8

Lipoproteins and atherogenesis

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General overview of the lipoprotein pathways and their contribution to lipid homeostasis and to atherosclerosis

The lipoprotein pathways

The transport of free cholesterol, cholesteryl esters, triglycerides, phospholipids and other lipids in the circulation is achieved by the packing of the lipid moieties into water-soluble lipoproteins. The plasma lipoproteins are either spherical particles or discoidal particles. The spherical particle has a core of non-polar neutral lipid consisting of cholesteryl esters (CE) and triglycerides (TG), and coats of relatively polar materials such as phospholipids, cholesterol, and proteins (Figure 8.1a).[1] The discoidal particle consists of mostly polar lipids and proteins, and is in a bilayer conformation (Figure 8.1a, Table 8.1).[1,2]

Plasma lipoproteins have traditionally been grouped into five major classes and various subclasses, based on their buoyant density (Figure 8.1a): chylomicrons, very low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Lipoprotein (a) (Lp(a)), which will be discussed later, floats in the LDL region.[2,3]

Lipoproteins are synthesized and catabolized in three distinct pathways: the chylomicron pathway, the VLDL/LDL/IDL pathway, and the HDL pathway, all of which are metabolically interrelated (Figure 8.1b–d). Several different proteins, including apolipoproteins, plasma enzymes, lipid transfer proteins, lipoprotein receptors, and lipid transporters, participate in these pathways and contribute to lipid homeostasis. The properties of the apolipoproteins, plasma enzymes and lipid transfer proteins, lipoprotein receptors, and receptors for modified lipoproteins (scavenger receptors) are shown in Tables 8.2, 8.3, 8.4, and 8.5, respectively.

The assembly of chylomicrons and VLDL occurs intracellularly, whereas that of HDL occurs extracellularly.[4,5] In the chylomicron pathway, synthesis of chylomicrons occurs in the intestine. Following food uptake, dietary lipids assemble with apoB-48 in intestinal epithelial cells to form chylomicrons. The assembly and subsequent secretion of chylomicrons in the lymph requires microsomal triglyceride transfer protein (MTP). In the absence of apoB-48 or MTP, chylomicrons are not formed. Following secretion, the TG of chylomicrons are hydrolyzed in plasma by lipoprotein lipase, which is anchored on the surface of microvascular endothelial cells and is activated by apolipoprotein CII (apoCII). Triglyceride hydrolysis converts chylomicrons to chylomicron remnants rich in CE. These remnants contain apoE on their surface and are cleared rapidly by the liver through the LDL receptor and possibly other members of the LDL receptor family members. This family includes the LDL receptor-related protein (LRR), the megalin /gp330, the apoE receptor-2 (apoER2), and the VLDL receptor (VLDLr), which are discussed later.

Known genetic alterations in the different steps of the chylomicron pathway, associated with human diseases, are shown schematically in Figure 8.1b (the numbers 1–9 shown in Figure 8.1b–d correspond to the different proteins of the pathways, and diseases associated with them), and have been extensively reviewed:[5–7]

Table 8.1 Properties and composition of major human plasma lipoproteins (modified from references 3 and 387)

Properties and compositions	*Chylomicrons*	*VLDL*	*LDL*	*HDL*	*Lp(a)*
Source	Intestine	Liver	VLDL	Liver and intestine	Liver
Size (Å)	750–12 000	300–800	180–300	50–120	250–300
Density (g/ml)	< 0.94	0.94–1.006	1.019–1.063	1.063–1.21	1.040–1.090
Molecular mass (kDa)	~400 000	10–80 000	2300	175–360	3000–8000
Triglycerides (%wt)	80–95	45–65	4–8	2–7	~1
Phospholipids (%wt)	3–6	15–20	18–24	26–32	~22
Free cholesterol (%wt)	1–3	4–8	6–8	3–5	~8
Esterified cholesterol (%wt)	2–4	16–22	45–50	15–20	~37
Protein (%wt)	1–2	6–10	18–22	45–55	~32
Major apolipoproteins	apoA-I, apoA-IV, apoB-48, apoCI, apoCIII, apoE	apoB-100, apoE, apoCI, apoCII, apoCIII	apoB-100	apoA-I, apoA-II	apoB-100, apo(a)
Minor apolipoproteins	apoA-II, apoCII	apoA-I, apoA-II, apoA-IV	apoCI, apoCII, apoCIII, apoE	apoCI, apoCII, apoCIII, apoD, apoE, apoJ	–

VLDL, very low-density lipoproteins; LDL, low-density lipoprotein; HDL, high-density lipoproteins;Lp(a), lipoprotein (a); apo, apolipoprotein

