The role of apolipoprotein CI in lipid metabolism and bacterial sepsis

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Chapter 1

General Introduction

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Contents General Introduction

Cardiovascular disease (CVD) is the primary cause of death in the Western world, accounting for up to 50% of all mortalities[1-3]. Atherosclerosis is the main cause of CVD, and is considered a chronic inflammatory disease, characterized by the focal accumulation of cells, fibrous tissue, lipids and inflammatory blood constituents in the vessel wall[4,5]. This results in narrowing of the arteries, which may subsequently cause clinical manifestations such as myocardial infarction and stroke. Several risk factors have been identified, such as dietary habits, age, gender, smoking, hypertension, stress, and physical inactivity[3,6,7].

Sepsis, another inflammatory disease, occurs when a subject is unable to successfully contain an infection with microorganisms. This uncontrolled infection will lead to an exaggerated inflammatory response by the host, with organ failure and finally septic shock or death as a result. A full panel of microorganisms, as bacteria, parasites, fungi, and viruses, can trigger the pathophysiological cascade leading to sepsis. Sepsis is the leading cause of death in medical and surgical intensive care units with mortality rates ranging from 15-80% in critically ill patients[8,9], and the incidence is still increasing, despite the development of new supportive therapies[10,11].

Atherosclerosis and sepsis are related to each other, in that in both diseases the immune system plays a central role. In both diseases the immune system serves initially as a protective factor, but in the same manner may initiate damaging processes. In atherosclerosis, it is a critical player in the repair of damaged tissues, whereas at the same time the atherosclerotic lesion develops. During infection, the immune system is critical to combat the infection, but is getting harmful when the infection cannot be contained and progresses into sepsis.

Several apolipoproteins, which are proteins on circulating lipid sphericals in the bloodstream, have been shown to be potent modulators of inflammatory processes[12-17]. The function of apolipoproteins in lipid metabolism, atherosclerosis, and sepsis will be outlined in more detail in this introduction.

1. Lipid Metabolism

Cholesterol and triglycerides (TG), the most common lipids of a diet, are of vital importance in many different cellular processes in the human body. Cholesterol is essential for biosynthesis of cellular membranes, steroid hormones, and bile acids. TG-derived free fatty acids (FFA) can be used as an energy source in cardiac and skeletal muscle or they can be stored in adipose tissue. Since cholesterol and TG are hydrophobic, they are packaged into water-soluble spherical particles for their transport in the circulation. These spherical particles

are composed of a lipid-rich core containing hydrophobic cholesteryl esters and TG surrounded by a polar surface monolayer of phospholipids, unesterified free cholesterol, and one ore more amphiphatic proteins termed apolipoproteins. These apolipoproteins facilitate the formation of lipoproteins, modulate the activity of enzymes and lipid transfer factors involved in lipoprotein remodelling in the circulation, and modulate receptor-mediated binding and endocytosis of lipoproteins and/or their remnants.

1.1. Exogenous Pathway

Dietary TG and cholesteryl esters that are absorbed in the intestine are packaged into chylomicrons, and are transported from the lymph to the blood circulation [23]. Nascent chylomicrons are very large particles that consist mainly of TG but

Table 1. Physical properties, lipid and apolipoprotein composition of human plasma lipoproteins.

Properties	Chylomicron	VLDL	IDL	LDL	HDL
Source	Intestine	Liver	VLDL	VLDL	Liver+intestine
Diameter (nm)	75-1200	30-80	25-35	18-25	5-12
Density (g/mL)	<0.96	0.96-1.006	1.006-1.019	1.019-1.063	1.063-1.210
Electrophoretic Mobility*	Origin	pre-β	slow pre-β	β	α
Composition**					
Triglycerides	87	54	27	11	10
Phospholipids	8	19	23	23	31
Cholesteryl esters	3	14	32	40	21
Free cholesterol	1	7	8	8	7
Protein	1	6	10	18	31
Apolipoproteins					
ApoA	AI, AII, AIV, AV	AV	-	-	AI,AII,AIV,AV
ApoB	B48	B100	B100	B100	-
ApoC	CI,CII,CIII,CIV	CI,CII,CIII,CIV	CI,CII,CIII,CIV	-	CI,CII,CIII,CIV
ApoE	E	E	E	-	E
Main function	Transport of exogenous cholesterol and TG	Transport of endogenous TG	Cholesterol transport to peripheral tissues	Cholesterol transport to peripheral tissues	Reverse cholesterol transport to liver

* According to electrophoretic mobility of plasma α- and β-globulins on agarose gel electrophoresis.
** Expressed as percentage of total weight.
Apo, apolipoproteins and TG, triglycerides. Modified from Gotto *et al.*[18].

also phospholipids, cholesterol, cholesteryl esters, and apolipoproteins (e.g. apoAI, apoAIV, apoB48, and apoCs) (Table 1). Upon entering the circulation, these chylomicrons are processed by lipoprotein lipase (LPL), thereby delivering FFA to peripheral tissues such as adipose tissue (for storage into TG), and skeletal muscle and heart (as energy source). The resulting cholesterol-enriched core remnants are subsequently taken up mainly via apoE-specific recognition sites on hepatocytes, including the LDL receptor (LDLr), LDLr-related protein (LRP), heparan sulphate proteoglycans (HSPG), and possibly also Scavenger receptor BI (SR-BI)[24] (**Figure 1**).

1.2. Endogenous Pathway

Hepatocytes secrete cholesterol and TG packaged into VLDL. These lipids are either derived from incoming chylomicron remnants, IDL, LDL, and HDL, or from *de novo* synthesis[25,26]. The formation of VLDL is described as a two-step process[27,28]. In the first step, apoB100, the major structural apolipoprotein of VLDL, associates with the core lipids during formation of the particle. The microsomal TG transfer protein (MTP) catalyzes the transfer of lipids toward apoB100 and is in this way an essential link in the assembly of VLDL[29,30]. Thereafter, the particle fuses with a lipid droplet to become a mature VLDL particle, which can be secreted into the blood[27,31]. Nascent VLDL consists of TG, phospholipids, cholesteryl esters, cholesterol, and apolipoproteins (*e.g.* apoB100 and apoE) (**Table 1**). Upon entering the circulation the particle is further enriched with apoE and apoCs. These TG-rich VLDL particles serve, similarly as chylomicrons, as a source of FFA for extrahepatic tissues predominantly under fasting conditions. Hydrolysis of VLDL-TG by LPL results in the formation of IDL, which is partly taken up by the liver as mediated by apoE[32]. The remainder is extensively processed by LPL and hepatic lipase (HL) to become cholesterol-rich LDL with apoB100 as its sole apolipoprotein, which is recognized by the LDLr on the liver and peripheral tissues[32] (**Figure 1**).

1.3. Reverse Cholesterol Pathway

To maintain cholesterol homeostasis, excess cholesterol in extrahepatic tissues is returned via HDL to the liver, which is classically known as the only organ capable of disposing cholesterol via the bile[22]. However, recent findings suggest that cholesterol is also secreted from the circulation directly into the intestine without the involvement of the liver (Groen AK, unpublished observations). In the liver and intestine, nascent discoidal HDL (HDL_3) is formed from apoAI and phospholipids[33]. In the blood, discoidal HDL matures into spherical HDL (HDL_1) by acquisition of phospholipids from chylomicrons and VLDL via phospholipid transfer protein (PLTP), and cholesterol from the liver and peripheral tissues via

ATP binding cassette transporter AI (ABCA1), SR-BI, and ABCG1. The cholesterol is subsequently esterified by lecithin:cholesterol acyltransferase (LCAT) into cholesteryl esters, which can then be taken up by the liver, either directly via SR-BI[34], or indirectly via the LDLr, LRP, and/or HSPGs after transfer to VLDL and LDL in exchange for TG by the cholesteryl ester transfer protein (CETP)[35,36] (**Figure 1**). It is important to note that rodents normally do not express CETP[37], and therefore in these species there is no bidirectional exchange of cholesteryl esters and TG between HDL and (V)LDL.

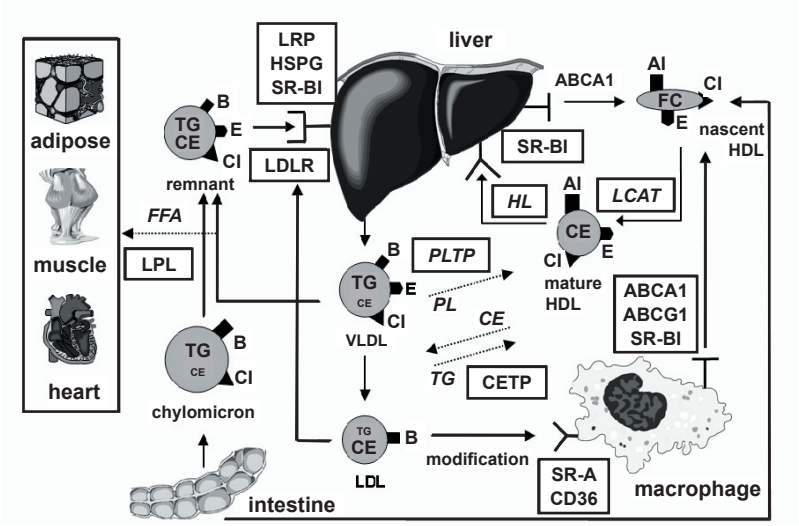

Figure 1. Lipoprotein metabolism. See text for explanation. AI, apolipoprotein AI; ABCA1/ABCG1, ATP-binding cassette transporter AI or GI; B, apolipoprotein B; CI, apolipoprotein CI; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; E, apolipoprotein E; FC, free cholesterol; FFA, free fatty acids; HSPG, heparan sulphate proteoglycans; LCAT, lecithin:cholesterol acyltransferase; LDLR, LDL receptor; LRP, LDL receptor-related protein; PL, phospholipids; PLTP, phospholipid transfer protein; SRA, scavenger receptor class A; SR-BI, scavenger receptor class B type I; TG, triglycerides. Modified from Berbée *et al*.[38].

2. Role of ApoE and ApoCI in TG-rich Lipoprotein Metabolism

2.1. Synthesis, Structure, and Function of ApoE

In 1973, Shore and Shore[39] identified apoE as a component of TG-enriched VLDL with a relatively high arginine content compared to other apolipoproteins known at that time, and referred to this protein as 'arginine-rich protein'. Consistent with the nomenclature of the other known apolipoproteins (apoA, apoB, apoC, and apoD) Utermann suggested the designation 'apoE' in 1975[40].

2.1.1. Synthesis and Structure of ApoE

The *APOE* gene, located on human chromosome 19 in the *APOE/APOC1/APOC4/APOC2* gene cluster, is 3.7 kb in length and contains four exons and three

introns[41]. The primary product of the *APOE* gene is a 317 amino acid prepeptide that gives rise to the 299 amino acid mature protein by cotranslational cleavage of an 18-amino acid signal peptide[42]. This 34.2 kDa apoE protein is synthesized in most organs, including the liver, spleen, lung, adrenal, ovary, kidney, and muscle, primarily by macrophages and in the liver also by hepatocytes[43,44]. ApoE is not expressed in the intestine. In the circulation apoE is mainly present on chylomicrons, VLDL, and HDL (**Table 1**) at total plasma levels of about 4-7 mg/dL[45,46]. The mouse *apoe* gene encodes a 285 amino acid mature protein, which has 70% homology with the human apoE protein[47]. Prediction of the secondary structure using the rules of Chou and Fasman[48], showed that the predicted structures of human and mice apoE are nearly identical with α-helical regions comprising two-thirds of the protein in 14 areas, and β-sheets comprising ~10% of the protein in three areas[47].

In the absence of lipids, apoE self-associates as a tetramer over a wide concentration range[50,51]. In contrast, self-association does not occur on lipid surfaces[52]. ApoE contains two domains that are joined by a protease-susceptible hinge region[50,53]. Thrombin digestion of apoE yields two fragments of which the 10 kDa C-terminal fragment harbours the lipid-binding domain, whereas the LDLr-binding domain (residues 139-153) is situated in the 22-kDa N-terminal fragment[54-56] (**Figure 2**). The C-terminal fragment contains three predicted α-helical regions, of which the third (amino acids 268-289) is critical for tetramer formation as well as lipoprotein association[57]. The N-terminal domain contains an antiparallel four-helix bundle, with the LDLr-binding domain is located in helix 4. The unusually high content of basic amino acids (Arg, Lys, His) within this LDLr-binding domain is important for binding to the LDLr, as has been demonstrated by chemical modifications[58,59]. Furthermore, apoE contains two heparin-binding sites of which one is located within the LDLr-binding site[60]. ApoE also interacts with HSPGs, which are suggested to be involved in both the secretion of apoE by hepatocytes and macrophages, as well as in the binding of lipoprotein-bound apoE[61].

2.1.2. Polymorphisms of ApoE

APOE has three common alleles known as ε2, ε3, and ε4[62]. This polymorphism results in six genotypes, three heterozygote (ε2/ε3, ε3/ε4, ε2/ε4) and three homozygote (ε2/ε2, ε3/ε3, ε4/ε4). In a typical Caucasian population, the frequency of the ε2, ε3, and ε4 alleles are approximately 8%, 80%, and 12%, respectively[46,63]. The encoded isoforms are distinguished from each other at two polymorphic sites: apoE2 (Arg^{112}, Arg^{158}), apoE3 (Cys^{112}, Arg^{158}), and apoE4 (Cys^{112}, Cys^{158})[62,64] (**Figure 2**). These isoforms differ in terms of their association with the various lipoproteins[65,66] and binding affinity for cell surface receptors

(*e.g.* LDLr)[67-70] and cell surface binding sites (*e.g.* HSPGs)[71,72]. While apoE3 and apoE2 are preferentially located on HDL, apoE4 preferentially interacts with large lipoproteins such as VLDL[65,66]. Furthermore, apoE2 exhibits lower affinity for the LDLr as compared to apoE3[67-70], resulting in dramatically reduced clearance of apoE and higher plasma apoE levels[46,63,73-75]. It is suggested that as a response the liver up-regulates the LDLr, resulting in lower cholesterol levels. Conversely, apoE4 is cleared more efficiently than apoE3, resulting in lower apoE levels, and concomitantly higher cholesterol levels[46,63,75].

Next to these common ε2, ε3, and ε4 alleles, several rare *APOE* variants have been reported (reviewed by Greenow *et al.*[76]). Most of these mutations are associated with hyperlipidemia (*e.g.* hypertriglyceridemia, hypercholesterolemia, type III hyperlipoproteinemia) as a result of defective LDLr binding[77-82] or as a result of apoE-deficiency[83-85].

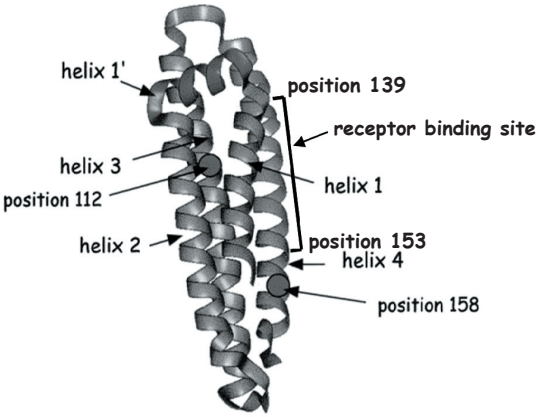

Figure 2. Ribbon model of the antiparallel N-terminal four-helix bundle of apoE. Highlighted are positions 112 and 158, which are either Arg or Cys residues in apoE2 (Arg^{112}, Arg^{158}), E3 (Cys^{112}, Arg^{158}), and E4 (Cys^{112}, Cys^{158}) isoforms (see text). The receptor-binding domain is located in helix 4 (residues 139-153). A short helix, helix 1', links helices 1 and 2. Adapted and modified from Weers *et al*.[49].

2.1.3. Functions of ApoE

ApoE is one of the most extensively studied apolipoproteins and appears to have numerous functions. The major role of apoE is the transport of (dietary) lipids within the blood circulation and determining the receptor-mediated uptake of these lipids as discussed above, and will be discussed in more detail in **section 2.3**. Furthermore, apoE has been proposed to play a role in intracellular lipid redistribution[86,87], VLDL-secretion[88], LPL inhibition[89] reverse cholesterol transport[80], atherosclerosis (discussed in **section 3.2**), and immunomodulation (discussed in **section 4.4.2**). In addition, apoE may have a role within the central nerve system. The *APOE4* gene is associated with familial and sporadic forms

of late-onset Alzheimer's disease, a neurodegenerative disorder associated with progressive dementia[90-92]. The reduced ability of the brain to respond to damage in ε4 carriers associated with not only the rate of progression and/or age of onset of Alzheimer's disease, but possibly also with other neurodegenerative disorders (*e.g.* Parkinson's disease, amyotrophic lateral sclerosis), as well as coma's length following traumatic brain injuries[93-95]. Interestingly, the *APOE4* polymorphism shows strong linkage-disequilibrium with the *Hpa* I polymorphism in the *APOC1* promotor[45,96,97], which is also associated with risk for Alzheimer's disease[98]. The consequence of this linkage-disequilibrium on causality for the above mentioned neurodegenerative disorders remains to be determined.

2.2. Synthesis, Structure, and Function of ApoCI

In the mid sixties, early seventies, three human apoCs were identified and characterized, apoCI[99-102], apoCII[99-101] and apoCIII[101]. They were initially referred to as apo-Val (later corrected to apo-Ser), apo-Glu and apo-Ala, respectively, as designated by their carboxyl terminal amino acids. These apoCs are often portrayed as members of one consistent protein family, because of their similar distributions among lipoprotein classes, their low molecular weights, and coincident purification. In 1995, Allan *et al.*[103] identified and characterized apoCIV as a fourth member of this human apoC-family, which was first discovered in mice[104]. ApoCIV is less well studied than the other three apoCs. It is undetectable in human plasma[103], and to date no major modulating role for apoCIV could be identified. The role of the other three apoCs, in particular as significant modulators of lipoprotein metabolism, has been extensively reviewed[105,106]. ApoCII is known as an essential cofactor of TG lipolysis by LPL[107-111], whereas apoCIII is primarily known as the main endogenous inhibitor of LPL[112-117]. The function of apoCI will be outlined in more detail below.

2.2.1. Synthesis and Structure of ApoCI

The human *APOC1* gene is located 4.3 kb downstream from the *APOE* gene on chromosome 19 in the same transcriptional orientation[118,119]. The *APOC1* gene is about 4.7 kb in size and is primarily expressed in the liver, but also at low levels in a wide variety of other tissues, including lung, skin, and spleen, where it is primarily expressed by macrophages within these tissues[118]. ApoCI is synthesized with a 26-residue signal peptide, which is co-translationally cleavaged, resulting in the formation of mature apoCI that consists of only 57 amino acids. With a molecular weight of 6.6 kDa mature apoCI is the smallest known apolipoprotein. The mouse *apoc1* cDNA contains an open reading frame encoding a protein of 88 amino acids, including a signal peptide of 26 amino acid residues, finally resulting in a mature apoCI of 62 amino acid residues[120]. The mature mouse

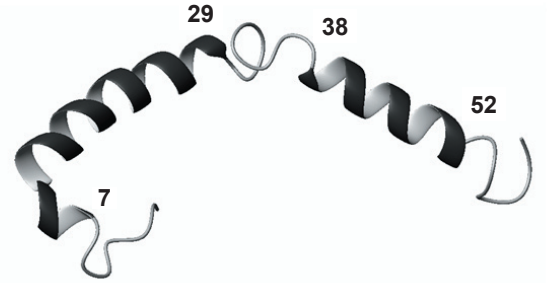

Figure 3. Structure of human apoCI. ApoCI consists of 2 α-helical structures, residues 7-29 (with a mobile hinge region involving residues 12-15) and residues 38-52, linked with a structurally unordered region (residues 30-37).

apoCI protein shares 67% homology with the human protein. Comparisons of amino acid sequence of apoCI from different species (human, baboon, mouse, rat, and dog) showed discrete regions with a high degree of conservation[120].

Just like apoCII and apoCIII, apoCI is mainly present on chylomicrons, VLDL and HDL (**table 1**), and circulates at levels in serum of about 8 mg/dL[45,121]. Predominantly due to its high lysine content (*i.e.* 16 mol%), human apoCI has the highest isoelectric point of all apolipoproteins (pI 6.5), a feature which is often used for the purification of the protein from other apolipoproteins[122-124]. Human apoCI has a boomerang shape, it contains two amphipathic α-helices, the N-terminal helix (residues 7-29) and the C-terminal helix (residues 38-52), separated by an unordered flexible linker[125] (**Figure 3**). The N-terminal domain contains a mobile hinge involving residues 12-15. The hydrophobic side chains cluster on the nonpolar face of both helices, thus forming two discrete lipid binding sites in the N-terminal helix and one in the C-terminal helix. The C-terminal helix is tightly lipid-bound, while the N-terminal helix has lower lipid-binding affinity, but is more flexible and able to adjust to the lipoprotein size and composition[125]. In other words, the C-terminal helix may act as a lipid anchor, while the N-terminal helix may be located more on the surface of the lipoprotein able to hinge off the lipid surface.

