM, Groot PH. Apolipoprotein E*3-Leiden transgenic mice as a test model for hypolipidaemic drugs. *Arzneimittelforschung.* 1998;48:396-402.
 168. Endo A, Kuroda M, Tanzawa K. Competitive inhibition of 3-hydroxy-3-methylglutaryl coenzyme A reductase by ML-236A and ML-236B fungal metabolites, having hypocholesterolemic activity. *FEBS Lett.* 1976;72:323-326.
 169. Davidson MH and Toth PP. Comparative effects of lipid-lowering therapies. *Prog Cardiovasc Dis.* 2004;47:73-104.
 170. Istvan ES and Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. *Science.* 2001;292:1160-1164.
 171. Sehayek E, Butbul E, Avner R, Levkovitz H, Eisenberg S. Enhanced cellular metabolism of very low density lipoprotein by simvastatin. A novel mechanism of action of HMG-CoA reductase inhibitors. *Eur J Clin Invest.* 1994;24:173-178.
 172. Arad Y, Ramakrishnan R, Ginsberg HN. Lovastatin therapy reduces low density lipoprotein apoB levels in subjects with combined hyperlipidemia by reducing the production of apoB-containing lipoproteins: implications for the pathophysiology of apoB production. *J Lipid Res.* 1990;31:567-582.
 173. Arad Y, Ramakrishnan R, Ginsberg HN. Effects of lovastatin therapy on very-low-density lipoprotein triglyceride metabolism in subjects with combined hyperlipidemia: evidence for reduced assembly and secretion of triglyceride-rich lipoproteins. *Metabolism.* 1992;41:487-493.
 174. Ginsberg HN, Le NA, Short MP, Ramakrishnan R, Desnick RJ. Suppression of apolipoprotein B production during treatment of cholesteryl ester storage disease with lovastatin. Implications for regulation of apolipoprotein B synthesis. *J Clin Invest.* 1987;80:1692-1697.
 175. Ginsberg HN. Effects of statins on triglyceride metabolism. *Am J Cardiol.* 1998;81:32B-35B.
 176. Sirtori CR. Pharmacology and mechanism of action of the new HMG-CoA reductase inhibitors. *Pharmacol Res.* 1990;22:555-563.
 177. Sirtori CR. Tissue selectivity of hydroxymethylglutaryl coenzyme A (HMG CoA) reductase inhibitors. *Pharmacol Ther.* 1993;60:431-459.
 178. Stancu C and Sima A. Statins: mechanism of action and effects. *J Cell Mol Med.* 2001;5:378-387.
 179. Stein EA, Lane M, Laskarzewski P. Comparison of statins in hypertriglyceridemia. *Am J Cardiol.* 1998;81:66B-

- 69B.
180. Wilt TJ, Bloomfield HE, MacDonald R, Nelson D, Rutks I, Ho M, Larsen G, McCall A, Pineros S, Sales A. Effectiveness of statin therapy in adults with coronary heart disease. *Arch Intern Med.* 2004;164:1427-1436.
 181. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA.* 2001;285:2486-2497.
 182. Cannon CP, Braunwald E, McCabe CH, Rader DJ, Rouleau JL, Belder R, Joyal SV, Hill KA, Pfeffer MA, Skene AM. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. *N Engl J Med.* 2004;350:1495-1504.
 183. Nissen SE, Tuzcu EM, Schoenhagen P, Brown BG, Ganz P, Vogel RA, Crowe T, Howard G, Cooper CJ, Brodie B, Grines CL, DeMaria AN. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. *JAMA.* 2004;291:1071-1080.
 184. Ahnadi CE, Berthezene F, Ponsin G. Simvastatin-induced decrease in the transfer of cholesterol esters from high density lipoproteins to very low and low density lipoproteins in normolipidemic subjects. *Atherosclerosis.* 1993;99:219-228.
 185. van Venrooij FV, Stolk RP, Banga JD, Sijmonsma TP, van Tol A, Erkelens DW, Dallinga-Thie GM. Common cholesteryl ester transfer protein gene polymorphisms and the effect of atorvastatin therapy in type 2 diabetes. *Diabetes Care.* 2003;26:1216-1223.
 186. Davignon J and Laaksonen R. Low-density lipoprotein-independent effects of statins. *Curr Opin Lipidol.* 1999;10:543-559.
 187. Kinlay S. Potential vascular benefits of statins. *Am J Med.* 2005;118 Suppl 12A:62-67.
 188. Forman BM, Chen J, Evans RM. Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors alpha and delta. *Proc Natl Acad Sci U S A.* 1997;94:4312-4317.
 189. Kliewer SA, Sundseth SS, Jones SA, Brown PJ, Wisely GB, Koble CS, Devchand P, Wahli W, Willson TM, Lenhard JM, Lehmann JM. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors alpha and gamma. *Proc Natl Acad Sci U S A.* 1997;94:4318-4323.
 190. Staels B, Dallongeville J, Auwerx J, Schoonjans K, Leitersdorf E, Fruchart JC. Mechanism of action of fibrates on lipid and lipoprotein metabolism. *Circulation.* 1998;98:2088-2093.