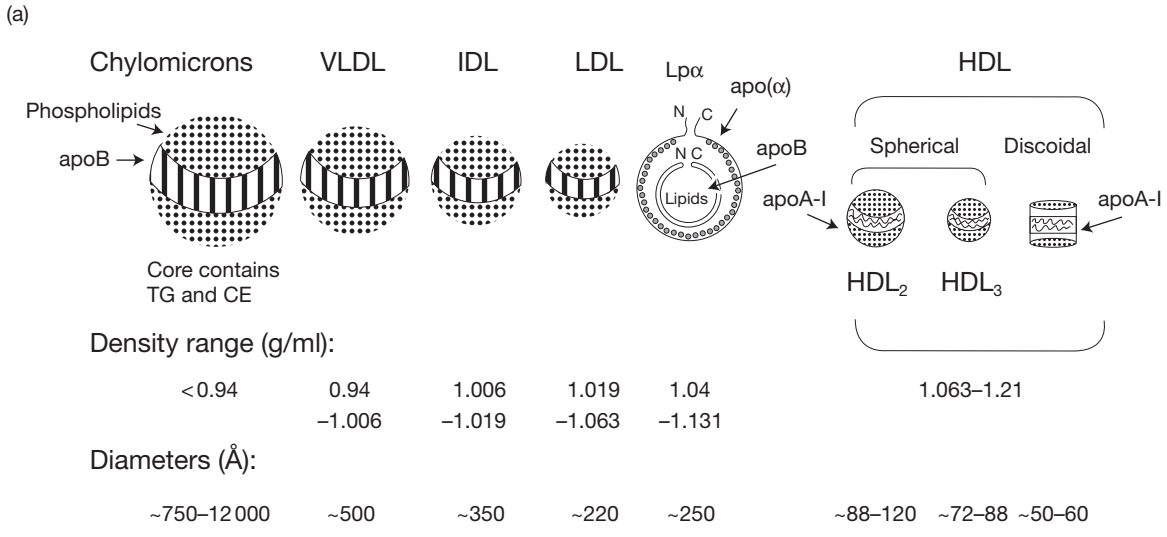
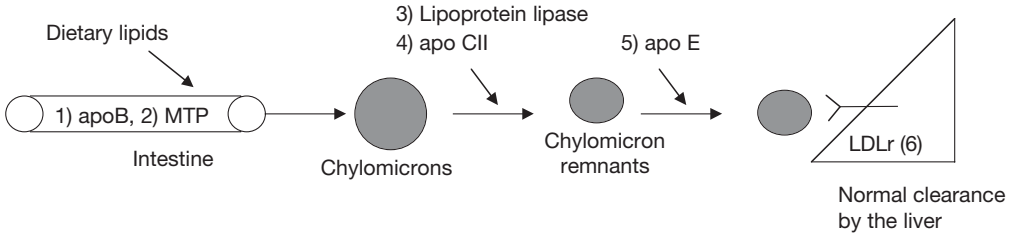


Figure 8.1 (a) Schematic representation of plasma lipoproteins and their metabolic pathways. The major apolipoproteins of low- and high-density lipoproteins (LDL and HDL) are depicted to shield the fatty acyl chains of the phospholipids. The schematic representation of lipoprotein(a) (LP(a)) is designed to depict the position of apolipoprotein B (apoB) and apo(a). TG, triglycerides; CE, cholesteryl esters

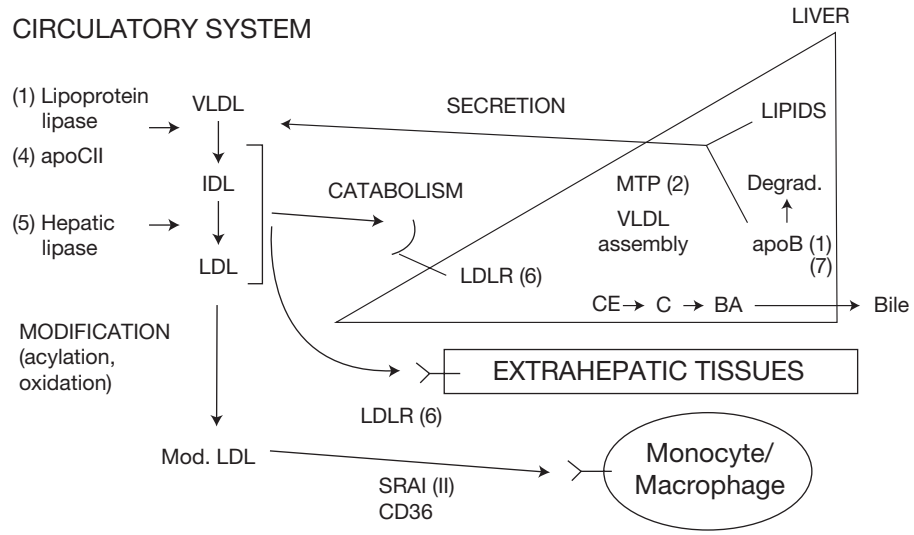
(b)