2.2.2. Polymorphisms of ApoCI

So far, no apoCI-deficient humans have been reported. Until recently, no human structural mutations had been reported as well, however Wroblewksi *et al.*[126] recently described the first structural polymorphism of apoCI. This polymorphism involves the substitution of a tyrosine on position 45 for a serine, and could be found only in persons of American Indian, or Mexican ancestry, and not in individuals with ancestry of Europe, Africa and Asia. Within these American Indian, or Mexican ancestry about 14% of the individuals were carrier for this

mutation. Initial studies suggest that the S45 variant has higher preference for VLDL and lower preference for HDL as compared to normal apoCI, but additional studies are necessary to confirm these findings and to unravel whether this polymorphism also leads to functional changes of apoCI.

In contrast, a common *Hpa* I polymorphism has been described already two decades ago[97,127,128]. This polymorphism is produced by a 4-bp CGTT insertion 317 bp upstream of the transcription initiation site of apoCI. *In vitro* studies showed that this polymorphism decreased the binding of a negatively acting transcription factor, leading to increased expression of apoCI[97]. Follow up studies in human populations did confirm that circulating apoCI levels were (at least partly) dependent on the *Hpa* I polymorphism, however conflicting results have been obtained, as both negative and positive significant associations have been reported (even within the same study)[45,121]. The results suggest that the biological impact of the *Hpa* I polymorphism is largely dependent on factors as gender, age, ethnicity, and hyperlipidemic state. As mentioned above, the *Hpa* I polymorphism is in almost complete linkage-disequilibrium with both the *APOE2* and *APOE4* alleles, but not the *APOE3* allele, which are located in the same gene cluster[45,96,97]. Interestingly the *Hpa* I polymorphism has been associated with increased risk for Alzheimer's disease[98].

2.2.3. Functions of ApoCI

The first role ascribed to apoCI was by Havel *et al.*[115], who showed that apoCI inhibited TG-hydrolysis by LPL. A few years later an inhibitory effect on HL activity was suggested as well[129], but these studies comprise *in vitro* findings and conformational *in vivo* studies were thus required. The inhibitory functions of apoCI on LPL and HL will be discussed in more detail in the next **section 2.3**. Others showed that apoCI was able to activate LCAT *in vitro*, resulting in increased formation of cholesteryl esters[130-132]. Importantly, both *in vitro* and *in vivo* studies showed that apoCI interferes with the apoE-mediated binding and/or uptake of TG-rich lipoproteins by lipoprotein receptors (*e.g.* LDLr and LRP)[133-136], which will be outlined in more detail in **section 2.3.3**. Furthermore, apoCI has been shown to inhibit CETP[137,138], and has been suggested to play a role in Alzheimer's disease[98], apoptosis of vascular smooth muscle cells[139], and atherosclerosis (the latter discussed in **section 3.3**).

2.3. Role of ApoE and apoCI in TG-rich Lipoprotein Processing

The metabolism of TG-rich lipoproteins, such as chylomicrons and VLDL, in the circulation is complex, and not yet fully understood. Indisputably, TG-rich lipoproteins are converted into lipoprotein remnants by size reduction via the hydrolysis of the core TG by lipases, primarily by LPL and HL. Subsequently,

these remnants are mainly taken up by the liver (~80%), but also by extrahepatic tissues, mediated via lipoprotein receptors. Most of the receptors participating in the uptake of TG-rich lipoproteins belong to the LDLr gene family (*e.g.* LDLr, VLDL-receptor (VLDLr) and LRP), but also binding sites outside this receptor family have been shown to be involved (*e.g.* HSPGs and SR-BI). Both apoE and apoCI have been proposed to play major roles in TG-rich lipoprotein processing.

2.3.1. Lipoprotein Lipase

As mentioned above, the hydrolysis of the core TG in chylomicrons and VLDL is an essential step in the processing, and the subsequent uptake, of these particles. The main enzyme responsible for this action is LPL, a member of a conserved lipase gene family, which included amongst others, HL, endothelial lipase, and pancreatic lipase[140]. By hydrolyzing TG, LPL liberates fatty acids, which can be used either directly as an energy source by the muscle and heart, or indirectly via storage as TG in adipose tissues. LPL is expressed in virtually all tissues, and is most abundant in adipose tissue, heart, and skeletal muscle[141-143]. LPL is not expressed in the adult liver[143]. Active LPL consists of a homodimer of two non-covalently linked glycoproteins of equal size[144,145].

The role of LPL in lipid metabolism goes beyond the hydrolizing properties of the enzyme. Once LPL is released from the cell membrane it circulates in plasma mainly as a monomer. As a monomer, LPL is able the enhance the binding and/or internalization of lipoproteins via the LDLr[146,147], LRP[148-151], VLDLr[152,153] and HSPGs[154,155], most likely by bridging the lipoprotein particle directly to the receptor[148].

LPL requires apoCII as a co-factor to be catalytically active[107-111]. Also other apolipoproteins have been shown to influence the lipolytic activity of LPL. The main endogenous inhibitor of LPL is apoCIII[112-117]. Studies in transgenic mice and gene-targeted mice have documented the physiologic significance of the action of apoCIII in decreasing lipolysis[156-161]. A few years ago, apoAV was discovered as a novel apolipoprotein[162,163]. Recent work indicates that apoAV increases the LPL-mediated hydrolysis of TG by guiding VLDL and chylomicrons to HSPG-bound LPL[164,165]. Since apoAV circulates in very low amounts in the human circulation (about 200-2000 ng/mL)[166,167], the physiological relevance of the LPL activation by apoAV requires further investigation.

Besides apoCIII and apoAV, also apoE and apoCI have been suggested to modulate the lipolysis of TG by LPL. ApoE was shown to directly stimulate LPL activity in the absence of apoCII[168,169], and was postulated to be required for the LPL-mediated metabolic conversion of VLDL into LDL[170]. However, in the

presence of the co-factor apoCII, apoE effectively inhibits LPL-mediated lipolysis of TG-rich particles *in vitro*[171,172] and *in vivo*[89]. The physiologic importance of apoE-mediated inhibition of LPL is still subject of discussion. The role of apoCI in modulating the LPL-activity is much less described. A few decades ago, apoCI was shown to inhibit LPL *in vitro*[115,173-176]. However, apoCI was not as efficient as apoCIII, leading apoCIII as the main focus of investigation. Eventually, in the nineties apoCI transgenic[133,159,177] and knockout[178] mice were generated. The predominant hypertriglyceridemia in the apoCI transgenic mice[133,159] is suggestive for apoCI-mediated inhibition of LPL *in vivo* as well; however no *in vivo* evidence has been reported so far.

2.3.2. Hepatic Lipase

Another enzyme that is postulated to be involved in remnant metabolism is HL. HL is primarily synthesized by hepatocytes, secreted, and mainly bound to the surface of parenchymal and hepatic endothelial cells associated with HSPG[179-181]. The functional unit is a monomer in the liver, and may be a dimer in other tissues as the adrenal gland and ovary[182]. LPL and HL differ in their substrate preference and specificity. While LPL is mainly responsible for the hydrolysis of plasma TG, HL efficiently hydrolyzes phospholipids and has lower preference for TG[183-187]. In line with this, the preferred enzymatic substrates are IDL and HDL, but HL is also capable of processing chylomicrons and VLDL[183,188-190]. HL does not have an absolute requirement for a cofactor in order to be enzymatically active, but the activity can be modulated by several apolipoproteins. The effects of HDL apolipoproteins on HL activity are well described *in vitro*[129,191,192]. ApoAI[191,192], apoCI[129,192], apoCII[129,193], and apoCIII[129,192] have been suggested to inhibit HL-mediated hydrolysis of TG, whereas for apoAII[191,192,194] and apoE[192,193,195,196] both inhibition and activation of HL-activity have been reported. apoE was suggested to activate HL-mediated hydrolysis of phospholipids in small HDL particles (with looser lipid packing), but not in larger VLDL particles (with tighter lipid packing), which could explain the inconsistencies found in earlier reports[197]. The physiologic relevance of HL-inhibition by apoCI *in vivo* remains to be elucidated. Recently it was suggested that apoCI-mediated inhibition of HL is responsible for the hypertriglyceridemic phenotype in apoCI transgenic mice[198]. Strikingly, HL-deficient mice do not show any sign of disturbed TG metabolism[199-201], arguing against HL-inhibition as a major determinant of the observed hypertriglyceridemic phenotype in *APOC1* mice.

2.3.3. TG-rich Lipoprotein Uptake Mechanisms

Lipoprotein receptors like the LDLr, LRP, and VLDLr play crucial roles in lipid homeostasis by mediating the cellular uptake of primarily TG-rich lipoproteins.

These receptors belong to the LDLr gene family, which represents a class of endocytic receptors that is present in both vertebrate and non-vertebrate species. In the last years, these receptors have been identified and characterized[202-204]. The members of this family exhibit several distinct functional domains: 1) an amino-terminal ligand binding domain; 2) an epidermal growth factor precursor homology domain; 3) an O-linked sugar domain; 4) a transmembrane domain that is required for anchoring the receptor to the plasma membrane; and 5) a cytoplasmatic region with a signal (Asp-Pro-X-Tyr) for receptor internalization via coated pits[205-207] (**Figure 4**).

2.3.3.1. LDLr

The LDLr (120 kDa) is the prototype of the LDLr family, is highly expressed in tissues that utilize lipoproteins, such as the liver and adrenals[208], and recognizes both apoB100 and apoE[209]. The LDLr can contribute to the clearance of both chylomicrons and VLDL (remnants) *in vivo* in animals as well as in humans. The clearance of these particles is mainly mediated via apoE, which binds to the LDLr via its LDLr-binding domain (residues 139-153; **Figure 2**)[58,59]. The LDLr also recognizes apoB100, the sole apolipoprotein of (mainly cholesterol-rich) LDL, and via this interaction the LDLr mediates the uptake of LDL from plasma (**Table 1**). ApoCI has been shown to inhibit the apoE-mediated hepatic uptake of TG-rich lipoprotein remnants by the LDLr, possibly by masking of the receptor binding domain of apoE[135], or via displacement of apoE from lipoprotein particles[136].

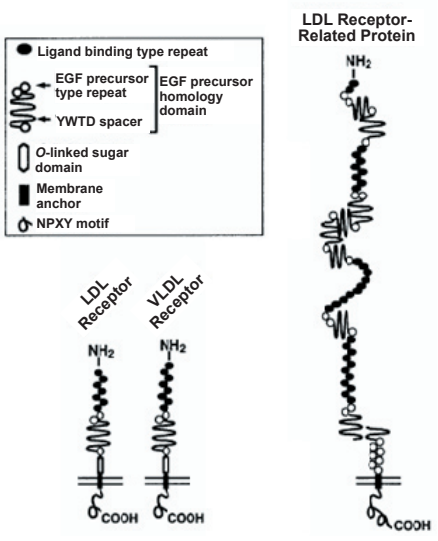

Figure 4. Schematic structures of several members of the LDLr family. See text for explanation. NPXY designates the tetraamino acid motif Asp-Pro-X-Tyr which directs the receptors into coated pits. EGF, endothelial growth factor. Adapted from Willnow *et al.*[204].

2.3.3.2. LRP

Besides the LDLr, also LRP (*i.e.* LRP1) plays a role in TG-rich lipoprotein uptake. LRP (also known as the α2-macroglobulin receptor) is the largest (*i.e.* 600 kDa) endocytotic receptor identified to date, and is expressed in a variety of tissues, such as the liver, intestine, lung, and brain, and in numerous cell types, such as fibroblasts, SMCs, monocytes/macrophages [210-212]. LRP1 is a heterodimer consisting of a 515 kDa extracellular and an 85 kDa membrane anchored subunit[213], and recognizes >50 structurally and functionally different ligands[214,215], including apoE-containing lipoproteins[216-219]. Similarly as for the LDLr, apoCI also inhibits the apoE-mediated uptake by LRP, probably via the same mechanism[136]. Studies form our group suggested that the inhibiting properties of apoCI towards LRP may exceed those towards the LDLr, because the apoCI-associated hyperlipidemia was substantially more pronounced on an LDLr-deficient background as compared to a wild-type background[133,134].

2.3.3.3. VLDLr

The member of the LDLr family that most closely resembles the LDLr is the VLDLr[220,221]. The VLDLr enhances the binding and uptake of apoE-containing lipoproteins, such as chylomicrons, VLDL, and IDL, but not LDL[152,220]. Similar as for the other members of the LDLr family, the apoE-mediated binding of TG-rich lipoproteins to the VLDLr is inhibited by apoCI[134]. The VLDLr is most abundantly expressed in tissues active in FFA metabolism, such as the heart, skeletal muscle, and adipose tissue[222-226], and only trace amounts are found in liver[221,227]. Within these tissues the VLDLr is mostly localized in endothelial cells and SMCs of arteries and veins[228,229]. Like LRP, the VLDLr is also a multiligand receptor, and is able to facilitate the uptake of fibrinolysis products[153] and extracellulair matrix proteins[230].

2.3.3.4. HSPG

HSPGs play also a role in TG-rich lipoprotein remnant clearance as part of the “HSPG/LRP pathway”[231-233]. HSPGs are components of the extracellular matrix within the Space of Disse, as well as collagen, fibronectin, laminin, and elastin. HSPGs are heterogenous, with respect to their number of chains per polypeptide, chain length, and extent of postpolymeric modifications, such as N-acetylation, N-sulfation, and O-sulfation[234,235]. Both *in vitro*[61,236] and *in vivo*[237,238] studies showed that HSPGs are involved in TG-rich lipoprotein clearance. It is envisioned that TG-rich lipoproteins may initially sequester within the Space of Disse through interaction with apoE bound to HSPGs, which are found on the microvilli of parenchymal cells[61,239-241]. HSPGs are thought not to be involved in the actual ligand uptake process, but to transfer the TG-rich lipoproteins to

an internalizing receptor, such as the LRP and the LDLr[242], although a direct low affinity HSPG-mediated internalisation of TG-rich particles has also been described[243].

2.3.3.5. SR-BI

Recently, SR-BI, which is well known for mediating selective uptake of cholesteryl esters from HDL without concomitant uptake of HDL protein[244], was shown to accelerate chylomicron metabolism[245]. Similar as for HSPGs, SR-BI probably mediates the initial capture of chylomicron remnants by the liver, whereby the subsequent internalization can be exerted by additional receptors like the LDLr and LRP. The role of apoE and other apolipoproteins in this SR-BI-mediated pathway still has to be elucidated.

3. Role of ApoE and ApoCI in Atherosclerosis

Atherosclerosis is the main cause of CVD such as myocardial infarction and stroke, and accounts for up to 50% of all mortality in Western countries[3]. Atherosclerosis has traditionally been viewed to simply reflect the deposition of lipids within the vessel wall. Classically, elevated cholesterol and/or TG levels, and in particular high LDL-cholesterol and low HDL-cholesterol levels, are the principal risk factors of atherosclerosis. However, nowadays atherosclerosis is considered not only as a disease of the lipids, but also as a chronic inflammatory disease of the intima, slowly developing in time starting from childhood, resulting in additional risk factors such as high C-reactive protein (CRP) for example[3,6,246]. Other risk factors for this multifactorial disease include age, gender, smoking, hypertension, stress, dietary habits, and physical inactivity[3,6,7].

3.1. The Pathogenesis of Atherosclerosis

Endothelial cells can be exposed to many forms of injury, including infectious, immunological, chemical, radiation and mechanical injury, which has an impact on their cellular structure and function[3,7]. As a result, markers such as vascular cell adhesion molecule 1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and selectins (*e.g.* E-selectin and L-selectin) are expressed. These adhesion molecules attract monocytes, which start rolling on the endothelium leading to their attachment and infiltration into the intima, initiating the formation of the lesion or plaque[247-249] (**Figure 5**). The presence of macrophage colony-stimulating factor in the vessel wall mediates the maturation of the infiltrated monocytes into macrophages, which are scavenging and antigen-presenting cells that secrete cytokines, growth-regulating molecules, chemokines, proteases, reactive oxygen radicals, and other inflammatory molecules[250,251]. These macrophages

may initially serve as a protecting factor, by playing a critical role in the repair and damaging processes while the lesion develops. However, scavenging of modified LDL results in lipid-laden macrophages, called foam-cells, which subsequently results in the formation of a fatty streak. This fatty streak is still completely reversible, but may progress into an advanced lesion by the influx of additional monocytes and T-cells, depending on the balance of proatherogenic and anti-atherogenic factors[4]. The resulting proatherogenic micro-environment in the lesion by the increased inflammation and tissue damage, stimulates migration of fibroproliferative vascular smooth muscle cells (SMCs), derived from the underlying media or circulating progenitor cells, to the endothelium to form a protective fibrous cap[3,252]. These SMCs are also capable of accumulating cholesterol and contribute to the foam cell formation. Further progression of the plaque includes the accumulation of foam cells and the formation of a lipid core. Also other immunocellular components as T-and B-cells, mast cells, natural killer cells, neutrophils and dendritic cells, present in the advanced atherosclerotic lesion are able to modulate the progression of the lesion. Subsequently, macrophage death by apoptosis or necrosis, as a consequence of cholesterol-toxicity, inflammatory cytokines, oxidative stress, and growth factor depletion, contributes to the formation of a necrotic core[253,254]. At this point the advanced fibrous lesion consists of a fibrous cap that covers a core of foam cells, macrophages and other inflammatory cells, SMCs, extracellular lipids, and a necrotic core. The plaque is stable when a uniform thick fibrous cap is formed. On the other hand,

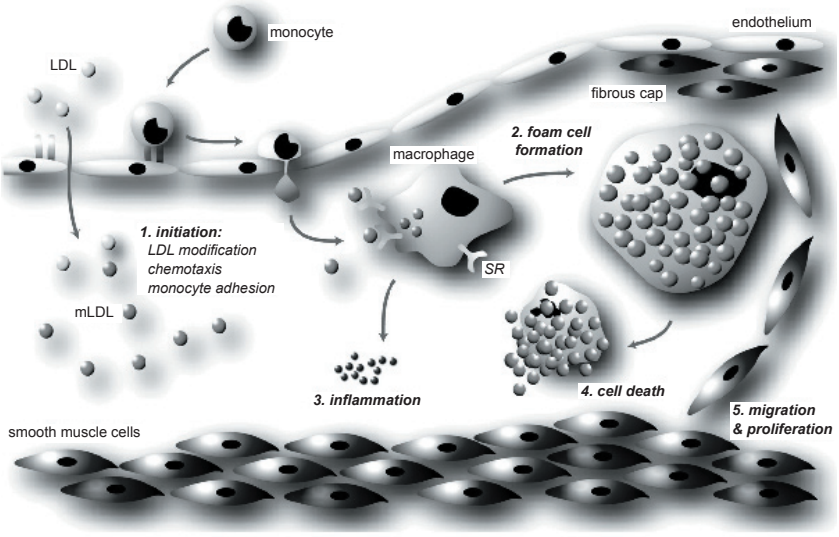

Figure 5. Schematic overview of atherogenesis from early to advanced atherosclerotic lesion formation. See text for explanation. mLDL, modified LDL; SR, scavenger receptor. Adapted from De Winther *et al*.[256].

continuous influx and activation of macrophages, releasing metalloproteinases and other proteolytic enzymes in the plaque can result in thinning of the fibrous cap[3,252,255]. The concomitant instable cap can lead to rupture of the plaque, which in worst case can occlude the vessel and result in cardiovascular events such as myocardial infarction and stroke.

3.2. ApoE in Atherosclerosis

A major focus of recent research on CVD has been to understand the molecular basis of atherosclerosis in detail, and has resulted in the identification of a key, but complex, role for apoE in this process. ApoE has been ascribed many anti-atherosclerotic functions (summarized in **Figure 6**), of which its central role in the regulation of lipid metabolism (discussed in **section 1 and 2**) is probably the most important and can be attributed to several actions: 1) Uptake and degradation of lipoprotein remnants by the liver[79,80,257], 2) stimulation of the reverse cholesterol transport[80,258,259], and 3) activation of enzymes involved in HDL-metabolism such as LCAT[260] and possibly HL (discussed in **section 2.3.2**). These above functions of apoE in lipid metabolism can all be considered as anti-atherogenic mechanisms. However, apoE may also have a pro-atherogenic function in lipid metabolism by: 1) stimulating the hepatic VLDL production[88,261], and 2) activating CETP[262], which is considered a pro-atherogenic lipid transfer protein[263-265]. Since mice normally lack expression of CETP[37] this is not relevant in mice, but may be of importance in the human situation.