 191. Auboeuf D, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M, Vidal H. Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes.* 1997;46:1319-1327.
 192. Schoonjans K, Peinado-Onsurbe J, Lefebvre AM, Heyman RA, Briggs M, Deeb S, Staels B, Auwerx J. PPARalpha and PPARgamma activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene. *EMBO J.* 1996;15:5336-5348.
 193. Malmendier CL, Lontie JF, Delcroix C, Dubois DY, Magot T, De Roy L. Apolipoproteins C-II and C-III metabolism in hypertriglyceridemic patients. Effect of a drastic triglyceride reduction by combined diet restriction and fenofibrate administration. *Atherosclerosis.* 1989;77:139-149.
 194. Staels B, Vu-Dac N, Kosykh VA, Saladin R, Fruchart JC, Dallongeville J, Auwerx J. Fibrates downregulate apolipoprotein C-III expression independent of induction of peroxisomal acyl coenzyme A oxidase. A potential mechanism for the hypolipidemic action of fibrates. *J Clin Invest.* 1995;95:705-712.
 195. Heller F and Harvengt C. Effects of clofibrate, bezafibrate, fenofibrate and probucol on plasma lipolytic enzymes in normolipemic subjects. *Eur J Clin Pharmacol.* 1983;25:57-63.
 196. Martin G, Schoonjans K, Lefebvre AM, Staels B, Auwerx J. Coordinate regulation of the expression of the fatty

- acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. *J Biol Chem.* 1997;272:28210-28217.
197. Schoonjans K, Watanabe M, Suzuki H, Mahfoudi A, Krey G, Wahli W, Grimaldi P, Staels B, Yamamoto T, Auwerx J. Induction of the acyl-coenzyme A synthetase gene by fibrates and fatty acids is mediated by a peroxisome proliferator response element in the C promoter. *J Biol Chem.* 1995;270:19269-19276.
198. Chapman MJ. Fibrates in 2003: therapeutic action in atherogenic dyslipidaemia and future perspectives. *Atherosclerosis.* 2003;171:1-13.
199. Ginsberg HN. Hypertriglyceridemia: new insights and new approaches to pharmacologic therapy. *Am J Cardiol.* 2001;87:1174-1180.
200. Hokanson JE and Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J Cardiovasc Risk.* 1996;3:213-219.
201. Assmann G, Cullen P, Schulte H. The Munster Heart Study (PROCAM). Results of follow-up at 8 years. *Eur Heart J.* 1998;19 Suppl A:2-11.
202. Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. Triglyceride concentration and ischemic heart disease: an eight-year follow-up in the Copenhagen Male Study. *Circulation.* 1998;97:1029-1036.
203. Birjmohun RS, Hutten BA, Kastelein JJ, Stroes ES. Efficacy and safety of high-density lipoprotein cholesterol-increasing compounds: a meta-analysis of randomized controlled trials. *J Am Coll Cardiol.* 2005;45:185-197.
204. Chinetti-Gbaguidi G, Fruchart JC, Staels B. Pleiotropic effects of fibrates. *Curr Atheroscler Rep.* 2005;7:396-401.
205. Berthou L, Duverger N, Emmanuel F, Langouet S, Auwerx J, Guillozo A, Fruchart JC, Rubin E, Denefle P, Staels B, Branellec D. Opposite regulation of human versus mouse apolipoprotein A-I by fibrates in human apolipoprotein A-I transgenic mice. *J Clin Invest.* 1996;97:2408-2416.
206. Bouly M, Masson D, Gross B, Jiang XC, Fievet C, Castro G, Tall AR, Fruchart JC, Staels B, Lagrost L, Luc G. Induction of the phospholipid transfer protein gene accounts for the high density lipoprotein enlargement in mice treated with fenofibrate. *J Biol Chem.* 2001;276:25841-25847.
207. Declercq V, Yeganeh B, Moshtaghi-Kashanian GR, Khademi H, Bahadori B, Moghadasian MH. Paradoxical effects of fenofibrate and nicotinic acid in apo E-deficient mice. *J Cardiovasc Pharmacol.* 2005;46:18-24.
208. Mardones P, Pilon A, Bouly M, Duran D, Nishimoto T, Arai H, Kozarsky KF, Altayo M, Miquel JF, Luc G, Clavey V, Staels B, Rigotti A. Fibrates down-regulate hepatic scavenger receptor class B type I protein expression in mice. *J Biol Chem.* 2003;278:7884-7890.
209. Guerin M, Le Goff W, Frisdal E, Schneider S, Milosavljevic D, Bruckert E, Chapman MJ. Action of ciprofibrate in type IIb hyperlipoproteinemia: modulation of the atherogenic lipoprotein phenotype and stimulation of high-density lipoprotein-mediated cellular cholesterol efflux. *J Clin Endocrinol Metab.* 2003;88:3738-3746.
210. Wasan KM and Cassidy SM. Role of plasma lipoproteins in modifying the biological activity of hydrophobic drugs. *J of Pharmaceutical Sciences.* 1998;87:411-424.