(1) Homozygous hypobetalipoproteinemia due to generation of truncated apoB forms (i.e. apoB-30 etc.)
(2) Abetalipoproteinemia due to mutations in MTP
(3) Familial hypertriglyceridemia due to lack of lipoprotein lipase
(4) Familial hypertriglyceridemia due to lack of apoCII
(5) Familial type III hyperlipoproteinemia due to mutations in apoE

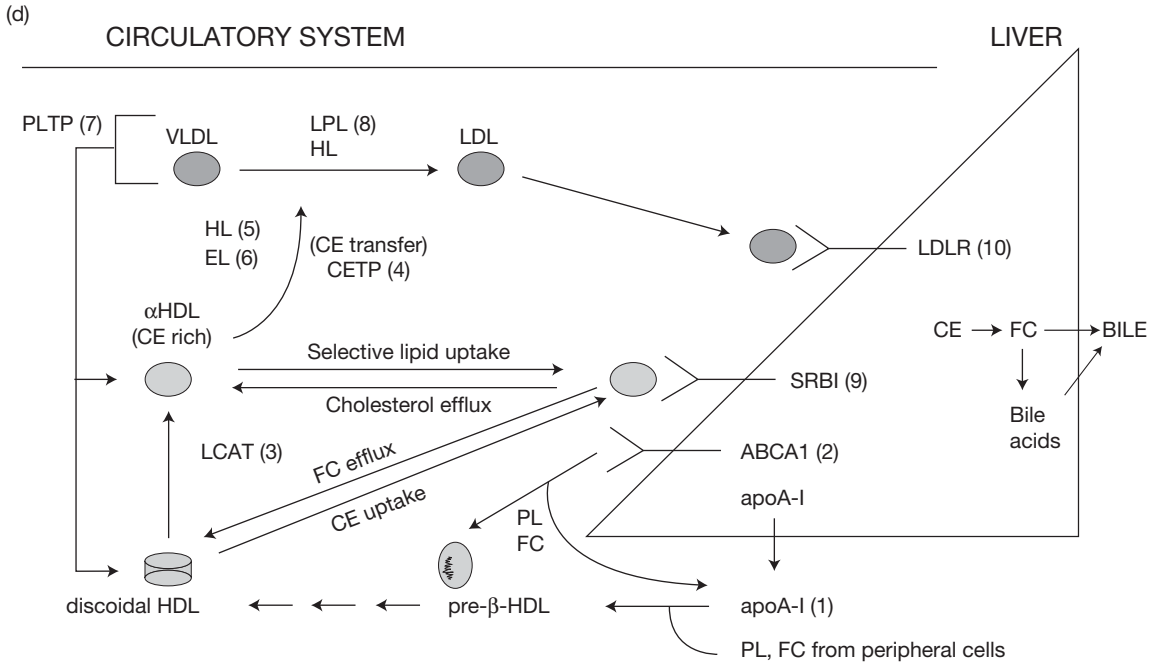
Figure 8.1 (b) Schematic representation of the pathway of the biosynthesis and catabolism of chylomicrons. Numbers 1–6 indicate the proteins of the pathway and their association with diseases. These are: (1) apoB, (2) microsomal triglyceride transfer protein (MTP), (3) lipoprotein lipase (LPL), (4) apoCII, (5) apoE, (6) LDL receptor (LDLr)

(c)



(1) Homozygous hypobetalipoproteinemia due to the generation of truncated apoB forms
(2) Abetalipoproteinemia due to mutations in MTP
(3) Familial hypertriglyceridemia due to mutations in lipoprotein lipase
(4) Familial hypertriglyceridemia due to mutations in apoCII
(5) Hepatic lipase deficiency due to mutations in hepatic lipase
(6) Familial hypercholesterolemia due to mutations in the LDL receptor
(7) Familial defective apoB-100 and other apoB mutations that affect the affinity of LDL for the LDL receptor

Figure 8.1 (c) Schematic representation of the pathway of the biosynthesis and catabolism of very low-density lipoprotein (VLDL). Numbers 1–6 indicate the proteins of the pathway and their association with diseases. These are: (1) apoB, (2) MTP, (3) LPL, (4) apoCII, (5) apoE, (6) LDLr



(1) ApoA-I deficiency due to chain termination mutations or deletions of the apoA-I gene
(2) Tangier disease due to mutations in the ABCA1 transporter
(3) LCAT deficiency (inability to esterify cholesterol on HDL and LDL) and fish eye disease (inability to esterify cholesterol on HDL only) due to mutations in LCAT
(4) CETP deficiency due to mutations in CETP
(5) Hepatic lipase
(6) Endothelial lipase
(7) Phospholipid transfer protein
(8) Lipoprotein lipase
(9) Scavenger receptor type BI
(10) LDL receptor