Additional anti-atherosclerotic functions of apoE have been identified which are mostly anti-inflammatory of nature. ApoE expressed by macrophages inhibits platelet aggregation by interacting with a specific cell surface receptor, the apoE receptor 2 (apoER2), initiating a signalling cascade leading to activation of nitric oxide (NO) synthase and the subsequent decrease in NO[266,267]. In addition, via the same mechanism apoE inhibits VCAM-1 expression on endothelial cells[268]. Furthermore, apoE inhibits T-cell activation and proliferation[269-271], and SMC migration and proliferation as induced by platelet-derived growth factor and oxidized LDL[272-274]. ApoE may prevent the accumulation of oxidized LDL by inhibiting lipid oxidation[275-277], and is suggested to inhibit endothelial cell proliferation by modulating the availability of cytokines and growth factors retained in the pericellular proteoglycan matrix[278]. Data also support an anti-inflammatory role for apoE in suppressing acute inflammation by lipopolysaccharide (LPS)[15,279-281] or bacteria (*e.g. Listeria monocytogenes*[282], *Klebsiella pneumoniae*[280,283] as will be discussed in **section 4.4.2**, which is likely to also have anti-atherogenic consequences.

In contrast to these anti-inflammatory properties, apoE has also proinflammatory properties, which thus may aggravate atherosclerosis

development. Van den Elzen *et al.*[16] showed that apoE is involved in enhancing the presentation of lipid antigens by dendritic cells. ApoE captures lipid antigens in the circulation, and, via an LDLr-mediated uptake route, these lipid antigens are subsequently presented on the surface of dendritic cells (also discussed in **section 4.4.2**).

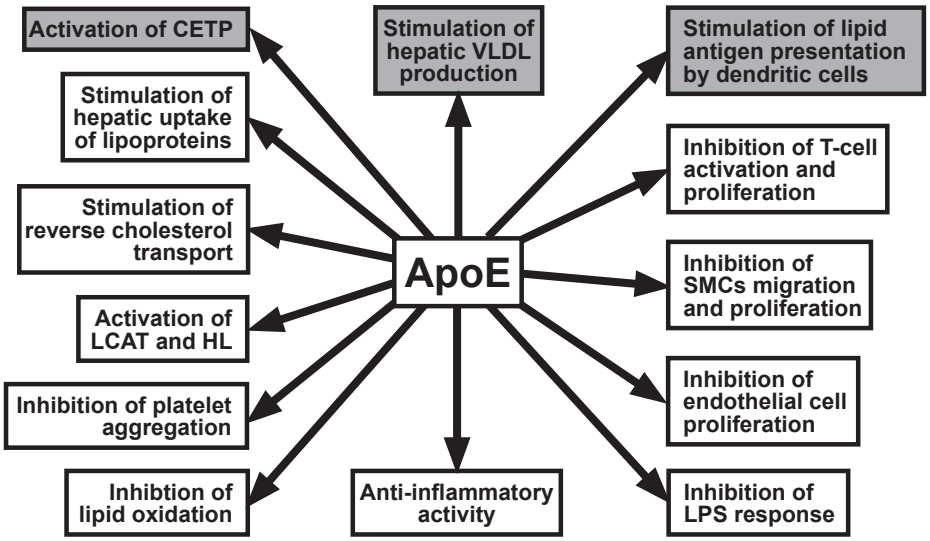

Figure 6. Proposed anti- (white boxes) and pro- (grey boxes) atherogenic roles of apoE. See text for explanation. CETP, cholesteryl ester transfer protein; HL, hepatic lipase; LCAT, lecitin:cholesterol acyltransferase; SMC, smooth muscle cell. Modified from Gilnow *et al.*[76].

Most of the understanding of the role of apoE in atherosclerosis as described above has been generated by the use of apoE-deficient mice. Disruption of the *apoe* gene in mice is associated with hypercholesterolemia and spontaneous, severe atherosclerosis that can be further enhanced by cholesterol feeding[284-286]. The development from the fatty streak to the advanced plaque in this model is to a certain extent similar as in the human plaque development, and, therefore, this model is widely used in atherosclerotic research.

In humans, apoE-deficiency (characterized by less than 1% of the normal apoE concentration in plasma) is associated with hyperlipidemia, mainly high VLDL, IDL, and LDL levels[83,85,287-292], increased lipid storage in monocyte/macrophages and phenotypic expression of xanthomas (massive foam-cell accumulation) early in life[83,85,291], and premature development of atherosclerosis[83,85,291]. Interestingly, heterozygous apoE-deficient subjects have normal lipid levels, although apoE concentrations are only approximately half of normal

levels[85]. Thus, only the nearly complete deficiency of apoE will result in increased risk to develop atherosclerosis.

A number of studies have investigated the impact of the common human apoE2, apoE3, and apoE4 isoforms on cardiovascular research[293-296]. These isoforms have distinct effects on lipid metabolism (discussed in **section 2.1.2**). ApoE2 is associated with higher plasma apoE levels, but lower cholesterol levels as compared to apoE3 subjects[46,63,73-75], whereas in apoE4 subjects lower plasma apoE levels and higher cholesterol levels are found[46,63,75]. The results on the association of the ε2 allele with CVD have yielded conflicting results; both harmful and protective associations have been found[293-296]. On the other hand, the ε4 allele has been consistently associated with an increased risk of CVD[293-296]. Importantly, irrespective of the *APOE* genotype, high plasma apoE levels are associated with increased cholesterol levels[63,73]. This may indicate that in humans high plasma apoE levels are associated with increased CVD irrespective of the *APOE* genotype, but studies addressing this hypothesis have not yet been reported.

3.3. ApoCI in Atherosclerosis

The role of apoCI in atherosclerosis has been far less studied than the role of apoE. It appears that researchers only recently started to really appreciate the significant role of apoCI in lipid metabolism and started studies on the role of apoCI in atherosclerosis development. ApoCI has potent hyperlipidemic effects by inhibiting the hepatic apoE-mediated uptake of (atherogenic) remnant particles, and possibly also by inhibiting the processing of TG-rich lipoproteins by LPL (discussed in **section 2**). Besides its hypertriglyceridemic effects, *in vitro* studies have indicated that apoCI may also promote plaque rupture by inducing apoptosis of aortic SMCs, via recruitment of neutral sphingomyelinase[139]. In contrast apoCI may also possess anti-atherosclerotic properties by promoting cholesterol efflux from macrophages via ABCA1[297], possibly via stabilisation of ABCA1. In addition, apoCI is the main endogenous inhibitor of CETP, which is a very promising anti-atherosclerotic characteristic[122,138]. Inhibition of this proatherogenic lipid transfer factor increases circulating HDL levels[298-300] and subsequently may decrease atherosclerosis risk.

Expression of human apoCI in mice, that naturally do not express CETP[37], aggravated atherosclerosis development[198]. To investigate the potentially important anti-atherosclerotic characteristic of apoCI as an inhibitor of CETP, studies using human CETP transgenic mice have been performed. Initial studies showed that human apoCI expression in CETP transgenic mice resulted in decreased specific CETP activity, but simultaneously increased total CETP mass as compared to

their controls[301]. Since the overall CETP activity was probably enhanced, this led to an even aggravated proatherogenic lipoprotein profile, with decreased HDL levels and markedly increased VLDL and LDL levels, indicating that the potential anti-atherogenic properties of apoCI are overruled by its proatherogenic properties in this model. However, this has to be confirmed in humans, since data on association of plasma apoCI levels with CVD or clinical endpoints are still lacking. However, human studies did reveal that the apoCI content of TG-rich lipoproteins in the postprandial state predicts early atherosclerosis in normolipidemic healthy men[302-304]. This proatherogenic effect of apoCI is most likely a result of reduced processing of the postprandial TG-rich lipoproteins by lipases and inhibited receptor binding, and the concomitant delayed uptake of (atherogenic) TG-rich lipoproteins, as evident from experimental studies (see also **section 2**). Recently, Kwiterovich *et al*.[305] described the presence of an elevated large HDL particle enriched in apoCI, in infants of lower birth weight and younger gestational age. Although this apoCI-enriched particle disappears soon after birth, these infants have increased risk of CVD in adulthood[306]. The molecular mechanism behind this association remains to be elucidated.

4. Role of ApoE and Other Apolipoproteins in Inflammation and Sepsis

Sepsis is a major cause of morbidity and mortality. It affects approximately 700,000 people annually and accounts for about 210,000 deaths per year in the United States[10,11]. In fact, it is the leading cause of death in medical and surgical intensive care units[8,9]. Due to advances in medical practice and technology, as the use of invasive equipment, implantation of prosthetic devices, and administration of corticosteroids and other immunosuppressive agents to patients with organ transplants or inflammatory diseases, the incidence is still rising at rates between 1.5% and 8% per year[10,11].

4.1. Infection, Sepsis, and Lipopolysaccharide

Sepsis is currently viewed as a complex dysregulation of the inflammatory response arising when the host is unable to successfully contain an infection with microorganisms, as bacteria, parasites, fungi, and viruses[11,307,308]. Infection with microorganisms first results in a proinflammatory response, during which proinflammatory cytokines (*e.g.* tumor necrosis factor-α (TNFα), interleukin-1 (IL-1), IL-6, and IL-12) are produced to effectively respond to the infection[309-311]. This first proinflammatory response is crucial to combat the bacterial infection in the early phase (**Figure 7**). When this initial proinflammatory response is inadequate and the invading microorganisms multiply, the proinflammatory

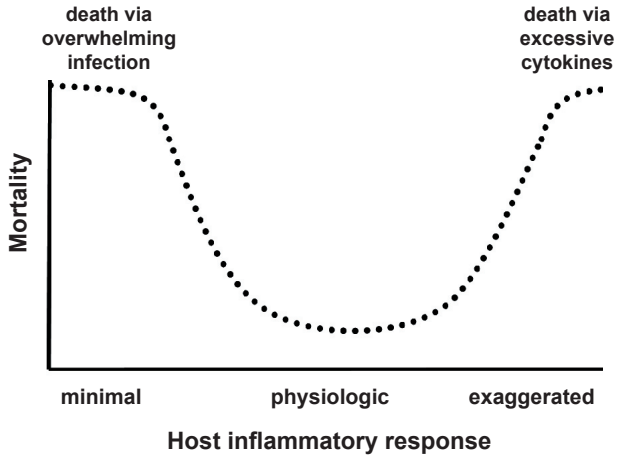

Figure 7. The U-shape relationship between the host inflammatory response and mortality. See text for explanation. Adapted from Cross *et al.* (*Cross AS, International Endotoxin Society Meeting, Kyoto, Japan, 2004*).

response enhances and develops into a systemic inflammatory response syndrome (SIRS). This response is counteracted by the compensatory anti-inflammatory response syndrome (CARS), during which IL-4, IL-10, IL-13, and other cytokines are produced. The correct balance of SIRS and CARS, as well as the intensity of these responses greatly influences host survival[309-311]. Imbalance between these responses can result in host damage (**Figure 7**). If the balance is shifted towards SIRS, excessive proinflammatory cytokine production will cause direct host damage. A shifted balance towards CARS will result in increased proliferation of the infection, eventually leading to an excessive proinflammatory response. Thus, an efficient proinflammatory response is crucial to prevent rapid multiplication of the invading microorganism and to surmount the first phase of infection, whereas in a later phase a high proinflammatory response is often harmful and may lead to tissue damage and organ failure.

Most cases of sepsis are caused by bacteria. The occurrence of Gram-positive sepsis increased over the last decades and accounts for 30-50% of all cases, whereas the incidence of Gram-negative sepsis is somewhat lower, but still accounts for 25-30% of all sepsis cases[307,308,312,313]. While Gram-positive bacteria contain a number of immunogenic cell wall components (*e.g.* M protein), in addition to often highly deleterious exotoxins, such as lipoteichoic acid (LTA) and peptidoglycan, Gram-negative bacteria share LPS as their main pathogenic component[314,315]. In fact, injection of LPS alone causes the same clinical features as can be seen in patients with Gram-negative sepsis[316]. LPS is essential for the growth and structural integrity of the bacteria[317-319], and, incorporated in the

bacterial membrane, activation of the immune cells is poor[320]. However, the release of LPS from the membrane during both cell division and death, exposes the toxic lipid A moiety to immune cells, evoking an immunological response[321,322]. The LPS molecule consists of 4 different parts: 1) lipid A, 2) the inner core, 3) the outer core, and 4) the O-antigen (**Figure 8**)[319,322,323].

The lipid A moiety is the toxic part of LPS. It is the lipid component of LPS and consists of 6 or more fatty acid residues linked to 2 phosphorylated glucosamine sugars. Despite the common architecture, lipid A of different bacterial origin varies in their fine structure. These variations are: 1) the acylation pattern; 2) length of the fatty acid residues; 3) the presence of 4-amino-deoxy-L-arabinose and/or phosphoethanolamine linked to the phosphor groups on the glucosamine sugars; and 4) the number of fatty acids (most bacteria contain 6 fatty acid residues).

The inner core of LPS consists of two or more 2-keto-3-deoxyoctonic acid (KDO) sugars linked to the lipid A glucosamine. To these KDO sugars, 2 or 3 heptose (L-glycero-D-manno-heptose) sugars are linked. Similar as for lipid A, the sugars of the inner core are also unique to bacteria. Re-LPS is the smallest LPS molecule produced by Gram-negative bacteria, and consists of lipid A with 1 or 2 KDO sugar units.

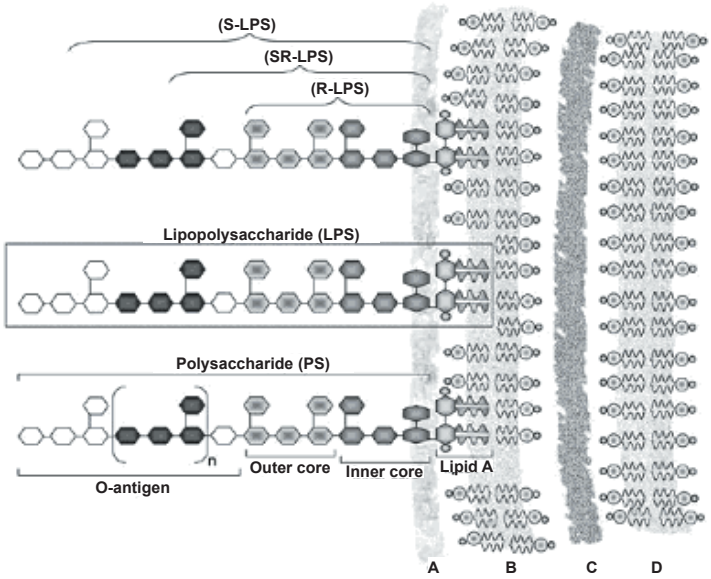

Figure 8. General simplified overview of lipopolysaccharide (LPS) on the outer membrane of Gram-negative bacteria. See text for explanation of the LPS components. Some bacterial species contain an outer capsule that protects the bacterium from host defenses such as complement, lysis, and phagocytosis (A). Outer lipid bilayer with LPS which is approximately 8 nm in width (B). Peptidoglycan layer (C). Inner bilipid membrane (D). S-LPS, smooth LPS; SR-LPS, semi-rough LPS; R-LPS, rough LPS. Adapted from Dixon *et al.*[324].

The outer core of LPS is more variable than the inner core and consists of common sugars. In most cases it is 3 sugars long with 1 or more covalently linked sugars as side chains. Serotypes of LPS that consist of lipid A, and the complete inner and outer core are denoted Ra-LPS, whereas the Rb- and Rc-LPS serotypes only contain a part of the outer core.

The O-antigen is attached to the outer core and is composed of repeating units of common sugars. The O-antigen extends from the bacterial surface, and is also immunogenic. The interspecies and interstrain variations in the composition and length of the O-antigen are huge, and can vary from 0 to 40 repeating units, but in general consist of 20-40 repeating units. O-antigen-containing LPS differ from O-antigen-lacking LPS by their smooth appearance on agar plates, and are therefore indicated as S-LPS, whereas O-antigen-lacking LPS have a rough (R) appearance.

4.2. Host Response to Lipopolysaccharide

The immune response to LPS and Gram-negative bacteria consists of the innate and adaptive immunity[325,326]. The innate immunity is evolutionary ancient, whereas the adaptive immunity provides specific recognition and immunological memory. The innate immunity is the first line of defense. In particular the mononuclear cells (*i.e.* monocytes and macrophages), neutrophils, and mast cells are of great significance, since these cells are activated by LPS to differentiate rapidly into short-lived effector cells to combat infection[325,327-329]. Immature dendritic cells which are specialized antigen-presenting cells involved in innate immunity, present internalized LPS on their surface to activate T-cells and to provide instructions about the nature of the microbe [325,328-333]. For complete activation of T-cells, dendritic cells need to deliver co-stimulatory signals, such as CD80 and CD86. The activated T-cells then start the adaptive immune response, in which cytotoxic T-cells, B-cells, and macrophages serve as effector cells. Macrophages play a major role in immunity, since these effector cells are thus involved in both the innate and adaptive immunity, as described above.

An important family of innate immune receptors are the Toll-like receptors (TLRs), named after the homologous Toll protein in *Drosophila melanogaster*[334]. These receptors can discriminate between different pathogens and self-antigens. To date, 10 different TLRs are described in humans and mice, which differ from each other in ligand specificities, expression patterns, and in the target genes they can induce[335-338]. Strict discrimination between pathogens and self-antigens by TLRs is required for a TLR response, because they play a crucial role in deciding whether to respond or not to an invading microorganism[339].

TLR4, together with its indispensable unit MD2, is essential for the recognition and inflammatory response towards LPS[340-344]. This LPS-signalling receptor was

identified after cloning of the defective gene in the LPS-unresponsive C3H/HeJ and C57BL/10ScCr mice[340,345,346]. Only few exceptions of LPS are known to be undetected by TLR4, these are the LPS of *Porphyromonas gingivalis*[347] and *Leptospira interrogans*[348] bacteria. The LPS of these bacteria differ in structure from other Gram-negative bacteria, and are recognized by TLR2. TLR4, however, is not the sole receptor involved in LPS recognition. Transport of LPS in the circulation is mainly mediated by LPS-binding protein (LBP), which catalyzes the movement of free LPS or LPS from the outer membrane of Gram-negative bacteria directly to HDL and other lipoprotein particles[349], but also to CD14[350,351]. CD14 is either expressed on the surface of myelomonocytic cells as a glycosyl phosphatidylinositol (GPI)-anchored molecule (membrane CD14, mCD14), or is present in the circulation as a soluble molecule (sCD14)[352]. CD14 then delivers the LPS to TLR4. CD14 appears not to be essential for LPS responses, but probably has a role in their amplification[353].

LPS processing by LBP and sCD14 does not necessarily result in LPS responses, but also in LPS clearance. Transfer of LPS to lipoproteins can result in neutralization and subsequently clearance via the liver[349,354-356] (discussed in **section 4.4**). Therefore, both LBP and CD14 have a dual role in LPS responses; they not only trigger LPS responses, but can also terminate them[357-359], which might explain the complex phenotypes of mice that lack these molecules. For instance, LBP-deficient mice are susceptible to Gram-negative bacterial infection, but are resistant to experimentally LPS-induced shock[349], while LPS-stimulated TNFα production *in vivo* is not impaired[360]. Similarly, CD14-deficient mice are highly resistant to LPS- and Gram-negative bacteria-induced shock[361]. Surprisingly, these mice also show reduced circulating bacteria levels, suggesting a role for CD14 in facilitating the dissemination of Gram-negative bacteria[362]. LPS clearance by CD14 might attenuate local inflammation and hereby could permit bacterial replication.