Figure 8.1 (d) Schematic representation of the pathway of biogenesis and catabolism of HDL by liver. Numbers 1–10 indicate key cell membrane or plasma proteins shown to influence HDL levels or composition and their association with diseases. These are: (1) apoA-I; (2) ATP-binding cassette A1 (ABCA1); (3) lecithin : cholesterol acyltransferase (LCAT); (4) cholesteryl ester transfer protein (CETP); (5) hepatic lipase (HL); (6) endothelial lipase (EL); (7) phospholipids transfer protein (PLTP); (8) LPL; (9) scavenger receptor type BI (SRBI); (10) LDLr

- *Homozygous hypobetalipoproteinemia* is characterized by lack of synthesis and secretion of chylomicrons, and is the result of chain termination mutations that generate truncated apoB forms.
- *Abetalipoproteinemia* is characterized by a lack of chylomicron synthesis and secretion, and is the result of mutations in MTP.
- *Familial hypertriglyceridemia* is characterized by high plasma triglyceride levels, and is the result of mutations in lipoprotein lipase or an inhibitor of lipoprotein lipase.
- *Familial hypertriglyceridemia* is characterized by high plasma triglyceride levels, and is the result of mutations in apoCII.
- *Familial type III hyperlipoproteinemia* is characterized by high plasma cholesterol and usually high triglyceride levels in the VLDL and IDL regions, and is the result of mutations in apoE.

Two additional conditions of unknown or uncertain molecular etiology are:

- *Type V hyperlipoproteinemia,* characterized by the presence of chylomicrons in fasting plasma, and moderate to severe hypertriglyceridemia.[6] This syndrome was described by Fredrickson and Lees,[8] and encompasses a diverse group of patients with primary and secondary causes of hypertriglyceridemia.[8,9]
- *Chylomicron retention disease* is characterized by defective secretion of chylomicrons and fat malabsorption.

Biogenesis of LDL occurs in the liver. In this pathway, apoB assembles intracellularly with lipids in the hepatocytes by the action of MTP to form VLDL, which is then secreted into the plasma. Lack of MTP or an availability of lipids leads to apoB degradation and inhibits VLDL assembly and secretion. More details on the assembly and secretion of VLDL, chylomicrons, and Lp(a) are provided in later chapters. Following secretion, the TG of VLDL are hydrolyzed by the action of lipoprotein lipase (which is activated by apoCII) to produce IDL, which is further converted to LDL by the action of hepatic lipase. IDL and LDL are recognized and catabolized by the LDL receptor (Figure 8.1c). An increase in the plasma LDL levels or the formation of small dense LDL particles is associated with an increased risk for atherosclerosis.[10] Mutations in the LDL pathway underlying human diseases are shown in Figure 8.1c and have been extensively reviewed:[5,6,11]

- *Homozygous hypobetalipoproteinemia.*
- *Abetalipoproteinemia.*
- *Familial hypertriglyceridemia.*
- *Familial hypertriglyceridemia.*
- *Hepatic lipase deficiency* is characterized by the accumulation of large-size HDL and LDL, and is the result of mutations in the hepatic lipase (HL).
- *Familial hypercholesterolemia* is characterized by high plasma cholesterol and xanthomatosis, and is the result of mutations in the LDL receptor.

Two additional diseases of unknown molecular etiology are:

- *Familial defective apoB-100 and other apoB mutations* that affect the affinity of LDL for the LDL receptor. They are characterized by moderate increases in the LDL levels.
- *Familial combined hyperlipidemia*, characterized by increased plasma cholesterol and in some cases increased triglyceride levels, and which is the result of overproduction of apoB.

The LDL pathway is discussed in more detail in a later chapter.

Biogenesis of HDL occurs mainly in the liver through a complex pathway. In the early steps of this pathway, apolipoprotein A-I (apoA-I) is secreted mostly lipid free by the liver, and acquires phospholipid and cholesterol via its interactions with the ATP-binding cassette A1 (ABCA1) lipid transporter and other processes. Through a series of intermediate steps that are poorly understood, apoA-I is gradually lipidated and proceeds to form discoidal particles that are converted to spherical particles by the action of lecithin: cholesterol acyl transferase (LCAT). Both the discoidal and the spherical HDL particles interact functionally with the HDL receptor/scavenger receptor class B type I (SRBI). The interactions of apoA-I with SRBI are important for the atheroprotective functions of HDL. The late steps of the HDL pathway involve the transfer of cholesteryl esters to VLDL/LDL for eventual catabolism by the LDL receptor, the hydrolysis of phospholipids and residual triglycerides by the various lipases (LPL, HL, and endothelial lipase (EL)), and the transfer of phospholipids from VLDL/LDL to HDL by the action of phospholipid transfer protein (PLTP) (Figure 8.1d and Table 8.3).

Known mutations in the HDL pathway that may affect the pathway are shown in Figure 8.1d and have been reviewed extensively.[4,6,12,13] They are listed below.

- *ApoA-I deficiency* is characterized by the absence of HDL, and is the result of chain determination mutations or deletions of the apoA-I gene.
- *Tangier disease* is characterized by the absence of HDL, and is the result of mutations in the ABCA1 transporter.
- *LCAT deficiency* (inability to esterify cholesterol on HDL and LDL, and the accumulation of discoidal HDL in plasma) and fish eye disease (inability to esterify cholesterol on HDL only), are both characterized by low HDL levels and caused by mutations in LCAT.

Table 8.2 Summary of apolipoprotein structure and functions[387]

Apolipo-protein	*Amino acids**	*Lipoprotein*	*Function*	*Association with disease*
ApoA-I	243	HDL	Activates LCAT, interacts with SRBI and promotes selective lipid uptake and cholesterol efflux, interacts with ABCA1 and promotes lipid efflux and HDL biogenesis, has atheroprotective functions in the arterial wall, antiatherogenic	Deletion of apoA-I, apoCIII, apoA-IV loci and inversion of the apoA-I, CIII, loci are associated with atherosclerosis
ApoA-II	77	HDL	Inhibits the activity of hepatic lipase Atherogenic in mice models	ApoA-II deficiency in humans does not affect HDL levels or susceptibility to coronary heart disease
ApoA-IV	376	d < 1.21 g/ml	Activates LCAT Is antiatherogenic in transgenic mice Has functional similarities with apoA-I Promotes cholesterol efflux, protects from atherosclerosis	Deletion of apoA-I, apoCIII, apoA-IV loci and inversion of the apoA-I, CIII, loci are associated with atherosclerosis
ApoB	4536	LDL	Ligand for the LDL receptor promotes the formation of nascent VLDL	Abetalipoproteinemia, hypobetalipoproteinemia, LDL receptor binding defects familial apoB-100 (deficiency)
ApoCI	57	VLDL, HDL	Activates LCAT moderately	
ApoCII	79	VLDL, HDL	Activates lipoprotein lipase	Familial type I hyperlipoproteinemia
ApoCIII	79	VLDL, HDL	Inhibits the catabolism of triglyceride-rich lipoproteins	Deletion of apoA-I, CIII, A-IV loci and inversion of apoA-I, CIII loci is associated with atherosclerosis
Apo CIV[388,389]	102	VLDL	Increased VLDL is expressed at very low levels in human liver, nearly undetectable May contribute to plasma triglyceride levels	None Overexpression increases plasma triglycerides
ApoAV[390]	345	VLDL, HDL	May contribute to plasma triglyceride homeostasis	Overexpression reduces plasma triglyceride levels
ApoE	299	VLDL, HDL	Ligand for the LDL receptor and other apoE-recognizing receptors	Familial type III hyperlipoproteinemia Late-onset of Alzheimer's disease
Apo(a)	4529	Lp(a)	Has atheroprotective functions in the arterial wall Has protease activity different from that of plasmin Interferes withfibrinolysis, induces proinflammatory and proliferative conditions in the vascular wall and inducesatherosclerosis in transgenic mice	Increase Lp(a) levels are associated with disease cardiovascular Lp(a) may contribute to thrombosis

* Refers to the sequence of the mature protein without the signal peptide.
HDL, high-density lipoprotein; apo, apolipoprotein; LCAT, lecithin : cholesterol acyltransferase; SRBI, scavenger receptor class B type I; ABCA1, ATP-binding cassette A1; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; Lp(a), lipoprotein (a)