4.3. Lipid Metabolism in Inflammation and Sepsis

Accumulating evidence indicates that lipoprotein metabolism is strongly influenced by inflammation, infection, and sepsis[363]. In human sepsis, plasma TG are increased[364-367], phospholipids are maintained at near normal levels[364], while total cholesterol is decreased[364,366-369]. The decrease in cholesterol, caused by a reduction in cholesteryl esters, and not unesterified cholesterol[364], can be attributed to reductions of LDL[365-367] and mainly HDL[365-367,369]. The decrease in HDL is accompanied by a loss of mainly large apoAI-containing particles, an almost total loss of apoCI, and an increase in apoE-containing HDL, which does not contain significant amounts of apoAI, apoAII, or apoCI[365]. In addition, apoAI-containing HDL also shows an increased content of the inflammation-associated

isoforms of SAA, which may inhibit the selective uptake of HDL-cholesteryl esters by SR-BI[370]. Also, plasma LBP is strongly increased, albeit that the disposition of LBP in the circulation (*i.e.* HDL versus apoB-containing lipoproteins) remains quite controversial. Initially, LBP was suggested to be bound to HDL based on removal of LBP from plasma by anti-apoAI immunochromatography[371]. However, more recent studies have indicated that LBP is mainly associated with apoB-containing lipoproteins as evidenced by co-migration during separation of lipoproteins by electrophoresis[365,372]. Intravenous infusion of a single dose of LPS into healthy volunteers can mimic many of the effects observed in septic patients, including a transient increase in plasma FFA and TG, and a decrease in cholesterol, LDL-cholesterol and apoB[373].

The mechanisms underlying the effects of sepsis on lipid metabolism have been mainly derived from studies in animals, by mimicking the septic conditions as seen in patients. Injection of LPS or bacteria into rats leads to a marked hyperlipidemic response, caused primarily by an increase of VLDL-TG[374]. Low doses of LPS increase the hepatic VLDL production, as related to an increased hepatic synthesis of TG[375] and cholesterol[376]. In contrast, high doses of LPS inhibit VLDL clearance[375], which may be related to a decrease in plasma LPL activity[377,378], VLDL-apoE content[379], or hepatic LDLr expression[380]. In addition, LPS has been shown to affect a wide range of HDL-associated apolipoproteins, plasma enzymes, lipid transfer proteins, and receptors that are involved in plasma HDL metabolism. Besides a decrease in HDL-associated apoAI and an increase in HDL-associated SAA, apoAIV, apoAV, apoE[363], and apoJ[381,382], LPS affects LCAT[378], HL[383], PLTP[384], CETP[385], macrophage ABCA1[386], macrophage SR-BI[387], and hepatic SR-BI[388], as summarized in **Figure 9**. Since PLTP is decreased in LPS-treated mice[384] and increased in human sepsis[365], the actual significance of some of these findings for human sepsis remains to be established. Nevertheless, despite some potential species' differences, both sepsis and LPS thus affect plasma lipoprotein levels by modulating lipoprotein production and clearance through their effect on apolipoproteins, lipolytic enzymes, lipid transfer factors, and lipoprotein receptors[363,389].

4.4. Lipoproteins and the Lipopolysaccharide Response

In addition to the effects of LPS and sepsis on lipid metabolism, all lipoprotein classes have been demonstrated to bind LPS. LPS, when added to whole human normal blood, mainly binds to HDL (60%), in addition to LDL (25%) and VLDL (12%)[364,390]. In septic blood, in which HDL levels are decreased, LPS binding shifts towards VLDL[364]. Biophysical studies showed that LPS interacts with HDL through its lipid A moiety, in particular involving the diglucosamine-

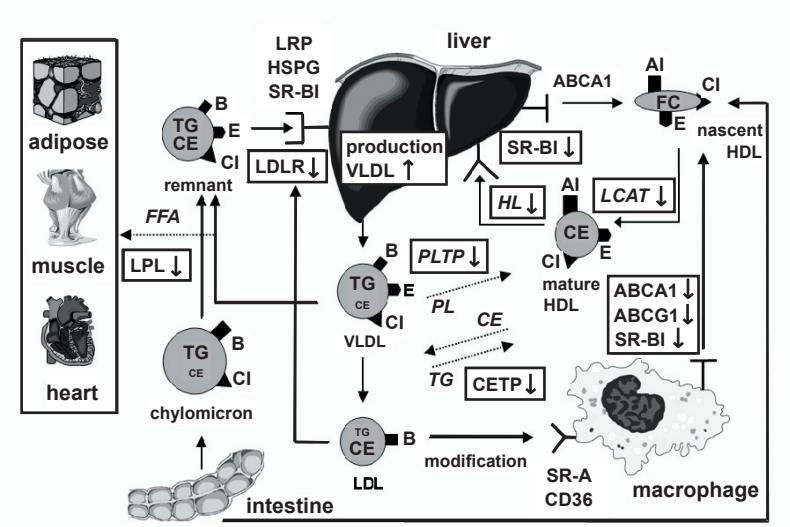

Figure 9. Effect of LPS on plasma lipoprotein metabolism. Animal studies have demonstrated that LPS affects the metabolism of VLDL, LDL, and HDL by influencing many processes involved in their formation, secretion, and clearance, as shown by the arrows in the boxed items. See text for explanation. AI, apolipoprotein AI; ABCA1/ABCG1, ATP-binding cassette transporter AI or GI; B, apolipoprotein B; CI, apolipoprotein CI; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; E, apolipoprotein E; FC, free cholesterol; FFA, free fatty acids; HSPG, heparan sulphate proteoglycans; LCAT, lecithin:cholesterol acyltransferase; LDLR, LDL receptor; LRP, LDL receptor-related protein; PL, phospholipids; PLTP, phospholipid transfer protein; SRA, scavenger receptor class A; SR-BI, scavenger receptor class B type I; TG, triglycerides. Modified from Berbée *et al*[38].

phosphate region[391]. However, the mode of interaction may strongly depend on the composition of HDL, especially with respect to its apolipoprotein pattern, which is drastically altered in inflammation and sepsis (as discussed in the previous **section 4.3**). The binding of LPS to lipoproteins modulates the biological response to LPS, as demonstrated *in vitro* and in rodents[392,393]. Both chylomicrons and VLDL were able to protect mice and rats against a lethal dose of LPS[354,393]. In fact, chylomicrons were able to prevent septic death resulting from cecal ligation and puncture (CLP) in rats[394]. TG-rich lipoproteins appeared to redirect LPS to hepatocytes[354,355], where LPS was secreted into the bile in a de-activated form, as shown in rats[356]. The cholesterol-rich lipoproteins LDL and HDL can also bind LPS and neutralize its biological activity[392]. Transgenic mice expressing human apoAI, resulting in a 2-fold elevated plasma HDL level, exhibited lower cytokine levels and improved survival rates after LPS challenge as compared to control mice[395]. Based on the LPS-neutralizing properties of HDL, it was hypothesized that reconstituted HDL (rHDL), built from phospholipids and apoAI, may constitute a valuable therapeutic agent in the protection and treatment of septic shock. Indeed, rHDL reduced LPS-induced TNFα production in mice[395], rabbits[396,397], and dogs[398]. In humans, infusion of rHDL reduced the

LPS-induced release of TNFα, IL-6, and IL-8, while the release of inhibitors of proinflammatory cytokines (*i.e.* IL-1 receptor antagonist, soluble TNF receptors, and IL-10) were only marginally affected[399].

4.4.1. Lipoprotein Constituents and the Lipopolysaccharide Response

The amphiphilic structure of LPS would suggest that LPS associates with lipoproteins merely by intercalation of the highly lipophylic lipid A moiety into the particle's phospholipid shell. Indeed it has been shown that the ability of the various lipoprotein classes to neutralize the bioactivity of LPS *in vitro* depends on their phospholipid content and not on their content of cholesterol or TG[400]. LPS can be directly transferred into phospholipids by LBP in the presence[401] or absence[402] of sCD14. The LBP-induced intercalation of LPS into phospholipid liposomes may also be enhanced by HDL[391]. Moreover, infusion of phospholipid-rich lipid emulsion has been shown to improve survival in a porcine model of septic peritonitis, as related to an increased phospholipid content of lipoproteins[403]. Finally, *in vitro* studies, in which LPS was added to the serum of septic patients, has shown that the distribution of lipoprotein-bound LPS among the major lipoprotein classes paralleled the phospholipid content of those classes[364].

On the other hand, it has also been reported that LPS aggregates do not intercalate into phospholipid membranes in a non-specific hydrophobic manner[404], indicating the necessity for proteins for association of LPS with lipoproteins. Although protein-free, TG-rich emulsion particles are able to bind LPS as shown by mobility shift assay on an agarose gel, the interaction between the emulsion particles and LPS is not strong enough to prevent dissociation of LPS in the blood[15]. Pre-incubation of high doses of emulsion particles with LPS before intravenous injection into rodents did not affect the kinetics of LPS [15] nor the proinflammatory reaction to LPS[281] (**Figure 10**). Likewise, continuous infusion of a commercial protein-free, TG-rich, lipid emulsion (*i.e.* Intralipid) did not attenuate inflammatory responses to LPS in humans, albeit that plasma TG levels were 9-fold elevated prior to LPS administration[405].

4.4.2. ApoE and the Lipopolysaccharide Response

The data thus indicate that the interaction between LPS and lipoproteins may not be mediated by simple intercalation of LPS into the amphiphilic phospholipid bilayer of lipoproteins, and that lipoprotein-associated apolipoproteins are important for the LPS binding properties of lipoproteins. Indeed, it was found that emulsions enriched with apoE, or even lipid-free apoE, avidly bind to LPS as evidenced by co-localization of ^{125}I-LPS and apoE upon agarose gel electrophoresis[15]. As with TG-rich lipoproteins[354-356], apoE-enriched, TG-rich, emulsion particles, but not protein-free, emulsion particles, were able to prevent the association of LPS with

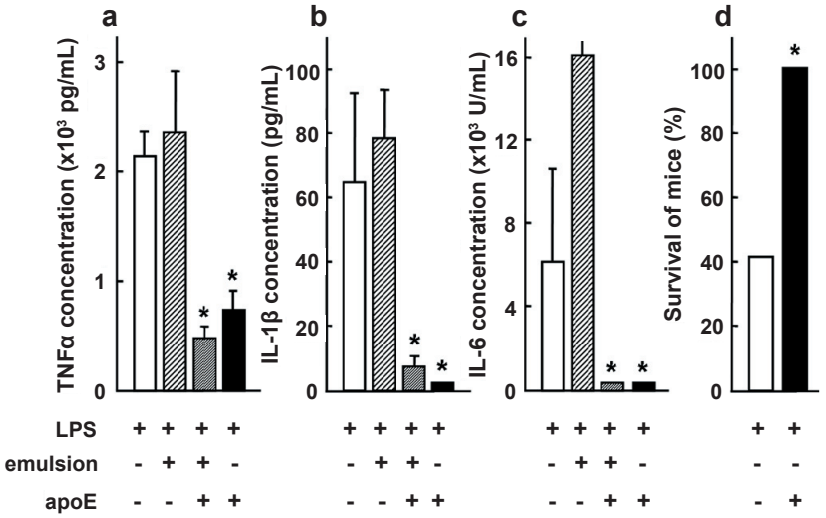

Figure 10. Effect of apoE on the biological response to LPS. ReLPS (*Salmonella Minnesota*; 25 μg/kg) was intravenously injected into rats, in the absence or presence of protein-free emulsion particles (20 mg triglycerides/kg) and/or human apoE (800 μg/kg). Concentrations of TNFα (A), IL-1β (B), and IL-6 (C) were determined in plasma samples taken at 60, 90, and 120 min, respectively. Data are means ± SEM (n=3). Alternatively, LPS (150 ng/kg) was intravenously injected with or without apoE (25 μg/kg) into mice (n=7 per group) that were sensitized to the effects of LPS by intraperitoneal injection of 20 mg of D-galactosamine, and survival of mice was determined over a 3-day period (D). Adapted from Van Oosten *et al*.[281]. Asterisks indicate significant differences as compared to the effects of LPS alone (P<0.05).

macrophages in the liver (*i.e.* Kupffer cells) and spleen, and enhance the uptake of LPS by hepatocytes[15]. Strikingly, the same effect was observed with lipid-free apoE[15], indicating that apoE may be a key lipoprotein component to mediate the protective effect of lipoproteins in endotoxemia and sepsis. Indeed, subsequent studies confirmed that apoE-emulsions and lipid-free apoE, but not protein-free emulsions, largely inhibited LPS-induced serum levels of proinflammatory mediators TNFα, IL-1α, and IL-6. In fact, apoE was able to protect mice against LPS-induced mortality (**Figure 10**)[281].

Accordingly, apoE-deficient mice showed increased plasma levels of TNFα[279-281], IL-6[279], IL-12[279], and interferon-γ (IFNγ)[279] on intravenous challenge with LPS, and increased plasma TNFα levels after intravenous *K. pneumoniae* injection[280]. Concomitantly, apoE-deficient mice were more susceptible to death from intravenous LPS or *K. pneumoniae*[280], the latter accompanied by increased bacterial outgrowth in their organs[283]. Since lipoprotein levels are severely increased in the apoE-deficient mice, as evident from 8-10-fold elevated plasma cholesterol levels, this indicated that increased lipoprotein levels *per se* do not necessarily lead to LPS neutralization. Interestingly, hypercholesterolemic LDLr/apobec-1 double knockout showed similar responses as wild-type mice[279],

whereas for LDLr single knockout mice even opposite results were reported as compared to apoE-deficient mice[406]. Since these mice accumulate apoE-rich lipoproteins in their plasma, it is tempting to speculate that protection of these mice against endotoxemia is at least partly mediated by apoE.

Besides attenuating inflammatory responses by direct binding to LPS, apoE may also exert direct immunomodulatory effects by initiating a signalling cascade in macrophages, thereby down-regulating macrophage activation after exposure to a variety of structurally diverse stimuli[279,407-409]. Recently, an unexpected proinflammatory property of apoE was described. Van den Elzen *et al.*[16] showed that apoE was able to deliver lipid antigens via LDLr-mediated uptake into endosomal compartments in antigen presenting dendritic cells. Via this route, apoE mediates the presentation of serum-borne lipid antigens to initiate immunological response to these antigens. Since dendritic cells are capable of presenting LPS, a lipid antigen, on their surface to activate T-cells[331-333], apoE may also stimulate the presentation of LPS by dendritic cells. However, if so, the anti-inflammatory effects of apoE overrule this proinflammatory action[279-281].

4.4.3. Other Apolipoproteins and the Lipopolysaccharide Response

Besides apoE, other apolipoproteins have now also been shown to modulate the response to LPS, either by direct binding to LPS or by modulating the function of LPS-responsive cells. For example apoAI was shown to inhibit the LPS-induced release of cytokines by monocytes[392]. Recently, apoAI was reported to bind LPS[13], reduce cytokine levels in serum[12,17,410], and improved survival rates after LPS challenge[12,13,17]. In addition, apoAI-deficient mice show increased LPS-induced serum levels of cytokines (*e.g.* TNFα, IL-1β, IL-4, IL-6, IL-10), but this could also be attributable to the almost complete lack of circulating HDL in these mice[410]. The effects of apoAI may be partially explained by direct effects of apoAI on LPS-responsive cells, since both apoAI[17,411,412] and apoAII[412] have been reported to down-regulate neutrophil adhesion, oxidative burst and degranulation. Another explanation for the *in vivo* findings with apoAI was proposed by Massamiri *et al.*[413], who showed that apoAI facilitates binding of the LBP-LPS complex to HDL. Blocking apoAI with antibodies directed against specific epitopes within apoAI reduced binding of LBP to HDL. In addition to apoAI itself, also apoAI mimetic peptides (*i.e.* L-4F) reduces LPS-induced cytokines, chemokines, and adhesion-molecules by macrophages *in vitro*[414].

Recently, it has been shown that apoAIV reduced the inflammatory response during experimental acute colitis (*i.e.* infection of the colon), presumably involving the inhibition of P-selectin-mediated leukocyte and platelet adhesive interactions[414,415]. Moreover, Recalde *et al.*[14,414] showed that apoAIV strongly inhibits monocyte activation as evident from a strong reduction in LPS-induced

TNFα production, while no effect of apoAI was detected under the applied experimental conditions[14,414]. Although the mechanism remains unclear as yet, expression of human apoAIV in hyperlipidemic apoE-deficient mice reduced the development of LPS-stimulated atherosclerosis, as related to a reduced production of proinflammatory cytokines[14].

5. Outline of this Thesis

In the first part of this thesis the role of apoCI in lipid metabolism will be further addressed. The severe hypertriglyceridemia, as observed in human apoCI transgenic mice[133,159,416], can not be completely explained by only the inhibitory effects of apoCI on the apoE-mediated uptake of TG-rich lipoproteins. Therefore, in **chapter 2** we aimed at elucidation of the main mechanism responsible for this apoCI-mediated severe hypertriglyceridemic phenotype. In **chapter 3**, we studied whether this main hypertriglyceridemic effect of apoCI was dependent of the VLDLr and/or apoCIII.

In the second part of this thesis the role of apoE in CVD in humans will be investigated. Human studies on apoE and CVD mortality have only focused on the role of the *APOE* genotype. However, independent of this genotype, plasma apoE levels are positively correlated with plasma cholesterol levels, indicating that irrespective of the *APOE* genotype high plasma apoE levels may be associated with increased CVD. Since the relation between plasma apoE levels and CVD mortality has not been studied yet, we investigated the association between plasma apoE levels and CVD independent of the *APOE* genotype in the Leiden 85-plus Study in **chapter 4**.

Finally, since we found by sequence alignment analysis that apoCI contains a putative LPS-binding domain in its C-terminal domain, we studied in the third part of this thesis the role of apoCI in Gram-negative sepsis. In **chapter 5** we assessed whether apoCI is indeed able to bind LPS, and whether apoCI is able to modulate the biological response towards LPS and survival in a murine Gram-negative sepsis model. These studies are extended in **chapter 6**, where we studied the structure-function relationship of apoCI with respect to modulating LPS by using apoCI-derived peptides. Since Barlage *et al.*[365] showed that apoCI was virtually absent from HDL during human sepsis, we studied in **chapter 7** the time course of plasma apoCI levels in severely septic patients and correlated these plasma apoCI levels with survival. In **chapter 8** we investigated whether high plasma apoCI levels could protect against mortality from infection in the Leiden 85-plus Study, a prospective population based follow-up study.

The results obtained from these studies as well as implications for future research are discussed in **chapter 9**.

6. References

1. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993;362:801-809.
2. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* 1997;349:1269-1276.
3. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999;340:115-126.
4. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W, Jr., Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1994;89:2462-2478.
5. Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W, Jr., Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb* 1994;14:840-856.
6. Murray CJ, Lopez AD. Regional patterns of disability-free life expectancy and disability-adjusted life expectancy: global Burden of Disease Study. *Lancet* 1997;349:1347-1352.
7. Stoll G, Bendszus M. Inflammation and atherosclerosis: novel insights into plaque formation and destabilization. *Stroke* 2006;37:1923-1932.
8. Tran DD, Groeneveld AB, Van der Meulen J, Nauta JJ, Strack van Schijndel RJ, Thijs LG. Age, chronic disease, sepsis, organ system failure, and mortality in a medical intensive care unit. *Crit Care Med* 1990;18:474-479.
9. Marshall JC, Cook DJ, Christou NV, Bernard GR, Sprung CL, Sibbald WJ. Multiple organ dysfunction score: a reliable descriptor of a complex clinical outcome. *Crit Care Med* 1995;23:1638-1652.
10. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med* 2001;29:1303-1310.
11. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003;348:1546-1554.
12. Imai T, Fujita T, Yamazaki Y. Beneficial effects of apolipoprotein A-I on endotoxemia. *Surg Today* 2003;33:684-687.
13. Ma J, Liao XL, Lou B, Wu MP. Role of apolipoprotein A-I in protecting against endotoxin toxicity. *Acta Biochim Biophys Sin (Shanghai)* 2004;36:419-424.
14. Recalde D, Ostos MA, Badell E, Garcia-Otin AL, Pidoux J, Castro G, Zakin MM, Scott-Algara D. Human apolipoprotein A-IV reduces secretion of proinflammatory cytokines and atherosclerotic effects of a chronic infection mimicked by lipopolysaccharide. *Arterioscler Thromb Vasc Biol* 2004;24:756-761.
15. Rensen PC, Oosten M, Bilt E, Eck M, Kuiper J, Berkel TJ. Human recombinant apolipoprotein E redirects lipopolysaccharide from Kupffer cells to liver parenchymal cells in rats In vivo. *J Clin Invest* 1997;99:2438-2445.
16. Van den Elzen P, Garg S, Leon L, Brigl M, Leadbetter EA, Gumperz JE, Dascher CC, Cheng TY, Sacks FM, Illarionov PA, Besra GS, Kent SC, Moody DB, Brenner MB. Apolipoprotein-mediated pathways of lipid antigen presentation. *Nature* 2005;437:906-910.
17. Yan YJ, Li Y, Lou B, Wu MP. Beneficial effects of ApoA-I on LPS-induced acute lung injury and endotoxemia in mice. *Life Sci* 2006;79:210-215.
18. Gotto AM, Jr., Pownall HJ, Havel RJ. Introduction to the plasma lipoproteins. *Methods Enzymol* 1986;128:3-41.
19. Ginsberg HN. Lipoprotein physiology. *Endocrinol Metab Clin North Am* 1998;27:503-519.
20. Mahley RW, Innerarity TL, Rall SC, Jr., Weisgraber KH. Plasma lipoproteins: apolipoprotein structure and function. *J Lipid Res* 1984;25:1277-1294.
21. Beisiegel U. Lipoprotein metabolism. *Eur Heart J* 1998;19 Suppl A:A20-A23.
22. Groen AK, Oude Elferink RP, Verkade HJ, Kuipers F. The ins and outs of reverse cholesterol transport. *Ann Med* 2004;36:135-145.
23. Mahley RW, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J Lipid Res* 1999;40:1-16.
24. 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.
25. Gibbons GF. Assembly and secretion of hepatic very-low-density lipoprotein. *Biochem J* 1990;268:1-13.