Table 8.3 Plasma enzymes and lipid transfer proteins

Enzyme/ protein	*Amino acids**	*Site of synthesis*	*Function*	*Association with disease*
Lipoprotein lipase (LPL)	448	High level in adipose tissue, heart, muscle, brain; low level in most other tissues Undetectable liver, spleen and white blood cells	Hydrolysis of 1 and 3 ester bonds of chylomicron and VLDL triglycerides	Type I hyperlipoproteinemia, (hypertriglyceridemia)
Hepatic lipase (HL)	476	Liver	Hydrolysis of lipoprotein mono- and diacylglycerol and phospholipids of HDL2 and IDL	Increased plasma cholesterol and triglycerides, abnormal lipoprotein profiles, premature atherosclerosis in some cases
Lecithin cholesterol acyltrans-ferase (LCAT)	416	Liver, brain, testis	Esterification of free cholesterol on HDL and LDL using the C2 acyl group of lecithin	Familial LCAT deficiency, fish eye disease In LCAT deficiency there is accumulation of discoidal HDL particles Kidney disease and atherosclerosis in some cases
Cholesteryl ester transfer protein (CETP)	476	Liver, small intestine, spleen, adipose tissue, muscle and adrenal, kidney (lesser extent)	Exchange or transfer of cholesteryl ester, triglycerides and phospholipids between lipoproteins CETP transfer primarily from VLDL to HDL	Familial hyperalphalipoproteinemia CHD is observed only in some populations with CETP mutations
Phospholipid transfer protein (PLTP)	476	Ubiquitously expressed; liver, ovary, thymus, placenta, and adipose tissue	Transfers phospholipid from chylomicrons and VLDL to HDL; HDL conversion	PLTP activity is correlated with the serum triglyceride levels
Endothelial lipase (EL)	482	Liver, lung, kidney, placenta (endothelial cells)	Phospholipase activity and limited triglyceride lipase activity HDL is the primary substrate	Overexpression decreases HDL EL deficiency increases HDL
Microsomal triglyceride transfer protein dimer of 35 kDa and 97 kDa subunits	876 aa (97 kDa subunit)	55 kDa disulfide isomerase subunit ubiquitous liver intestine	Assembly of VLDL and chylomicrons	Mutations in MTP are associated with αβ-lipoproteinemia

VLDL, very low-density lipoprotein; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein

Table 8.4 Summary of low-density lipoprotein (LDL) receptor family members (modified from reference 391)

Name	*Amino acids**	*Tissue-specific*	*Ligand*	*Recognition*	*Function*
LDL receptor	839	Ubiquitous	LDL, remnants	ApoB, apoE	Binds and internalizes lipoproteins that contain apoB and apoE on their surface (LDL, β-VLDL, IDL, HDL with apoE); is subjected to feedback regulation by cholesterol levels.
ApoER2	922	Brain, testis, ovary, placenta	β-VLDL apoE-containing lipoproteins	ApoE	Binds apoE-containing lipoproteins such as β-VLDL and other apoE-containing lipoproteins; may play an important role in cholesterol homeostasis in the brain
VLDL receptor	819	Heart, muscle, adipose tissue	VLDL, β-VLDL, IDL	ApoE	Binds VLDL, β-VLDL, and IDL (does not bind LDL)
LRP	4525	Liver, brain, placenta	Multiligand receptor binds apoE-enriched β-VLDL, other ligands	ApoE	Physiological function unknown in activation of the LRP gene leads to embryonic lethality Binds and internalizes apoE-containing lipoproteins such as β-VLDL α_2-macroglobulin–protease complex plasminogen activator Inhibitor complex lactopherin lipoprotein lipase

* Refers to the mature sequence without the signal peptide.
Apo, apolipoprotein; VLDL, very low-density lipoprotein; IDL, intermediate-density lipoprotein; HDL, high-desnity lipoprotein; LRP, LDL receptor-related protein

- *Cholesteryl ester transfer protein (CETP) deficiency* is characterized by an increase in HDL, and is the result of two mutations in CETP.
- *Hepatic lipase.*

Based on studies in animal models that are discussed later, HDL structure and function can also be affected by defects in:

- *Endothelial lipase*
- *Phospholipid transfer protein*
- *Lipoprotein lipase*
- *Scavenger receptor BI.*

Finally, low levels of HDL and apoA-I predispose to atherosclerosis.[10]

Apolipoproteins

The protein components of lipoproteins are called apolipoproteins and have been named apoA-I, apoA-II, apoA-IV, apoB, apoCI, apoCII, apoCIII, apoCIV, apoD, apoE, apo(a), and apoJ, etc.[1,14] A hallmark of most of the apolipoproteins, such as apoA-I, apoA-IV, and apoE, is the presence of amphipathic α-helices consisting of either 22 or 11 residues in their secondary structure.[15] These amphipathic helices contribute to the lipid-binding properties and possibly some other functions of the apolipoproteins. ApoB is characterized by the presence of extensive regions of amphipathic β sheet(s), which contribute to lipid binding.[16] Examples of how the apolipoproteins apoB and apoA-I assemble with lipids and form LDL and HDL are discussed later.