26. Spring DJ, Chen-Liu LW, Chatterton JE, Elovson J, Schumaker VN. Lipoprotein assembly. Apolipoprotein B size determines lipoprotein core circumference. *J Biol Chem* 1992;267:14839-14845.
27. Alexander CA, Hamilton RL, Havel RJ. Subcellular localization of B apoprotein of plasma lipoproteins in rat liver. *J Cell Biol* 1976;69:241-263.
28. Davis RA. Cell and molecular biology of the assembly and secretion of apolipoprotein B-containing lipoproteins by the liver. *Biochim Biophys Acta* 1999;1440:1-31.
29. Raabe M, Flynn LM, Zlot CH, Wong JS, Veniant MM, Hamilton RL, Young SG. Knockout of the abetalipoproteinemia gene in mice: reduced lipoprotein secretion in heterozygotes and embryonic lethality in homozygotes. *Proc Natl Acad Sci U S A* 1998;95:8686-8691.
30. Tietge UJ, Bakillah A, Maugeais C, Tsukamoto K, Hussain M, Rader DJ. Hepatic overexpression of microsomal triglyceride transfer protein (MTP) results in increased in vivo secretion of VLDL triglycerides and apolipoprotein B. *J Lipid Res* 1999;40:2134-2139.
31. Ginsberg HN. Synthesis and secretion of apolipoprotein B from cultured liver cells. *Curr Opin Lipidol* 1995;6:275-280.
32. Brown MS, Kovanen PT, Goldstein JL. Regulation of plasma cholesterol by lipoprotein receptors. *Science* 1981;212:628-635.
33. 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.
34. Rigotti A, Trigatti B, Babitt J, Penman M, Xu S, Krieger M. Scavenger receptor BI--a cell surface receptor for high density lipoprotein. *Curr Opin Lipidol* 1997;8:181-188.
35. Rader DJ. Regulation of reverse cholesterol transport and clinical implications. *Am J Cardiol* 2003;92:42J-49J.
36. Tall AR. Plasma high density lipoproteins. Metabolism and relationship to atherogenesis. *J Clin Invest* 1990;86:379-384.
37. Jiao S, Cole TG, Kitchens RT, Pfleger B, Schonfeld G. Genetic heterogeneity of lipoproteins in inbred strains of mice: analysis by gel-permeation chromatography. *Metabolism* 1990;39:155-160.
38. Berbee JF, Havekes LM, Rensen PC. Apolipoproteins modulate the inflammatory response to lipopolysaccharide. *J Endotoxin Res* 2005;11:97-103.
39. Shore VG, Shore B. Heterogeneity of human plasma very low density lipoproteins. Separation of species differing in protein components. *Biochemistry* 1973;12:502-507.
40. Utermann G. Isolation and partial characterization of an arginine-rich apolipoprotein from human plasma very-low-density lipoproteins: apolipoprotein E. *Hoppe Seylers Z Physiol Chem* 1975;356:1113-1121.
41. Paik YK, Chang DJ, Reardon CA, Davies GE, Mahley RW, Taylor JM. Nucleotide sequence and structure of the human apolipoprotein E gene. *Proc Natl Acad Sci U S A* 1985;82:3445-3449.
42. McLean JW, Elshourbagy NA, Chang DJ, Mahley RW, Taylor JM. Human apolipoprotein E mRNA. cDNA cloning and nucleotide sequencing of a new variant. *J Biol Chem* 1984;259:6498-6504.
43. Elshourbagy NA, Liao WS, Mahley RW, Taylor JM. Apolipoprotein E mRNA is abundant in the brain and adrenals, as well as in the liver, and is present in other peripheral tissues of rats and marmosets. *Proc Natl Acad Sci U S A* 1985;82:203-207.
44. Srivastava RA, Bhasin N, Srivastava N. Apolipoprotein E gene expression in various tissues of mouse and regulation by estrogen. *Biochem Mol Biol Int* 1996;38:91-101.
45. Cohn JS, Tremblay M, Boulet L, Jacques H, Davignon J, Roy M, Bernier L. Plasma concentration and lipoprotein distribution of ApoC-I is dependent on ApoE genotype rather than the Hpa I ApoC-I promoter polymorphism. *Atherosclerosis* 2003;169:63-70.
46. Smit M, De Knijff P, Rosseneu M, Bury J, Klasen E, Frants R, Havekes L. Apolipoprotein E polymorphism in The Netherlands and its effect on plasma lipid and apolipoprotein levels. *Hum Genet* 1988;80:287-292.
47. Rajavashisth TB, Kaptein JS, Reue KL, Lusis AJ. Evolution of apolipoprotein E: mouse sequence and evidence for an 11-nucleotide ancestral unit. *Proc Natl Acad Sci U S A* 1985;82:8085-8089.
48. Chou PY, Fasman GD. Empirical predictions of protein conformation. *Annu Rev Biochem* 1978;47:251-276.
49. Weers PM, Narayanaswami V, Choy N, Luty R, Hicks L, Kay CM, Ryan RO. Lipid binding ability of human apolipoprotein E N-terminal domain isoforms: correlation with protein stability? *Biophys Chem* 2003;100:481-492.
50. Aggerbeck LP, Wetterau JR, Weisgraber KH, Wu CS, Lindgren FT. Human apolipoprotein E3 in aqueous solution. II. Properties of the amino- and carboxyl-terminal domains. *J Biol Chem*

1988;263:6249-6258.
51. Yokoyama S, Kawai Y, Tajima S, Yamamoto A. Behavior of human apolipoprotein E in aqueous solutions and at interfaces. *J Biol Chem* 1985;260:16375-16382.
52. Yokoyama S. Self-associated tetramer of human apolipoprotein E does not lead to its accumulation on a lipid particle. *Biochim Biophys Acta* 1990;1047:99-101.
53. Wetterau JR, Aggerbeck LP, Rall SC, Jr., Weisgraber KH. Human apolipoprotein E3 in aqueous solution. I. Evidence for two structural domains. *J Biol Chem* 1988;263:6240-6248.
54. Innerarity TL, Friedlander EJ, Rall SC, Jr., Weisgraber KH, Mahley RW. The receptor-binding domain of human apolipoprotein E. Binding of apolipoprotein E fragments. *J Biol Chem* 1983;258:12341-12347.
55. Weisgraber KH, Innerarity TL, Harder KJ, Mahley RW, Milne RW, Marcel YL, Sparrow JT. The receptor-binding domain of human apolipoprotein E. Monoclonal antibody inhibition of binding. *J Biol Chem* 1983;258:12348-12354.
56. Weisgraber KH. Apolipoprotein E: structure-function relationships. *Adv Protein Chem* 1994;45:249-302.
57. Westerlund JA, Weisgraber KH. Discrete carboxyl-terminal segments of apolipoprotein E mediate lipoprotein association and protein oligomerization. *J Biol Chem* 1993;268:15745-15750.
58. Mahley RW, Innerarity TL, Pitas RE, Weisgraber KH, Brown JH, Gross E. Inhibition of lipoprotein binding to cell surface receptors of fibroblasts following selective modification of arginyl residues in arginine-rich and B apoproteins. *J Biol Chem* 1977;252:7279-7287.
59. 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.
60. Weisgraber KH, Rall SC, Jr., Mahley RW, Milne RW, Marcel YL, Sparrow JT. Human apolipoprotein E. Determination of the heparin binding sites of apolipoprotein E3. *J Biol Chem* 1986;261:2068-2076.
61. 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.
62. 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.
63. Haddy N, De Bacquer D, Chemaly MM, Maurice M, Ehnholm C, Evans A, Sans S, Do Carmo MM, De Backer G, Siest G, Visvikis S. The importance of plasma apolipoprotein E concentration in addition to its common polymorphism on inter-individual variation in lipid levels: results from Apo Europe. *Eur J Hum Genet* 2002;10:841-850.
64. Zannis VI, Breslow JL. Human very low density lipoprotein apolipoprotein E isoprotein polymorphism is explained by genetic variation and posttranslational modification. *Biochemistry* 1981;20:1033-1041.
65. 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.
66. Dong LM, 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.
67. Bohnet K, Pillot T, Visvikis S, Sabolovic N, Siest G. Apolipoprotein (apo) E genotype and apoE concentration determine binding of normal very low density lipoproteins to HepG2 cell surface receptors. *J Lipid Res* 1996;37:1316-1324.
68. Mamotte CD, Sturm M, Foo JI, Van Bockxmeer FM, Taylor RR. Comparison of the LDL-receptor binding of VLDL and LDL from apoE4 and apoE3 homozygotes. *Am J Physiol* 1999;276:E553-E557.
69. Miettinen TA. Impact of apo E phenotype on the regulation of cholesterol metabolism. *Ann Med* 1991;23:181-186.
70. Weisgraber KH, Innerarity TL, Mahley RW. Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cysteine-arginine interchange at a single site. *J Biol Chem* 1982;257:2518-2521.
71. 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.
72. 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.
73. Boerwinkle E, Utermann G. Simultaneous effects of the apolipoprotein E polymorphism on apolipoprotein E, apolipoprotein B, and cholesterol metabolism. *Am J Hum Genet* 1988;42:104-112.
74. Sing CF, Davignon J. Role of the apolipoprotein E polymorphism in determining normal plasma lipid and lipoprotein variation. *Am J Hum Genet* 1985;37:268-285.
75. Utermann G, Pruin N, Steinmetz A. Polymorphism of apolipoprotein E. III. Effect of a single polymorphic gene locus on plasma lipid levels in man. *Clin Genet* 1979;15:63-72.
76. Greenow K, Pearce NJ, Ramji DP. The key role of apolipoprotein E in atherosclerosis. *J Mol Med* 2005;83:329-342.
77. Hoffer MJ, Niththyananthan S, Naoumova RP, Kibirige MS, Frants RR, Havekes LM, Thompson GR. Apolipoprotein E1-Hammersmith (Lys146-->Asn;Arg147-->Trp), due to a dinucleotide substitution, is associated with early manifestation of dominant type III hyperlipoproteinaemia. *Atherosclerosis* 1996;124:183-189.
78. Lohse P, Rader DJ, Brewer HB, Jr. Heterozygosity for apolipoprotein E-4Philadelphia(Glu13----Lys, Arg145----Cys) is associated with incomplete dominance of type III hyperlipoproteinemia. *J Biol Chem* 1992;267:13642-13646.
79. Mahley RW, Huang Y, Rall SC, Jr. Pathogenesis of type III hyperlipoproteinemia (dysbetalipoproteinemia). Questions, quandaries, and paradoxes. *J Lipid Res* 1999;40:1933-1949.
80. Mahley RW, Rall SC, Jr. Apolipoprotein E: far more than a lipid transport protein. *Annu Rev Genomics Hum Genet* 2000;1:507-537.
81. Rall SC, Jr., Mahley RW. The role of apolipoprotein E genetic variants in lipoprotein disorders. *J Intern Med* 1992;231:653-659.
82. 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.
83. Feussner G. Severe xanthomatosis associated with familial apolipoprotein E deficiency. *J Clin Pathol* 1996;49:985-989.
84. Gabelli C, Gregg RE, Zech LA, Manzato E, Brewer HB, Jr. Abnormal low density lipoprotein metabolism in apolipoprotein E deficiency. *J Lipid Res* 1986;27:326-333.
85. Schaefer EJ, Gregg RE, Ghiselli G, Forte TM, Ordovas JM, Zech LA, Brewer HB, Jr. Familial apolipoprotein E deficiency. *J Clin Invest* 1986;78:1206-1219.
86. Heeren J, Beisiegel U, Grewal T. Apolipoprotein E recycling: implications for dyslipidemia and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2006;26:442-448.
87. Schwiegelshohn B, Presley JF, Gorecki M, Vogel T, Carpentier YA, Maxfield FR, Deckelbaum RJ. Effects of apoprotein E on intracellular metabolism of model triglyceride-rich particles are distinct from effects on cell particle uptake. *J Biol Chem* 1995;270:1761-1769.
88. Kuipers F, Jong MC, Lin Y, Eck M, Havinga R, Bloks V, Verkade HJ, Hofker MH, Moshage H, Berkel TJ, Vonk RJ, Havekes LM. Impaired secretion of very low density lipoprotein-triglycerides by apolipoprotein E- deficient mouse hepatocytes. *J Clin Invest* 1997;100:2915-2922.
89. Rensen PC, Van Berkel TJ. Apolipoprotein E effectively inhibits lipoprotein lipase-mediated lipolysis of chylomicron-like triglyceride-rich lipid emulsions in vitro and in vivo. *J Biol Chem* 1996;271:14791-14799.
90. Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S. Apolipoprotein E polymorphism and Alzheimer's disease. *Lancet* 1993;342:697-699.
91. Strittmatter WJ, Saunders AM, Schmechel D, Pericak-Vance M, Enghild J, Salvesen GS, Roses AD. Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc Natl Acad Sci U S A* 1993;90:1977-1981.
92. Van Duijn CM, De Knijff P, Cruts M, Wehnert A, Havekes LM, Hofman A, Van Broeckhoven C. Apolipoprotein E4 allele in a population-based study of early-onset Alzheimer's disease. *Nat Genet* 1994;7:74-78.
93. Kunst CB. Complex genetics of amyotrophic lateral sclerosis. *Am J Hum Genet* 2004;75:933-947.
94. Nicoll JA, Roberts GW, Graham DI. Apolipoprotein E epsilon 4 allele is associated with deposition of amyloid beta-protein following head injury. *Nat Med* 1995;1:135-137.
95. Wright AF. Neurogenetics II: complex disorders. *J Neurol Neurosurg Psychiatry* 2005;76:623-631.
96. 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.
97. 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.
98. Drigalenko E, Poduslo S, Elston R. Interaction of the apolipoprotein E and CI loci in predisposing to late-onset Alzheimer's disease. *Neurology* 1998;51:131-135.
99. Gustafson A, Alaupovic P, Furman RH. STUDIES OF THE COMPOSITION AND STRUCTURE OF SERUM LIPOPROTEINS: PHYSICAL-CHEMICAL CHARACTERIZATION OF PHOSPHOLIPID-PROTEIN RESIDUES OBTAINED FROM VERY-LOW-DENSITY HUMAN SERUM LIPOPROTEINS. *Biochim Biophys Acta* 1964;84:767-769.
100. Gustafson A, Alaupovic P, Furman RH. Studies of the composition and structure of serum lipoproteins. Separation and characterization of phospholipid-protein residues obtained by partial delipidization of very low density lipoproteins of human serum. *Biochemistry* 1966;5:632-640.
101. Seidel D, Alaupovic P, Furman RH, McConathy WJ. A lipoprotein characterizing obstructive jaundice. II. Isolation and partial characterization of the protein moieties of low density lipoproteins. *J Clin Invest* 1970;49:2396-2407.
102. Shulman RS, Herbert PN, Wehrly K, Fredrickson DS. Thf complete amino acid sequence of C-I (apoLp-Ser), an apolipoprotein from human very low density lipoproteins. *J Biol Chem* 1975;250:182-190.
103. 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.
104. Van Eck MM, Hoffer MJ, Havekes LM, Frants RR, Hofker MH. The apolipoprotein C2-linked (Acl) gene: a new gene within the mouse apolipoprotein e-c1-c2 gene cluster. *Genomics* 1994;21:110-115.
105. 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.
106. Shachter NS. Apolipoproteins C-I and C-III as important modulators of lipoprotein metabolism. *Curr Opin Lipidol* 2001;12:297-304.
107. Breckenridge WC, Little JA, Steiner G, Chow A, Poapst M. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. *N Engl J Med* 1978;298:1265-1273.
108. Cox DW, Breckenridge WC, Little JA. Inheritance of apolipoprotein C-II deficiency with hypertriglyceridemia and pancreatitis. *N Engl J Med* 1978;299:1421-1424.
109. Fojo SS, Brewer HB. Hypertriglyceridaemia due to genetic defects in lipoprotein lipase and apolipoprotein C-II. *J Intern Med* 1992;231:669-677.
110. Santamarina-Fojo S, Dugi KA. Structure, function and role of lipoprotein lipase in lipoprotein metabolism. *Curr Opin Lipidol* 1994;5:117-125.
111. Wang CS. Structure and functional properties of apolipoprotein C-II. *Prog Lipid Res* 1991;30:253-258.
112. Brown WV, Baginsky ML. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem Biophys Res Commun* 1972;46:375-382.
113. Ekman R, Nilsson-Ehle P. Effects of apolipoproteins on lipoprotein lipase activity of human adipose tissue. *Clin Chim Acta* 1975;63:29-35.
114. Ginsberg HN, Le NA, Goldberg IJ, Gibson JC, Rubinstein A, Wang-Iverson P, Norum R, Brown WV. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *J Clin Invest* 1986;78:1287-1295.
115. 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.
116. Krauss RM, Herbert PN, Levy RI, Fredrickson DS. Further observations on the activation and inhibition of lipoprotein lipase by apolipoproteins. *Circ Res* 1973;33:403-411.
117. 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.
118. 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.
119. Myklebost O, Rogne S. The gene for human apolipoprotein CI is located 4.3 kilobases away from the apolipoprotein E gene on chromosome 19. *Hum Genet* 1986;73:286-289.

120. Hoffer MJ, Van Eck MM, Havekes LM, Hofker MH, Frants RR. The mouse apolipoprotein C1 gene: structure and expression. *Genomics* 1993;18:37-42.
121. Shachter NS, Rabinowitz D, Stohl S, Conde-Knape K, Cohn JS, Deckelbaum RJ, Berglund L, Shea S. The common insertional polymorphism in the APOC1 promoter is associated with serum apolipoprotein C-I levels in Hispanic children. *Atherosclerosis* 2005;179:387-393.
122. 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.
123. Marcel YL, Bergseth M, Nestruck AC. Preparative isoelectric focussing of apolipoproteins C and E from human very low density lipoproteins. *Biochim Biophys Acta* 1979;573:175-183.
124. Tournier JF, Bayard F, Tauber JP. Rapid purification and activity of apolipoprotein C1 on the proliferation of bovine vascular endothelial cells in vitro. *Biochim Biophys Acta* 1984;804:216-220.
125. Rozek A, Sparrow JT, Weisgraber KH, Cushley RJ. Conformation of human apolipoprotein C-I in a lipid-mimetic environment determined by CD and NMR spectroscopy. *Biochemistry* 1999;38:14475-14484.
126. Wroblewski MS, Wilson-Grady JT, Martinez MB, Kasthuri RS, McMillan KR, Flood-Urdangarin C, Nelsestuen GL. A functional polymorphism of apolipoprotein C1 detected by mass spectrometry. *FEBS J* 2006;273:4707-4715.
127. Klasen EC, Talmud PJ, Havekes L, De Wit E, Kooij-Meijs E, Smit M, Hansson G, Humphries SE. A common restriction fragment length polymorphism of the human apolipoprotein E gene and its relationship to type III hyperlipidaemia. *Hum Genet* 1987;75:244-247.
128. Nillesen WM, Smeets HJ, Van Oost BA. Human ApoCI HpaI restriction site polymorphism revealed by the polymerase chain reaction. *Nucleic Acids Res* 1990;18:3428.
129. Kinnunen PK, 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.
130. Liu M, Subbaiah PV. Activation of plasma lysolecithin acyltransferase reaction by apolipoproteins A-I, C-I and E. *Biochim Biophys Acta* 1993;1168:144-152.
131. 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.
132. Steyrer E, 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.
133. 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.
134. Jong MC, Van Dijk KW, Dahlmans VE, Van der Boom H, 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.
135. Sehayek E, 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.
136. 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.
137. 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.
138. 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.
139. Kolmakova A, Kwiterovich P, Virgil D, Alaupovic P, Knight-Gibson C, Martin SF, Chatterjee S. Apolipoprotein C-I induces apoptosis in human aortic smooth muscle cells via recruiting neutral sphingomyelinase. *Arterioscler Thromb Vasc Biol* 2004;24:264-269.
140. Wong H, Schotz MC. The lipase gene family. *J Lipid Res* 2002;43:993-999.

141. Cryer A. Tissue lipoprotein lipase activity and its action in lipoprotein metabolism. *Int J Biochem* 1981;13:525-541.
142. Chajek T, Stein O, Stein Y. Pre- and post-natal development of lipoprotein lipase and hepatic triglyceride hydrolase activity in rat tissues. *Atherosclerosis* 1977;26:549-561.
143. Merkel M, Eckel RH, Goldberg IJ. Lipoprotein lipase: genetics, lipid uptake, and regulation. *J Lipid Res* 2002;43:1997-2006.
144. Iverius PH, Ostlund-Lindqvist AM. Lipoprotein lipase from bovine milk. Isolation procedure, chemical characterization, and molecular weight analysis. *J Biol Chem* 1976;251:7791-7795.
145. Olivecrona T, Bengtsson-Olivecrona G, Osborne JC, Jr., Kempner ES. Molecular size of bovine lipoprotein lipase as determined by radiation inactivation. *J Biol Chem* 1985;260:6888-6891.
146. Medh JD, Bowen SL, Fry GL, Ruben S, Andracki M, Inoue I, Lalouel JM, Strickland DK, Chappell DA. Lipoprotein lipase binds to low density lipoprotein receptors and induces receptor-mediated catabolism of very low density lipoproteins in vitro. *J Biol Chem* 1996;271:17073-17080.
147. Salinelli S, Lo JY, Mims MP, Zsigmond E, Smith LC, Chan L. Structure-function relationship of lipoprotein lipase-mediated enhancement of very low density lipoprotein binding and catabolism by the low density lipoprotein receptor. Functional importance of a properly folded surface loop covering the catalytic center. *J Biol Chem* 1996;271:21906-21913.
148. Beisiegel U, Weber W, Bengtsson-Olivecrona G. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc Natl Acad Sci U S A* 1991;88:8342-8346.
149. Chappell DA, Fry GL, Waknitz MA, Muhonen LE, Pladet MW, Iverius PH, Strickland DK. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor in vitro. A process facilitated by cell-surface proteoglycans. *J Biol Chem* 1993;268:14168-14175.
150. Williams SE, Inoue I, Tran H, Fry GL, Pladet MW, Iverius PH, Lalouel JM, Chappell DA, Strickland DK. The carboxyl-terminal domain of lipoprotein lipase binds to the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor (LRP) and mediates binding of normal very low density lipoproteins to LRP. *J Biol Chem* 1994;269:8653-8658.
151. Nykjaer A, Bengtsson-Olivecrona G, Lookene A, Moestrup SK, Petersen CM, Weber W, Beisiegel U, Gliemann J. The alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein binds lipoprotein lipase and beta-migrating very low density lipoprotein associated with the lipase. *J Biol Chem* 1993;268:15048-15055.
152. 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.
153. Argraves KM, Battey FD, MacCalman CD, McCrae KR, Gafvels M, Kozarsky KF, Chappell DA, Strauss JF, III, Strickland DK. The very low density lipoprotein receptor mediates the cellular catabolism of lipoprotein lipase and urokinase-plasminogen activator inhibitor type I complexes. *J Biol Chem* 1995;270:26550-26557.
154. Mulder M, Lombardi P, Jansen H, Van Berkel TJ, Frants RR, Havekes LM. Heparan sulphate proteoglycans are involved in the lipoprotein lipase-mediated enhancement of the cellular binding of very low density and low density lipoproteins. *Biochem Biophys Res Commun* 1992;185:582-587.
155. 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.
156. 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.
157. Aalto-Setala 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.
158. Ebara T, Ramakrishnan R, Steiner G, Shachter NS. Chylomicronemia due to apolipoprotein CIII overexpression in apolipoprotein E-null mice. Apolipoprotein CIII-induced hypertriglyceridemia is not mediated by effects on apolipoprotein E. *J Clin Invest* 1997;99:2672-2681.
159. 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.
160. 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.
161. Jong MC, Rensen PC, Dahlmans VE, Van der Boom H, Van Berkel TJ, Havekes LM. Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. *J Lipid Res* 2001;42:1578-1585.
162. 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.
163. 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.
164. Schaap FG, Rensen PC, Voshol PJ, Vrins 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.
165. 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.
166. Schaap FG, Nierman MC, Berbee JF, Hattori H, Talmud PJ, Vaessen SF, Rensen PC, Chamuleau RA, Kuivenhoven JA, Groen AK. Evidence for a complex relationship between apoA-V and apoC-III in patients with severe hypertriglyceridemia. *J Lipid Res* 2006;47:2333-2339.
167. Henneman P, Schaap FG, Havekes LM, Rensen PC, Frants RR, Van Tol A, Hattori H, Smelt AH, Van Dijk KW. Plasma apoAV levels are markedly elevated in severe hypertriglyceridemia and positively correlated with the APOA5 S19W polymorphism. *Atherosclerosis* 2006.
168. Yamada N, Murase T. Modulation, by apolipoprotein E, of lipoprotein lipase activity. *Biochem Biophys Res Commun* 1980;94:710-715.
169. Clark AB, Quarfordt SH. Apolipoprotein effects on the lipolysis of perfused triglyceride by heparin-immobilized milk lipase. *J Biol Chem* 1985;260:4778-4783.
170. Havel RJ. The formation of LDL: mechanisms and regulation. *J Lipid Res* 1984;25:1570-1576.
171. McConathy WJ, Wang CS. Inhibition of lipoprotein lipase by the receptor-binding domain of apolipoprotein E. *FEBS Lett* 1989;251:250-252.
172. Gomez-Coronado D, Saez GT, Lasuncion MA, Herrera E. Different hydrolytic efficiencies of adipose tissue lipoprotein lipase on very-low-density lipoprotein subfractions separated by heparin-Sepharose chromatography. *Biochim Biophys Acta* 1993;1167:70-78.
173. Kortz WJ, Schirmer BD, Mansbach CM, Shelburne F, Toglia MR, Quarfordt SH. Hepatic uptake of chylomicrons and triglyceride emulsions in rats fed diets of differing fat content. *J Lipid Res* 1984;25:799-804.
174. Oswald B, Quarfordt S. Effect of apoE on triglyceride emulsion interaction with hepatocyte and hepatoma G2 cells. *J Lipid Res* 1987;28:798-809.
175. Quarfordt SH, Michalopoulos G, Schirmer B. The effect of human C apolipoproteins on the in vitro hepatic metabolism of triglyceride emulsions in the rat. *J Biol Chem* 1982;257:14642-14647.
176. Windler E, Havel RJ. Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. *J Lipid Res* 1985;26:556-565.
177. Simonet WS, Bucay N, Pitas RE, Lauer SJ, Taylor JM. Multiple tissue-specific elements control the apolipoprotein E/C-I gene locus in transgenic mice. *J Biol Chem* 1991;266:8651-8654.
178. Van Ree JH, Hofker MH, Van den Broek WJ, Van Deursen JM, Van der Boom H, Frants RR, Wieringa B, Havekes LM. Increased response to cholesterol feeding in apolipoprotein C1-deficient mice. *Biochem J* 1995;305 (Pt 3):905-911.
179. Jansen H, Kalkman C, Zonneveld AJ, Hulsmann WC. Secretion of triacylglycerol hydrolase activity by isolated parenchymal rat liver cells. *FEBS Lett* 1979;98:299-302.
180. Jansen H, Van Berkel TJ, Hulsmann WC. Properties of binding of lipases to non-parenchymal rat liver cells. *Biochim Biophys Acta* 1980;619:119-128.
181. Sanan DA, Fan J, Bensadoun A, Taylor JM. Hepatic lipase is abundant on both hepatocyte and endothelial cell surfaces in the liver. *J Lipid Res* 1997;38:1002-1013.
182. Schoonderwoerd K, Hom ML, Luthjens LH, Vieira vB, Jansen H. Functional molecular mass of rat hepatic lipase in liver, adrenal gland and ovary is different. *Biochem J* 1996;318 (Pt 2):463-467.
183. Deckelbaum RJ, Ramakrishnan R, Eisenberg S, Olivecrona T, Bengtsson-Olivecrona G.

Triacylglycerol and phospholipid hydrolysis in human plasma lipoproteins: role of lipoprotein and hepatic lipase. *Biochemistry* 1992;31:8544-8551.
184. Dugi KA, Dichek HL, Santamarina-Fojo S. Human hepatic and lipoprotein lipase: the loop covering the catalytic site mediates lipase substrate specificity. *J Biol Chem* 1995;270:25396-25401.
185. Cohen JC, Wang Z, Grundy SM, Stoesz MR, Guerra R. Variation at the hepatic lipase and apolipoprotein AI/CIII/AIV loci is a major cause of genetically determined variation in plasma HDL cholesterol levels. *J Clin Invest* 1994;94:2377-2384.
186. Bamberger M, Lund-Katz S, Phillips MC, Rothblat GH. Mechanism of the hepatic lipase induced accumulation of high-density lipoprotein cholesterol by cells in culture. *Biochemistry* 1985;24:3693-3701.
187. Collet X, Perret BP, Simard G, Vieu C, Douste-Blazy L. Behaviour of phospholipase-modified HDL towards cultured hepatocytes. I. Enhanced transfers of HDL sterols and apoproteins. *Biochim Biophys Acta* 1990;1043:301-310.
188. Hegele RA, Little JA, Vezina C, Maguire GF, Tu L, Wolever TS, Jenkins DJ, Connelly PW. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. *Arterioscler Thromb* 1993;13:720-728.
189. Barrans A, Collet X, Barbaras R, Jaspard B, Manent J, Vieu C, Chap H, Perret B. Hepatic lipase induces the formation of pre-beta 1 high density lipoprotein (HDL) from triacylglycerol-rich HDL2. A study comparing liver perfusion to in vitro incubation with lipases. *J Biol Chem* 1994;269:11572-11577.
190. Applebaum-Bowden D. Lipases and lecithin: cholesterol acyltransferase in the control of lipoprotein metabolism. *Curr Opin Lipidol* 1995;6:130-135.
191. Kubo M, Matsuzawa Y, Yokoyama S, Tajima S, Ishikawa K, Yamamoto A, Tarui S. Mechanism of inhibition of hepatic triglyceride lipase from human postheparin plasma by apolipoproteins A-I and A-II. *J Biochem (Tokyo)* 1982;92:865-870.
192. Shinomiya M, Sasaki N, Barnhart RL, Shirai K, Jackson RL. Effect of apolipoproteins on the hepatic lipase-catalyzed hydrolysis of human plasma high density lipoprotein2-triacylglycerols. *Biochim Biophys Acta* 1982;713:292-299.
193. Thuren T, Wilcox RW, Sisson P, Waite M. Hepatic lipase hydrolysis of lipid monolayers. Regulation by apolipoproteins. *J Biol Chem* 1991;266:4853-4861.
194. Jahn CE, Osborne JC, Jr., Schaefer EJ, Brewer HB, Jr. In vitro activation of the enzymic activity of hepatic lipase by apoA-II. *FEBS Lett* 1981;131:366-368.
195. Landis BA, Rotolo FS, Meyers WC, Clark AB, Quarfordt SH. Influence of apolipoprotein E on soluble and heparin-immobilized hepatic lipase. *Am J Physiol* 1987;252:G805-G810.
196. Vainio P, Virtanen JA, Kinnunen PK, Voyta JC, Smith LC, Gotto AM, Jr., Sparrow JT, Pattus F, Verger R. Action of lipoprotein lipase on phospholipid monolayers. Activation by apolipoprotein C-II. *Biochemistry* 1983;22:2270-2275.
197. Thuren T. Hepatic lipase and HDL metabolism. *Curr Opin Lipidol* 2000;11:277-283.
198. Conde-Knape K, Bensadoun A, Sobel JH, Cohn JS, Shachter NS. Overexpression of apoC-I in apoE-null mice: severe hypertriglyceridemia due to inhibition of hepatic lipase. *J Lipid Res* 2002;43:2136-2145.
199. Applebaum-Bowden D, Kobayashi J, Kashyap VS, Brown DR, Berard A, Meyn S, Parrott C, Maeda N, Shamburek R, Brewer HB, Jr., Santamarina-Fojo S. Hepatic lipase gene therapy in hepatic lipase-deficient mice. Adenovirus-mediated replacement of a lipolytic enzyme to the vascular endothelium. *J Clin Invest* 1996;97:799-805.
200. Homanics GE, De Silva HV, Osada J, Zhang SH, Wong H, Borensztajn J, Maeda N. Mild dyslipidemia in mice following targeted inactivation of the hepatic lipase gene. *J Biol Chem* 1995;270:2974-2980.
201. Mezdour H, Jones R, Dengremont C, Castro G, Maeda N. Hepatic lipase deficiency increases plasma cholesterol but reduces susceptibility to atherosclerosis in apolipoprotein E-deficient mice. *J Biol Chem* 1997;272:13570-13575.
202. Strickland DK, Gonias SL, Argraves WS. Diverse roles for the LDL receptor family. *Trends Endocrinol Metab* 2002;13:66-74.
203. Howell BW, Herz J. The LDL receptor gene family: signaling functions during development. *Curr Opin Neurobiol* 2001;11:74-81.
204. Willnow TE. The low-density lipoprotein receptor gene family: multiple roles in lipid metabolism. *J Mol Med* 1999;77:306-315.
205. Yamamoto T, Davis CG, Brown MS, Schneider WJ, Casey ML, Goldstein JL, Russell DW. The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA. *Cell* 1984;39:27-38.

206. Davis CG, Goldstein JL, Sudhof TC, Anderson RG, Russell DW, Brown MS. Acid-dependent ligand dissociation and recycling of LDL receptor mediated by growth factor homology region. *Nature* 1987;326:760-765.
207. Chen WJ, Goldstein JL, Brown MS. NPXY, a sequence often found in cytoplasmic tails, is required for coated pit-mediated internalization of the low density lipoprotein receptor. *J Biol Chem* 1990;265:3116-3123.
208. Hussain MM, Strickland DK, Bakillah A. The mammalian low-density lipoprotein receptor family. *Annu Rev Nutr* 1999;19:141-172.
209. Russell DW, Brown MS, Goldstein JL. Different combinations of cysteine-rich repeats mediate binding of low density lipoprotein receptor to two different proteins. *J Biol Chem* 1989;264:21682-21688.
210. Herz J, Hamann U, Rogne S, Myklebost O, Gausepohl H, Stanley KK. Surface location and high affinity for calcium of a 500-kd liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO J* 1988;7:4119-4127.
211. Zheng G, Bachinsky DR, Stamenkovic I, Strickland DK, Brown D, Andres G, McCluskey RT. Organ distribution in rats of two members of the low-density lipoprotein receptor gene family, gp330 and LRP/alpa 2MR, and the receptor-associated protein (RAP). *J Histochem Cytochem* 1994;42:531-542.
212. Moestrup SK, Kaltoft K, Petersen CM, Pedersen S, Gliemann J, Christensen EI. Immunocytochemical identification of the human alpha 2-macroglobulin receptor in monocytes and fibroblasts: monoclonal antibodies define the receptor as a monocyte differentiation antigen. *Exp Cell Res* 1990;190:195-203.
213. Herz J, Kowal RC, Goldstein JL, Brown MS. Proteolytic processing of the 600 kd low density lipoprotein receptor-related protein (LRP) occurs in a trans-Golgi compartment. *EMBO J* 1990;9:1769-1776.
214. Herz J, Strickland DK. LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest* 2001;108:779-784.
215. Lillis AP, Mikhailenko I, Strickland DK. Beyond endocytosis: LRP function in cell migration, proliferation and vascular permeability. *J Thromb Haemost* 2005;3:1884-1893.
216. Beisiegel U, Weber W, Ihrke G, Herz J, Stanley KK. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature* 1989;341:162-164.
217. Rohlmann A, Gotthardt M, Hammer RE, Herz J. Inducible inactivation of hepatic LRP gene by cre-mediated recombination confirms role of LRP in clearance of chylomicron remnants. *J Clin Invest* 1998;101:689-695.
218. Willnow TE, Armstrong SA, Hammer RE, Herz J. Functional expression of low density lipoprotein receptor-related protein is controlled by receptor-associated protein in vivo. *Proc Natl Acad Sci U S A* 1995;92:4537-4541.
219. Martins IJ, Hone E, Chi C, Seydel U, Martins RN, Redgrave TG. Relative roles of LDLr and LRP in the metabolism of chylomicron remnants in genetically manipulated mice. *J Lipid Res* 2000;41:205-213.
220. Takahashi S, Kawarabayasi Y, Nakai T, Sakai J, Yamamoto T. Rabbit very low density lipoprotein receptor: a low density lipoprotein receptor-like protein with distinct ligand specificity. *Proc Natl Acad Sci U S A* 1992;89:9252-9256.
221. Sakai J, Hoshino A, Takahashi S, Miura Y, Ishii H, Suzuki H, Kawarabayasi Y, Yamamoto T. Structure, chromosome location, and expression of the human very low density lipoprotein receptor gene. *J Biol Chem* 1994;269:2173-2182.
222. Gafvels ME, Caird M, Britt D, Jackson CL, Patterson D, Strauss JF, III. Cloning of a cDNA encoding a putative human very low density lipoprotein/apolipoprotein E receptor and assignment of the gene to chromosome 9pter-p23. *Somat Cell Mol Genet* 1993;19:557-569.
223. Gafvels ME, Paavola LG, Boyd CO, Nolan PM, Wittmaack F, Chawla A, Lazar MA, Bucan M, Angelin BO, Strauss JF, III. Cloning of a complementary deoxyribonucleic acid encoding the murine homolog of the very low density lipoprotein/apolipoprotein-E receptor: expression pattern and assignment of the gene to mouse chromosome 19. *Endocrinology* 1994;135:387-394.
224. Oka K, Ishimura-Oka K, Chu MJ, Sullivan M, Krushkal J, Li WH, Chan L. Mouse very-low-density-lipoprotein receptor (VLDLR) cDNA cloning, tissue-specific expression and evolutionary relationship with the low-density-lipoprotein receptor. *Eur J Biochem* 1994;224:975-982.
225. Tiebel O, Oka K, Robinson K, Sullivan M, Martinez J, Nakamuta M, Ishimura-Oka K, Chan L. Mouse very low-density lipoprotein receptor (VLDLR): gene structure, tissue-specific expression and dietary and developmental regulation. *Atherosclerosis* 1999;145:239-251.
226. Webb JC, Patel DD, Jones MD, Knight BL, Soutar AK. Characterization and tissue-specific