In addition to their lipid-binding functions, the apolipoproteins have several other specific functions. Lipid-bound apoA-I is a ligand for SRBI.[17] Lipid-free apoA-I is a ligand for ABCA1.[18] Both lipid-bound and lipid-free apoA-I are ligands for cubilin, which is an HDL receptor.[19] ApoA-I, and to a lesser extent apoA-IV, apoCII, and apoE, are activators of LCAT,[20,21] and apoCII is an activator of LPL.[22]

Table 8.5 Summary of scavenger receptors (SR)[392]

Name	*Amino acids**	*Character*	*Tissue-specific*	*Ligands known*	*Function*
Class A					
SRAI	453		Macrophages	AcLDL, oxLDL, modified BSA, mal-BSA, fucoidan, dextran sulfate, poly G/ poly I, Gram-negative, apoptotic, silica, LPS, LTA, Aβ-peptide, AGE-modified proteins	Plays a role in phagocytosis
SRAII	349	Lacks the 106 C-terminal residues	Macrophages	AcLDL, oxLDL, modified BSA, mal-BSA, fucoidan, dextran sulfate, poly G/poly I, Gram-negative, apoptotic, silica, LPS, LTA, Aβ-peptide, AGE-modified proteins	Mediates macrophage adhesion in vitro
Class B					
SRBI	509	SRBI has a variant called SRBII, arising from alternative splicing	Liver and steroidogenic tissues	AcLDL, oxLDL, LDL, HDL, mBSA, phosphatidylserine, apoptotic cells	Recognize HDL particles via apoA-I and promotes selective lipid uptake and cholesterol efflux The affinity of the ligand is apoA-I~ pre-β1-HDL <<HDL<rHDL[apoA-I]
SRBII[393]	506	Differs from SRBI in the C-terminal cytoplasmic tail Generated by alternative splicing	Liver and steroidogenic tissues	HDL, LDL and modified LDL	Has 25% of selective lipid uptake and cholesterol efflux capacity of SRBI
CD36	471		Platelets, microvascular endothelial cells, erythroid precursors, adipocytes, striated muscle, breast, retina, monocyte/ macrophages	Thrombospordin, modified LDL, oxidized lipids Apoptotic cells *Plasmodium*-infected erythrocytes	Mediates macrophage scavenging of modified lipoproteins of senescent polymorphonuclear cells Long-chain fatty acid transporter Primary receptor for platelet adhesion in muscle and heart CD36 deficiency in humans is associated with reduced uptake of oxLDL

*Refers to the mature sequence without the signal peptide
Ac, acetylated; ox, oxidized; LDL, low-density lipoprotein; BSA, bovine serum albumin; mal, maleylated; LPS,; LTA,; AGE, advanced glycation end products; HDL, high-density lipoprotein

ApoB-100 and apoE are ligands for the LDL receptor (LDLr).[7,23] ApoE is a ligand for apoE receptor 2 (apoER2), LDLr-related protein (LRP), gp 330/megalin, VLDL receptor (VLDLr), and other lipoprotein receptors.[1,23] Apolipoprotein functions and their associations with human disease are summarized in Table 8.2.

Enzymes and lipid transfer proteins

Following biosynthesis, lipoproteins are modified in plasma by the action of plasma enzymes and lipid transfer proteins, and are subsequently recognized by different types of cell receptor. These modifications are very important for the function and catabolism of lipoproteins. The enzymes and lipid transfer proteins involved in these modifications are LPL, HL, LCAT, CETP, and PLTP.[4,6,12] Their properties, sites of expression, and associations with human disease are summarized in Table 8.3.

LPL

LPL is a glycoprotein synthesized mainly by adipose tissue, cardiac and skeletal muscle, and monocyte-derived macrophages. It hydrolyzes preferentially the 1- and 3-ester bonds of the triglycerides of chylomicron and VLDL, generating free fatty acids and mainly 2-monoglycerides. LPL is secreted and binds to endothelial cells via its heparin-binding sites. The enzyme is activated by apoCII[6] and inhibited by 1 M NaCl. Deficiency in LPL is associated with severe hypertriglyceridemia.[6]

HL

HL is a glycoprotein synthesized by the liver. It is secreted and binds to the surfaces of sinusoidal endothelial cells.[6] HL hydrolyzes mono- and diacylglycerols and phospholipids of IDL and HDL, resulting in the generation of more dense lipoprotein particles.[24] In vivo and in vitro studies have shown that HL hydrolyzes large, apoE-enriched HDL particles.[6] Deficiency of HL in human and experimental animals is associated with increased levels of triglycerides and phospholipids in HDL and IDL. Studies in mice deficient in both HL and apoA-II suggest that apoA-II may inhibit the action of HL on HDL metabolism.[1] Variation in the activity of HL due to genetic or environmental factors. Cholesterol-lowering and other treatments may affect the size of LDL and HDL.[25]

EL

EL is structurally and functionally related to the other members of the lipase gene family. Similar to HL, EL has primarily phospholipase activity and limited triglyceride lipase activity.[26–28] EL is expressed by endothelial cells, macrophages, liver, and other tissues, including lung, kidney, testis, and placenta.[26–28] Overexpression of HL dramatically reduces the HDL and apoA-I levels and causes only a small decrease in non-HDL cholesterol levels.[26,27,29,30] In contrast, inactivation of EL in mice decreased clearance of HDL and increased plasma HDL and apoA-I levels.[29,30] The findings indicate that EL affects the structure, concentration, and metabolism of HDL. The role of this novel lipase in atherogenesis has yet to be determined.