expression of the human 'very low density lipoprotein (VLDL) receptor' mRNA. *Hum Mol Genet* 1994;3:531-537.
227. Jokinen EV, Landschulz KT, Wyne KL, Ho YK, Frykman PK, Hobbs HH. Regulation of the very low density lipoprotein receptor by thyroid hormone in rat skeletal muscle. *J Biol Chem* 1994;269:26411-26418.
228. Multhaupt HA, Gafvels ME, Kariko K, Jin H, Arenas-Elliot C, Goldman BI, Strauss JF, III, Angelin B, Warhol MJ, McCrae KR. Expression of very low density lipoprotein receptor in the vascular wall. Analysis of human tissues by in situ hybridization and immunohistochemistry. *Am J Pathol* 1996;148:1985-1997.
229. Wyne KL, Pathak K, Seabra MC, Hobbs HH. Expression of the VLDL receptor in endothelial cells. *Arterioscler Thromb Vasc Biol* 1996;16:407-415.
230. Hiesberger T, Trommsdorff M, Howell BW, Goffinet A, Mumby MC, Cooper JA, Herz J. Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation. *Neuron* 1999;24:481-489.
231. 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.
232. Mahley RW, Ji ZS, Brecht WJ, Miranda RD, He D. Role of heparan sulfate proteoglycans and the LDL receptor-related protein in remnant lipoprotein metabolism. *Ann N Y Acad Sci* 1994;737:39-52.
233. Mahley RW, Nathan BP, Pitas RE. Apolipoprotein E. Structure, function, and possible roles in Alzheimer's disease. *Ann N Y Acad Sci* 1996;777:139-145.
234. Kjellen L, Lindahl U. Proteoglycans: structures and interactions. *Annu Rev Biochem* 1991;60:443-475.
235. David G. Integral membrane heparan sulfate proteoglycans. *FASEB J* 1993;7:1023-1030.
236. Ji ZS, Brecht WJ, Miranda RD, Hussain MM, Innerarity TL, Mahley RW. Role of heparan sulfate proteoglycans in the binding and uptake of apolipoprotein E-enriched remnant lipoproteins by cultured cells. *J Biol Chem* 1993;268:10160-10167.
237. Ji ZS, Sanan DA, Mahley RW. Intravenous heparinase inhibits remnant lipoprotein clearance from the plasma and uptake by the liver: in vivo role of heparan sulfate proteoglycans. *J Lipid Res* 1995;36:583-592.
238. Sinnis P, Willnow TE, Briones MR, Herz J, Nussenzweig V. Remnant lipoproteins inhibit malaria sporozoite invasion of hepatocytes. *J Exp Med* 1996;184:945-954.
239. Hamilton RL, Wong JS, Guo LS, Krisans S, Havel RJ. Apolipoprotein E localization in rat hepatocytes by immunogold labeling of cryothin sections. *J Lipid Res* 1990;31:1589-1603.
240. Hamilton RL, Moorehouse A, Havel RJ. Isolation and properties of nascent lipoproteins from highly purified rat hepatocytic Golgi fractions. *J Lipid Res* 1991;32:529-543.
241. Shimano H, Namba Y, Ohsuga J, Kawamura M, Yamamoto K, Shimada M, Gotoda T, Harada K, Yazaki Y, Yamada N. Secretion-recapture process of apolipoprotein E in hepatic uptake of chylomicron remnants in transgenic mice. *J Clin Invest* 1994;93:2215-2223.
242. Herz J, Qiu SQ, Oesterle A, DeSilva HV, Shafi S, Havel RJ. Initial hepatic removal of chylomicron remnants is unaffected but endocytosis is delayed in mice lacking the low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 1995;92:4611-4615.
243. Al Haideri M, Goldberg IJ, Galeano NF, Gleeson A, Vogel T, Gorecki M, Sturley SL, Deckelbaum RJ. Heparan sulfate proteoglycan-mediated uptake of apolipoprotein E-triglyceride-rich lipoprotein particles: a major pathway at physiological particle concentrations. *Biochemistry* 1997;36:12766-12772.
244. Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 1996;271:518-520.
245. Out R, Hoekstra M, De Jager SC, De Vos P, Van der Westhuyzen DR, Webb NR, Van Eck M, Biessen EA, Van Berkel TJ. Adenovirus-mediated hepatic overexpression of scavenger receptor class B type I accelerates chylomicron metabolism in C57BL/6J mice. *J Lipid Res* 2005;46:1172-1181.
246. Napoli C, D'Armiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G, Palinski W. Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia. Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. *J Clin Invest* 1997;100:2680-2690.
247. Nakashima Y, Raines EW, Plump AS, Breslow JL, Ross R. Upregulation of VCAM-1 and ICAM-1 at atherosclerosis-prone sites on the endothelium in the ApoE-deficient mouse. *Arterioscler Thromb Vasc Biol* 1998;18:842-851.
248. Eriksson EE, Xie X, Werr J, Thoren P, Lindbom L. Importance of primary capture and L-selectin-dependent secondary capture in leukocyte accumulation in inflammation and atherosclerosis

in vivo. *J Exp Med* 2001;194:205-218.
249. Cybulsky MI, Gimbrone MA, Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. *Science* 1991;251:788-791.
250. Folcik VA, Aamir R, Cathcart MK. Cytokine modulation of LDL oxidation by activated human monocytes. *Arterioscler Thromb Vasc Biol* 1997;17:1954-1961.
251. Hansson GK. Immune mechanisms in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2001;21:1876-1890.
252. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005;352:1685-1695.
253. Getz GS. Thematic review series: the immune system and atherogenesis. Immune function in atherogenesis. *J Lipid Res* 2005;46:1-10.
254. Guyton JR, Klemp KF. Development of the lipid-rich core in human atherosclerosis. *Arterioscler Thromb Vasc Biol* 1996;16:4-11.
255. Fuster V, Badimon L, Badimon JJ, Chesebro JH. The pathogenesis of coronary artery disease and the acute coronary syndromes (1). *N Engl J Med* 1992;326:242-250.
256. De Winther MP, Kanters E, Kraal G, Hofker MH. Nuclear factor kappaB signaling in atherogenesis. *Arterioscler Thromb Vasc Biol* 2005;25:904-914.
257. Linton MF, Hasty AH, Babaev VR, Fazio S. Hepatic apo E expression is required for remnant lipoprotein clearance in the absence of the low density lipoprotein receptor. *J Clin Invest* 1998;101:1726-1736.
258. Hayek T, Oiknine J, Brook JG, Aviram M. Role of HDL apolipoprotein E in cellular cholesterol efflux: studies in apo E knockout transgenic mice. *Biochem Biophys Res Commun* 1994;205:1072-1078.
259. Shimano H, Ohsuga J, Shimada M, Namba Y, Gotoda T, Harada K, Katsuki M, Yazaki Y, Yamada N. Inhibition of diet-induced atheroma formation in transgenic mice expressing apolipoprotein E in the arterial wall. *J Clin Invest* 1995;95:469-476.
260. De Pauw M, Vanloo B, Weisgraber K, Rosseneu M. Comparison of lipid-binding and lecithin: cholesterol acyltransferase activation of the amino- and carboxyl-terminal domains of human apolipoprotein E3. *Biochemistry* 1995;34:10953-10966.
261. Huang Y, Liu XQ, Rall SC, Jr., Taylor JM, von Eckardstein A, Assmann G, Mahley RW. Overexpression and accumulation of apolipoprotein E as a cause of hypertriglyceridemia. *J Biol Chem* 1998;273:26388-26393.
262. Kinoshita M, Arai H, Fukasawa M, Watanabe T, Tsukamoto K, Hashimoto Y, Inoue K, Kurokawa K, Teramoto T. Apolipoprotein E enhances lipid exchange between lipoproteins mediated by cholesteryl ester transfer protein. *J Lipid Res* 1993;34:261-268.
263. 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.
264. Klerkx AH, El Harchaoui K, Van der Steeg WA, Boekholdt SM, Stroes ES, Kastelein JJ, Kuivenhoven JA. Cholesteryl ester transfer protein (CETP) inhibition beyond raising high-density lipoprotein cholesterol levels: pathways by which modulation of CETP activity may alter atherogenesis. *Arterioscler Thromb Vasc Biol* 2006;26:706-715.
265. Forrester JS, Makkar R, Shah PK. Increasing high-density lipoprotein cholesterol in dyslipidemia by cholesteryl ester transfer protein inhibition: an update for clinicians. *Circulation* 2005;111:1847-1854.
266. Riddell DR, Vinogradov DV, Stannard AK, Chadwick N, Owen JS. Identification and characterization of LRP8 (apoER2) in human blood platelets. *J Lipid Res* 1999;40:1925-1930.
267. Riddell DR, Graham A, Owen JS. Apolipoprotein E inhibits platelet aggregation through the L-arginine:nitric oxide pathway. Implications for vascular disease. *J Biol Chem* 1997;272:89-95.
268. Sacre SM, Stannard AK, Owen JS. Apolipoprotein E (apoE) isoforms differentially induce nitric oxide production in endothelial cells. *FEBS Lett* 2003;540:181-187.
269. Avila EM, Holdsworth G, Sasaki N, Jackson RL, Harmony JA. Apoprotein E suppresses phytohemagglutinin-activated phospholipid turnover in peripheral blood mononuclear cells. *J Biol Chem* 1982;257:5900-5909.
270. Hui DY, Harmony JA. Inhibition of Ca2+ accumulation in mitogen-activated lymphocytes: role of membrane-bound plasma lipoproteins. *Proc Natl Acad Sci U S A* 1980;77:4764-4768.
271. Kelly ME, Clay MA, Mistry MJ, Hsieh-Li HM, Harmony JA. Apolipoprotein E inhibition of proliferation of mitogen-activated T lymphocytes: production of interleukin 2 with reduced biological activity. *Cell Immunol* 1994;159:124-139.
272. Ishigami M, Swertfeger DK, Hui MS, Granholm NA, Hui DY. Apolipoprotein E inhibition of vascular smooth muscle cell proliferation but not the inhibition of migration is mediated through

activation of inducible nitric oxide synthase. *Arterioscler Thromb Vasc Biol* 2000;20:1020-1026.
273. Swertfeger DK, Hui DY. Apolipoprotein E receptor binding versus heparan sulfate proteoglycan binding in its regulation of smooth muscle cell migration and proliferation. *J Biol Chem* 2001;276:25043-25048.
274. Kothapalli D, Fuki I, Ali K, Stewart SA, Zhao L, Yahil R, Kwiatkowski D, Hawthorne EA, FitzGerald GA, Phillips MC, Lund-Katz S, Pure E, Rader DJ, Assoian RK. Antimitogenic effects of HDL and APOE mediated by Cox-2-dependent IP activation. *J Clin Invest* 2004;113:609-618.
275. Miyata M, Smith JD. Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and beta-amyloid peptides. *Nat Genet* 1996;14:55-61.
276. Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL. ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde-lysine in serum. *Arterioscler Thromb* 1994;14:605-616.
277. Hayek T, Oiknine J, Brook JG, Aviram M. Increased plasma and lipoprotein lipid peroxidation in apo E-deficient mice. *Biochem Biophys Res Commun* 1994;201:1567-1574.
278. Vogel T, Guo NH, Guy R, Drezlich N, Krutzsch HC, Blake DA, Panet A, Roberts DD. Apolipoprotein E: a potent inhibitor of endothelial and tumor cell proliferation. *J Cell Biochem* 1994;54:299-308.
279. Ali K, Middleton M, Pure E, Rader DJ. Apolipoprotein E suppresses the type I inflammatory response in vivo. *Circ Res* 2005;97:922-927.
280. De Bont N, Netea MG, Demacker PN, Verschueren I, Kullberg BJ, Van Dijk KW, Van der Meer JW, Stalenhoef AF. Apolipoprotein E knock-out mice are highly susceptible to endotoxemia and Klebsiella pneumoniae infection. *J Lipid Res* 1999;40:680-685.
281. Van Oosten M, Rensen PC, Van Amersfoort ES, Van Eck M, Van Dam AM, Breve JJ, Vogel T, Panet A, Van Berkel TJ, Kuiper J. Apolipoprotein E protects against bacterial lipopolysaccharide-induced lethality. A new therapeutic approach to treat gram-negative sepsis. *J Biol Chem* 2001;276:8820-8824.
282. Roselaar SE, Daugherty A. Apolipoprotein E-deficient mice have impaired innate immune responses to Listeria monocytogenes in vivo. *J Lipid Res* 1998;39:1740-1743.
283. De Bont N, Netea MG, Demacker PN, Kullberg BJ, Van der Meer JW, Stalenhoef AF. Apolipoprotein E-deficient mice have an impaired immune response to Klebsiella pneumoniae. *Eur J Clin Invest* 2000;30:818-822.
284. Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG, Rubin EM, Breslow JL. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 1992;71:343-353.
285. Zhang SH, Reddick RL, Piedrahita JA, Maeda N. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science* 1992;258:468-471.
286. Zhang SH, Reddick RL, Burkey B, Maeda N. Diet-induced atherosclerosis in mice heterozygous and homozygous for apolipoprotein E gene disruption. *J Clin Invest* 1994;94:937-945.
287. Cladaras C, Hadzopoulou-Cladaras M, Felber BK, Pavlakis G, Zannis VI. The molecular basis of a familial apoE deficiency. An acceptor splice site mutation in the third intron of the deficient apoE gene. *J Biol Chem* 1987;262:2310-2315.
288. Ghiselli G, Schaefer EJ, Gascon P, Breser HB, Jr. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science* 1981;214:1239-1241.
289. Kurosaka D, Teramoto T, Matsushima T, Yokoyama T, Yamada A, Aikawa T, Miyamoto Y, Kurokawa K. Apolipoprotein E deficiency with a depressed mRNA of normal size. *Atherosclerosis* 1991;88:15-20.
290. Lohse P, Brewer HB, III, Meng MS, Skarlatos SI, LaRosa JC, Brewer HB, Jr. Familial apolipoprotein E deficiency and type III hyperlipoproteinemia due to a premature stop codon in the apolipoprotein E gene. *J Lipid Res* 1992;33:1583-1590.
291. Mabuchi H, Itoh H, Takeda M, Kajinami K, Wakasugi T, Koizumi J, Takeda R, Asagami C. A young type III hyperlipoproteinemic patient associated with apolipoprotein E deficiency. *Metabolism* 1989;38:115-119.
292. Zannis VI, Ordovas JM, Cladaras C, Cole FS, Forbes G, Schaefer EJ. mRNA and apolipoprotein E synthesis abnormalities in peripheral blood monocyte macrophages in familial apolipoprotein E deficiency. *J Biol Chem* 1985;260:12891-12894.
293. Wilson PW, Schaefer EJ, Larson MG, Ordovas JM. Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. *Arterioscler Thromb Vasc Biol* 1996;16:1250-1255.
294. Song Y, Stampfer MJ, Liu S. Meta-analysis: apolipoprotein E genotypes and risk for coronary

heart disease. *Ann Intern Med* 2004;141:137-147.
295. Eichner JE, Dunn ST, Perveen G, Thompson DM, Stewart KE, Stroehla BC. Apolipoprotein E polymorphism and cardiovascular disease: a HuGE review. *Am J Epidemiol* 2002;155:487-495.
296. Kolovou GD, Daskalova DC, Hatzivassiliou M, Yiannakouris N, Pilatis ND, Elisaf M, Mikhailidis DP, Cariolou MA, Cokkinos DV. The epsilon 2 and 4 alleles of apolipoprotein E and ischemic vascular events in the Greek population--implications for the interpretation of similar studies. *Angiology* 2003;54:51-58.
297. Remaley AT, Stonik JA, Demosky SJ, Neufeld EB, Bocharov AV, Vishnyakova TG, Eggerman TL, Patterson AP, Duverger NJ, Santamarina-Fojo S, Brewer HB, Jr. Apolipoprotein specificity for lipid efflux by the human ABCAI transporter. *Biochem Biophys Res Commun* 2001;280:818-823.
298. Clark RW, Sutfin TA, Ruggeri RB, Willauer AT, Sugarman ED, Magnus-Aryitey G, Cosgrove PG, Sand TM, Wester RT, Williams JA, Perlman ME, Bamberger MJ. Raising high-density lipoprotein in humans through inhibition of cholesteryl ester transfer protein: an initial multidose study of torcetrapib. *Arterioscler Thromb Vasc Biol* 2004;24:490-497.
299. Evans GF, Bensch WR, Apelgren LD, Bailey D, Kauffman RF, Bumol TF, Zuckerman SH. Inhibition of cholesteryl ester transfer protein in normocholesterolemic and hypercholesterolemic hamsters: effects on HDL subspecies, quantity, and apolipoprotein distribution. *J Lipid Res* 1994;35:1634-1645.
300. Van der Steeg WA, El Harchaoui K, Kuivenhoven JA, Kastelein JJ. Increasing high-density lipoprotein cholesterol through cholesteryl Ester transfer protein inhibition: a next step in the fight against cardiovascular disease? *Curr Drug Targets Cardiovasc Haematol Disord* 2005;5:481-488.
301. Gautier T, Masson D, Jong MC, Pais de Barros JP, Duverneuil L, Le Guern N, Deckert V, Dumont L, Bataille A, Zak Z, Jiang XC, Havekes LM, Lagrost L. Apolipoprotein CI overexpression is not a relevant strategy to block cholesteryl ester transfer protein (CETP) activity in CETP transgenic mice. *Biochem J* 2005;385:189-195.
302. Bjorkegren J, Boquist S, Samnegard A, Lundman P, Tornvall P, Ericsson CG, Hamsten A. Accumulation of apolipoprotein C-I-rich and cholesterol-rich VLDL remnants during exaggerated postprandial triglyceridemia in normolipidemic patients with coronary artery disease. *Circulation* 2000;101:227-230.
303. Bjorkegren J, Silveira A, Boquist S, Tang R, Karpe F, Bond MG, De Faire U, Hamsten A. Postprandial enrichment of remnant lipoproteins with apoC-I in healthy normolipidemic men with early asymptomatic atherosclerosis. *Arterioscler Thromb Vasc Biol* 2002;22:1470-1474.
304. Hamsten A, Silveira A, Boquist S, Tang R, Bond MG, De Faire U, Bjorkegren J. The apolipoprotein CI content of triglyceride-rich lipoproteins independently predicts early atherosclerosis in healthy middle-aged men. *J Am Coll Cardiol* 2005;45:1013-1017.
305. Kwiterovich PO, Jr., Cockrill SL, Virgil DG, Garrett ES, Otvos J, Knight-Gibson C, Alaupovic P, Forte T, Zhang L, Farwig ZN, Macfarlane RD. A large high-density lipoprotein enriched in apolipoprotein C-I: a novel biochemical marker in infants of lower birth weight and younger gestational age. *JAMA* 2005;293:1891-1899.
306. Godfrey KM, Barker DJ. Fetal nutrition and adult disease. *Am J Clin Nutr* 2000;71:1344S-1352S.
307. Alberti C, Brun-Buisson C, Burchardi H, Martin C, Goodman S, Artigas A, Sicignano A, Palazzo M, Moreno R, Boulme R, Lepage E, Le Gall R. Epidemiology of sepsis and infection in ICU patients from an international multicentre cohort study. *Intensive Care Med* 2002;28:108-121.
308. Annane D, Aegerter P, Jars-Guincestre MC, Guidet B. Current epidemiology of septic shock: the CUB-Rea Network. *Am J Respir Crit Care Med* 2003;168:165-172.
309. Bone RC, Grodzin CJ, Balk RA. Sepsis: a new hypothesis for pathogenesis of the disease process. *Chest* 1997;112:235-243.
310. Cohen J. The immunopathogenesis of sepsis. *Nature* 2002;420:885-891.
311. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *N Engl J Med* 2003;348:138-150.
312. Annane D, Sebille V, Charpentier C, Bollaert PE, Francois B, Korach JM, Capellier G, Cohen Y, Azoulay E, Troche G, Chaumet-Riffaut P, Bellissant E. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 2002;288:862-871.
313. Bernard GR, Vincent JL, Laterre PF, LaRosa SP, Dhainaut JF, Lopez-Rodriguez A, Steingrub JS, Garber GE, Helterbrand JD, Ely EW, Fisher CJ, Jr. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001;344:699-709.

314. Opal SM, Cohen J. Clinical gram-positive sepsis: does it fundamentally differ from gram-negative bacterial sepsis? *Crit Care Med* 1999;27:1608-1616.
315. Sriskandan S, Cohen J. Gram-positive sepsis. Mechanisms and differences from gram-negative sepsis. *Infect Dis Clin North Am* 1999;13:397-412.
316. Parrillo JE. Pathogenetic mechanisms of septic shock. *N Engl J Med* 1993;328:1471-1477.
317. Zhou Z, White KA, Polissi A, Georgopoulos C, Raetz CR. Function of Escherichia coli MsbA, an essential ABC family transporter, in lipid A and phospholipid biosynthesis. *J Biol Chem* 1998;273:12466-12475.
318. Doerrler WT, Reedy MC, Raetz CR. An Escherichia coli mutant defective in lipid export. *J Biol Chem* 2001;276:11461-11464.
319. Raetz CR, Whitfield C. Lipopolysaccharide endotoxins. *Annu Rev Biochem* 2002;71:635-700.
320. Kitchens RL, Munford RS. CD14-dependent internalization of bacterial lipopolysaccharide (LPS) is strongly influenced by LPS aggregation but not by cellular responses to LPS. *J Immunol* 1998;160:1920-1928.
321. Hellman J, Loiselle PM, Tehan MM, Allaire JE, Boyle LA, Kurnick JT, Andrews DM, Sik KK, Warren HS. Outer membrane protein A, peptidoglycan-associated lipoprotein, and murein lipoprotein are released by Escherichia coli bacteria into serum. *Infect Immun* 2000;68:2566-2572.
322. Rietschel ET, Kirikae T, Schade FU, Mamat U, Schmidt G, Loppnow H, Ulmer AJ, Zahringer U, Seydel U, Di Padova F, . Bacterial endotoxin: molecular relationships of structure to activity and function. *FASEB J* 1994;8:217-225.
323. Lugtenberg B, Van Alphen L. Molecular architecture and functioning of the outer membrane of Escherichia coli and other gram-negative bacteria. *Biochim Biophys Acta* 1983;737:51-115.
324. Dixon DR, Darveau RP. Lipopolysaccharide heterogeneity: innate host responses to bacterial modification of lipid a structure. *J Dent Res* 2005;84:584-595.
325. Janeway CA, Jr., Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002;20:197-216.
326. Hoffmann JA, Kafatos FC, Janeway CA, Ezekowitz RA. Phylogenetic perspectives in innate immunity. *Science* 1999;284:1313-1318.
327. Hume DA. The mononuclear phagocyte system. *Curr Opin Immunol* 2006;18:49-53.
328. Hemmi H, Akira S. TLR signalling and the function of dendritic cells. *Chem Immunol Allergy* 2005;86:120-135.
329. Miyake K. Innate recognition of lipopolysaccharide by Toll-like receptor 4-MD-2. *Trends Microbiol* 2004;12:186-192.
330. Pearce EJ, Kane CM, Sun J. Regulation of dendritic cell function by pathogen-derived molecules plays a key role in dictating the outcome of the adaptive immune response. *Chem Immunol Allergy* 2006;90:82-90.
331. Rescigno M, Urbano M, Rimoldi M, Valzasina B, Rotta G, Granucci F, Ricciardi-Castagnoli P. Toll-like receptor 4 is not required for the full maturation of dendritic cells or for the degradation of Gram-negative bacteria. *Eur J Immunol* 2002;32:2800-2806.
332. Verhasselt V, Buelens C, Willems F, De Groote D, Haeffner-Cavaillon N, Goldman M. Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells: evidence for a soluble CD14-dependent pathway. *J Immunol* 1997;158:2919-2925.
333. Svensson M, Pfeifer J, Stockinger B, Wick MJ. Bacterial antigen delivery systems: phagocytic processing of bacterial antigens for MHC-I and MHC-II presentation to T cells. *Behring Inst Mitt* 1997;197-211.
334. Medzhitov R, Preston-Hurlburt P, Janeway CA, Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997;388:394-397.
335. Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to Drosophila Toll. *Proc Natl Acad Sci U S A* 1998;95:588-593.
336. Takeda K, Akira S. TLR signaling pathways. *Semin Immunol* 2004;16:3-9.
337. Yang IA, Fong KM, Holgate ST, Holloway JW. The role of Toll-like receptors and related receptors of the innate immune system in asthma. *Curr Opin Allergy Clin Immunol* 2006;6:23-28.
338. Takeda K, Akira S. Microbial recognition by Toll-like receptors. *J Dermatol Sci* 2004;34:73-82.
339. Medzhitov R, Janeway CA, Jr. Decoding the patterns of self and nonself by the innate immune system. *Science* 2002;296:298-300.
340. Poltorak A, He X, Smirnova I, Liu MY, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutler B. Defective LPS signaling in

C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998;282:2085-2088.
341. Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F. Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem* 1999;274:10689-10692.
342. Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S. Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 1999;162:3749-3752.
343. Lien E, Means TK, Heine H, Yoshimura A, Kusumoto S, Fukase K, Fenton MJ, Oikawa M, Qureshi N, Monks B, Finberg RW, Ingalls RR, Golenbock DT. Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J Clin Invest* 2000;105:497-504.
344. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000;25:187-191.
345. Poltorak A, Smirnova I, He X, Liu MY, Van Huffel C, McNally O, Birdwell D, Alejos E, Silva M, Du X, Thompson P, Chan EK, Ledesma J, Roe B, Clifton S, Vogel SN, Beutler B. Genetic and physical mapping of the Lps locus: identification of the toll-4 receptor as a candidate gene in the critical region. *Blood Cells Mol Dis* 1998;24:340-355.
346. Qureshi ST, Lariviere L, Leveque G, Clermont S, Moore KJ, Gros P, Malo D. Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4). *J Exp Med* 1999;189:615-625.
347. Hirschfeld M, Weis JJ, Toshchakov V, Salkowski CA, Cody MJ, Ward DC, Qureshi N, Michalek SM, Vogel SN. Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infect Immun* 2001;69:1477-1482.
348. Werts C, Tapping RI, Mathison JC, Chuang TH, Kravchenko V, Saint G, I, Haake DA, Godowski PJ, Hayashi F, Ozinsky A, Underhill DM, Kirschning CJ, Wagner H, Aderem A, Tobias PS, Ulevitch RJ. Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nat Immunol* 2001;2:346-352.
349. Jack RS, Fan X, Bernheiden M, Rune G, Ehlers M, Weber A, Kirsch G, Mentel R, Furll B, Freudenberg M, Schmitz G, Stelter F, Schutt C. Lipopolysaccharide-binding protein is required to combat a murine gram-negative bacterial infection. *Nature* 1997;389:742-745.
350. Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, Tobias PS, Ulevitch RJ. Structure and function of lipopolysaccharide binding protein. *Science* 1990;249:1429-1431.
351. Hailman E, Lichenstein HS, Wurfel MM, Miller DS, Johnson DA, Kelley M, Busse LA, Zukowski MM, Wright SD. Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *J Exp Med* 1994;179:269-277.
352. Wright SD, Ramos RA, Tobias PS, Ulevitch RJ, Mathison JC. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990;249:1431-1433.
353. Haziot A, Lin XY, Zhang F, Goyert SM. The induction of acute phase proteins by lipopolysaccharide uses a novel pathway that is CD14-independent. *J Immunol* 1998;160:2570-2572.
354. Harris HW, Grunfeld C, Feingold KR, Read TE, Kane JP, Jones AL, Eichbaum EB, Bland GF, Rapp JH. Chylomicrons alter the fate of endotoxin, decreasing tumor necrosis factor release and preventing death. *J Clin Invest* 1993;91:1028-1034.
355. Harris HW, Rockey DC, Chau P. Chylomicrons alter the hepatic distribution and cellular response to endotoxin in rats. *Hepatology* 1998;27:1341-1348.
356. Read TE, Harris HW, Grunfeld C, Feingold KR, Calhoun MC, Kane JP, Rapp JH. Chylomicrons enhance endotoxin excretion in bile. *Infect Immun* 1993;61:3496-3502.
357. Gutsmann T, Muller M, Carroll SF, MacKenzie RC, Wiese A, Seydel U. Dual role of lipopolysaccharide (LPS)-binding protein in neutralization of LPS and enhancement of LPS-induced activation of mononuclear cells. *Infect Immun* 2001;69:6942-6950.
358. Thompson PA, Tobias PS, Viriyakosol S, Kirkland TN, Kitchens RL. Lipopolysaccharide (LPS)-binding protein inhibits responses to cell-bound LPS. *J Biol Chem* 2003;278:28367-28371.
359. Heumann D, Lauener R, Ryffel B. The dual role of LBP and CD14 in response to Gram-negative bacteria or Gram-negative compounds. *J Endotoxin Res* 2003;9:381-384.
360. Wurfel MM, Monks BG, Ingalls RR, Dedrick RL, Delude R, Zhou D, Lamping N, Schumann RR, Thieringer R, Fenton MJ, Wright SD, Golenbock D. Targeted deletion of the lipopolysaccharide (LPS)-binding protein gene leads to profound suppression of LPS responses ex vivo, whereas in vivo responses remain intact. *J Exp Med* 1997;186:2051-2056.
361. Haziot A, Ferrero E, Kontgen F, Hijiya N, Yamamoto S, Silver J, Stewart CL, Goyert SM. Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14-deficient mice. *Immunity* 1996;4:407-414.
362. Haziot A, Hijiya N, Gangloff SC, Silver J, Goyert SM. Induction of a novel mechanism of accelerated bacterial clearance by lipopolysaccharide in CD14-deficient and Toll-like receptor

4-deficient mice. *J Immunol* 2001;166:1075-1078.
363. Khovidhunkit W, Kim MS, Memon RA, Shigenaga JK, Moser AH, Feingold KR, Grunfeld C. Effects of infection and inflammation on lipid and lipoprotein metabolism: mechanisms and consequences to the host. *J Lipid Res* 2004;45:1169-1196.
364. Kitchens RL, Thompson PA, Munford RS, O'Keefe GE. Acute inflammation and infection maintain circulating phospholipid levels and enhance lipopolysaccharide binding to plasma lipoproteins. *J Lipid Res* 2003;44:2339-2348.
365. Barlage S, Frohlich D, Bottcher A, Jauhiainen M, Muller HP, Noetzel F, Rothe G, Schutt C, Linke RP, Lackner KJ, Ehnholm C, Schmitz G. ApoE-containing high density lipoproteins and phospholipid transfer protein activity increase in patients with a systemic inflammatory response. *J Lipid Res* 2001;42:281-290.
366. Gordon BR, Parker TS, Levine DM, Saal SD, Wang JC, Sloan BJ, Barie PS, Rubin AL. Low lipid concentrations in critical illness: implications for preventing and treating endotoxemia. *Crit Care Med* 1996;24:584-589.
367. Van Leeuwen HJ, Heezius EC, Dallinga GM, Van Strijp JA, Verhoef J, Van Kessel KP. Lipoprotein metabolism in patients with severe sepsis. *Crit Care Med* 2003;31:1359-1366.
368. Fraunberger P, Schaefer S, Werdan K, Walli AK, Seidel D. Reduction of circulating cholesterol and apolipoprotein levels during sepsis. *Clin Chem Lab Med* 1999;37:357-362.
369. Alvarez C, Ramos A. Lipids, lipoproteins, and apoproteins in serum during infection. *Clin Chem* 1986;32:142-145.
370. Cai L, De Beer MC, De Beer FC, Van der Westhuyzen DR. Serum amyloid A is a ligand for scavenger receptor class B type I and inhibits high density lipoprotein binding and selective lipid uptake. *J Biol Chem* 2005;280:2954-2961.
371. Wurfel MM, Kunitake ST, Lichenstein H, Kane JP, Wright SD. Lipopolysaccharide (LPS)-binding protein is carried on lipoproteins and acts as a cofactor in the neutralization of LPS. *J Exp Med* 1994;180:1025-1035.
372. Vreugdenhil AC, Snoek AM, Van ', V, Greve JW, Buurman WA. LPS-binding protein circulates in association with apoB-containing lipoproteins and enhances endotoxin-LDL/VLDL interaction. *J Clin Invest* 2001;107:225-234.
373. Hudgins LC, Parker TS, Levine DM, Gordon BR, Saal SD, Jiang XC, Seidman CE, Tremaroli JD, Lai J, Rubin AL. A single intravenous dose of endotoxin rapidly alters serum lipoproteins and lipid transfer proteins in normal volunteers. *J Lipid Res* 2003;44:1489-1498.
374. Scholl RA, Lang CH, Bagby GJ. Hypertriglyceridemia and its relation to tissue lipoprotein lipase activity in endotoxemic, Escherichia coli bacteremic, and polymicrobial septic rats. *J Surg Res* 1984;37:394-401.
375. Feingold KR, Staprans I, Memon RA, Moser AH, Shigenaga JK, Doerrler W, Dinarello CA, Grunfeld C. Endotoxin rapidly induces changes in lipid metabolism that produce hypertriglyceridemia: low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J Lipid Res* 1992;33:1765-1776.
376. Feingold KR, Hardardottir I, Memon R, Krul EJ, Moser AH, Taylor JM, Grunfeld C. Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters. *J Lipid Res* 1993;34:2147-2158.
377. Kawakami M, Cerami A. Studies of endotoxin-induced decrease in lipoprotein lipase activity. *J Exp Med* 1981;154:631-639.
378. Auerbach BJ, Parks JS. Lipoprotein abnormalities associated with lipopolysaccharide-induced lecithin: cholesterol acyltransferase and lipase deficiency. *J Biol Chem* 1989;264:10264-10270.
379. Lanza-Jacoby S, Wong SH, Tabares A, Baer D, Schneider T. Disturbances in the composition of plasma lipoproteins during gram-negative sepsis in the rat. *Biochim Biophys Acta* 1992;1124:233-240.
380. Liao W, Rudling M, Angelin B. Endotoxin suppresses mouse hepatic low-density lipoprotein-receptor expression via a pathway independent of the toll-like receptor 4. *Hepatology* 1999;30:1252-1256.
381. Hardardottir I, Sipe J, Moser AH, Fielding CJ, Feingold KR, Grunfeld C. LPS and cytokines regulate extra hepatic mRNA levels of apolipoproteins during the acute phase response in Syrian hamsters. *Biochim Biophys Acta* 1997;1344:210-220.
382. Van Lenten BJ, Wagner AC, Nayak DP, Hama S, Navab M, Fogelman AM. High-density lipoprotein loses its anti-inflammatory properties during acute influenza a infection. *Circulation* 2001;103:2283-2288.
383. Feingold KR, Memon RA, Moser AH, Shigenaga JK, Grunfeld C. Endotoxin and interleukin-1 decrease hepatic lipase mRNA levels. *Atherosclerosis* 1999;142:379-387.

384. Jiang XC, Bruce C. Regulation of murine plasma phospholipid transfer protein activity and mRNA levels by lipopolysaccharide and high cholesterol diet. *J Biol Chem* 1995;270:17133-17138.
385. Hardardottir I, Moser AH, Fuller J, Fielding C, Feingold K, Grunfeld C. Endotoxin and cytokines decrease serum levels and extra hepatic protein and mRNA levels of cholesteryl ester transfer protein in syrian hamsters. *J Clin Invest* 1996;97:2585-2592.
386. Khovidhunkit W, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. Endotoxin down-regulates ABCG5 and ABCG8 in mouse liver and ABCA1 and ABCG1 in J774 murine macrophages: differential role of LXR. *J Lipid Res* 2003;44:1728-1736.
387. Baranova I, Vishnyakova T, Bocharov A, Chen Z, Remaley AT, Stonik J, Eggerman TL, Patterson AP. Lipopolysaccharide down regulates both scavenger receptor B1 and ATP binding cassette transporter A1 in RAW cells. *Infect Immun* 2002;70:2995-3003.
388. Khovidhunkit W, Moser AH, Shigenaga JK, Grunfeld C, Feingold KR. Regulation of scavenger receptor class B type I in hamster liver and Hep3B cells by endotoxin and cytokines. *J Lipid Res* 2001;42:1636-1644.
389. Hardardottir I, Grunfeld C, Feingold KR. Effects of endotoxin on lipid metabolism. *Biochem Soc Trans* 1995;23:1013-1018.
390. Levels JH, Abraham PR, Van den Elzen A, Van Deventer SJ. Distribution and kinetics of lipoprotein-bound endotoxin. *Infect Immun* 2001;69:2821-2828.
391. Brandenburg K, Jurgens G, Andra J, Lindner B, Koch MH, Blume A, Garidel P. Biophysical characterization of the interaction of high-density lipoprotein (HDL) with endotoxins. *Eur J Biochem* 2002;269:5972-5981.
392. Flegel WA, Baumstark MW, Weinstock C, Berg A, Northoff H. Prevention of endotoxin-induced monokine release by human low- and high-density lipoproteins and by apolipoprotein A-I. *Infect Immun* 1993;61:5140-5146.
393. Harris HW, Grunfeld C, Feingold KR, Rapp JH. Human very low density lipoproteins and chylomicrons can protect against endotoxin-induced death in mice. *J Clin Invest* 1990;86:696-702.
394. Read TE, Grunfeld C, Kumwenda ZL, Calhoun MC, Kane JP, Feingold KR, Rapp JH. Triglyceride-rich lipoproteins prevent septic death in rats. *J Exp Med* 1995;182:267-272.
395. Levine DM, Parker TS, Donnelly TM, Walsh A, Rubin AL. In vivo protection against endotoxin by plasma high density lipoprotein. *Proc Natl Acad Sci U S A* 1993;90:12040-12044.
396. Casas AT, Hubsch AP, Rogers BC, Doran JE. Reconstituted high-density lipoprotein reduces LPS-stimulated TNF alpha. *J Surg Res* 1995;59:544-552.
397. Hubsch AP, Casas AT, Doran JE. Protective effects of reconstituted high-density lipoprotein in rabbit gram-negative bacteremia models. *J Lab Clin Med* 1995;126:548-558.
398. Quezado ZM, Natanson C, Banks SM, Alling DW, Koev CA, Danner RL, Elin RJ, Hosseini JM, Parker TS, Levine DM, . Therapeutic trial of reconstituted human high-density lipoprotein in a canine model of gram-negative septic shock. *J Pharmacol Exp Ther* 1995;272:604-611.
399. Pajkrt D, Doran JE, Koster F, Lerch PG, Arnet B, Van der Poll T, Ten Cate JW, Van Deventer SJ. Antiinflammatory effects of reconstituted high-density lipoprotein during human endotoxemia. *J Exp Med* 1996;184:1601-1608.
400. Parker TS, Levine DM, Chang JC, Laxer J, Coffin CC, Rubin AL. Reconstituted high-density lipoprotein neutralizes gram-negative bacterial lipopolysaccharides in human whole blood. *Infect Immun* 1995;63:253-258.
401. Wurfel MM, Wright SD. Lipopolysaccharide-binding protein and soluble CD14 transfer lipopolysaccharide to phospholipid bilayers: preferential interaction with particular classes of lipid. *J Immunol* 1997;158:3925-3934.
402. Schromm AB, Brandenburg K, Rietschel ET, Flad HD, Carroll SF, Seydel U. Lipopolysaccharide-binding protein mediates CD14-independent intercalation of lipopolysaccharide into phospholipid membranes. *FEBS Lett* 1996;399:267-271.
403. Goldfarb RD, Parker TS, Levine DM, Glock D, Akhter I, Alkhudari A, McCarthy RJ, David EM, Gordon BR, Saal SD, Rubin AL, Trenholme GM, Parrillo JE. Protein-free phospholipid emulsion treatment improved cardiopulmonary function and survival in porcine sepsis. *Am J Physiol Regul Integr Comp Physiol* 2003;284:R550-R557.
404. Schromm AB, Lien E, Henneke P, Chow JC, Yoshimura A, Heine H, Latz E, Monks BG, Schwartz DA, Miyake K, Golenbock DT. Molecular genetic analysis of an endotoxin nonresponder mutant cell line: a point mutation in a conserved region of MD-2 abolishes endotoxin-induced signaling. *J Exp Med* 2001;194:79-88.
405. Van der Poll T, Braxton CC, Coyle SM, Boermeester MA, Wang JC, Jansen PM, Montegut WJ, Calvano SE, Hack CE, Lowry SF. Effect of hypertriglyceridemia on endotoxin responsiveness

in humans. *Infect Immun* 1995;63:3396-3400.
406. Netea MG, Demacker PN, Kullberg BJ, Boerman OC, Verschueren I, Stalenhoef AF, Van der Meer JW. Low-density lipoprotein receptor-deficient mice are protected against lethal endotoxemia and severe gram-negative infections. *J Clin Invest* 1996;97:1366-1372.
407. Lynch JR, Tang W, Wang H, Vitek MP, Bennett ER, Sullivan PM, Warner DS, Laskowitz DT. APOE genotype and an ApoE-mimetic peptide modify the systemic and central nervous system inflammatory response. *J Biol Chem* 2003;278:48529-48533.
408. Laskowitz DT, Thekdi AD, Thekdi SD, Han SK, Myers JK, Pizzo SV, Bennett ER. Downregulation of microglial activation by apolipoprotein E and apoE-mimetic peptides. *Exp Neurol* 2001;167:74-85.
409. Barger SW, Harmon AD. Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature* 1997;388:878-881.
410. Moore RE, Navab M, Millar JS, Zimetti F, Hama S, Rothblat GH, Rader DJ. Increased atherosclerosis in mice lacking apolipoprotein A-I attributable to both impaired reverse cholesterol transport and increased inflammation. *Circ Res* 2005;97:763-771.
411. Liao XL, Lou B, Ma J, Wu MP. Neutrophils activation can be diminished by apolipoprotein A-I. *Life Sci* 2005;77:325-335.
412. Furlaneto CJ, Ribeiro FP, Hatanaka E, Souza GM, Cassatella MA, Campa A. Apolipoproteins A-I and A-II downregulate neutrophil functions. *Lipids* 2002;37:925-928.
413. Massamiri T, Tobias PS, Curtiss LK. Structural determinants for the interaction of lipopolysaccharide binding protein with purified high density lipoproteins: role of apolipoprotein A-I. *J Lipid Res* 1997;38:516-525.
414. Gupta H, Dai L, Datta G, Garber DW, Grenett H, Li Y, Mishra V, Palgunachari MN, Handattu S, Gianturco SH, Bradley WA, Anantharamaiah GM, White CR. Inhibition of lipopolysaccharide-induced inflammatory responses by an apolipoprotein AI mimetic peptide. *Circ Res* 2005;97:236-243.
415. Vowinkel T, Mori M, Krieglstein CF, Russell J, Saijo F, Bharwani S, Turnage RH, Davidson WS, Tso P, Granger DN, Kalogeris TJ. Apolipoprotein A-IV inhibits experimental colitis. *J Clin Invest* 2004;114:260-269.
416. Jong MC, Gijbels MJ, Dahlmans VE, Gorp PJ, Koopman SJ, Ponec M, Hofker MH, Havekes LM. Hyperlipidemia and cutaneous abnormalities in transgenic mice overexpressing human apolipoprotein C1. *J Clin Invest* 1998;101:145-152.