LCAT

LCAT is a glycoprotein synthesized by the liver. Following secretion, it associates with HDL and LDL. It is responsible for the esterification of free cholesterol of HDL and LDL, using the fatty acyl group on the position C-2 of lecithin as the acyl donor.[13] Mutations in LCAT are associated with LCAT deficiency, characterized by the inability of the enzyme to esterify the cholesterol of HDL and LDL,[13] or with fish eye disease, characterized by the inability of the enzyme to esterify cholesterol on HDL, but not on LDL.[31]

CETP

CETP is a highly hydrophobic glycoprotein synthesized by the liver, the small intestine, and several other tissues.[32] It catalyzes an exchange of neutral lipids, particularly triglyceride and cholesteryl esters, between all the major lipoprotein classes. Net transport of CE by the action of CETP depends mainly on the availability of suitable triglyceride-rich acceptor particles.[1] Cholesteryl ester transport to VLDL and LDL is associated with reciprocal but not equimolar transport of triglycerides.[33] CETP deficiency in humans is characterized by increased HDL levels and a low prevalence of coronary heart disease (CHD), whereas heterozygotes for another CETP mutation have increased risk for CHD.[4]

PLTP

PLTP is a glycoprotein synthesized principally by the liver and the adipose tissue. PLTP facilitates the

exchange and net transfer of phospholipids from VLDL to HDL, and does not have cholesteryl ester or triglyceride transfer activity. PLTP can also remodel the HDL.[34,35] PLTP activity is increased in diabetes mellitus, in obesity, and in insulin resistance.[34] The role of the lipases and lipid transfer proteins in atherogenesis is discussed later, as they appear in the different lipoprotein pathways.

Under normal conditions, existing homeostatic mechanisms in the body help to maintain physiological concentrations of the various classes of lipoproteins and their derivatives in plasma. When the function or regulation of synthesis of one or more proteins of the lipoprotein system is altered, the concentration or the function(s) of one of the lipoproteins may be altered. These changes may affect the levels of cholesterol and/or triglycerides in plasma and, in some instances, promote atherosclerosis and other complications.

Lipoproteins and atherogenesis

Factors contributing to atherogenesis

Our ability to understand better the role of apolipoproteins, plasma enzymes, lipid transfer proteins, and lipoprotein receptors in the homeostasis of cholesterol and other lipids and their contribution to atherogenesis was assessed first in human studies. This knowledge has been greatly enhanced during the last 15 years by the generation of animal models in which one or more protein(s) have been altered by the addition or subtraction of the corresponding gene(s). Following alterations of one or more gene(s) of the pathway of interest, the parameters analyzed are the lipid and lipoprotein profile, the pathogenesis of atherosclerosis, or other physiological changes. The combined knowledge from the study of human subjects and animal models is summarized in Tables 8.6, 8.7, 8.8, and 8.9, and is discussed in further detail in later sections.

Atherosclerosis is a focal disease of the arterial wall that appears usually in areas of disturbed blood flow where gene expression is altered,[36] and affects large- and medium-sized arteries. In response to proatherogenic conditions, such as those created by hypercholesterolemia, monocytes bind to adhesion molecules on the endothelial cell surface and migrate to the subendothelial space, where they differentiate to macrophages. Induction of adhesion molecules is promoted by proinflammatory stimuli.[37] Recruitment and migration of monocytes into the subendothelial space is promoted by oxidized LDL, as well as by monocyte chemattractant factor (MCP-1), which binds to the MCP-1 receptor CCR2.[38] These proteins are expressed by endothelial cells, smooth muscle cells, and monocyte/macrophages, and are induced in hypercholesterolemia.[39] These cells, through the scavenger receptors (SRAI, SRAII, and CD36)[40,41] and possibly other processes, are loaded with cholesteryl ester, which is later deposited in the site of the lesion and contributes to the evolution of the atherosclerotic plaque.[42]

The initial lesion created by macrophages is called the fatty streak, and is reversible.[43–46] The lesions may progress with the recruitment of additional monocytes and T cells and migration into the intima (Figure 8.2).[47] Signals secreted by the blood-borne cells, as well as by the activated endothelial cells,[48] promote migration of smooth muscle cells from the media into the intima, which subsequently proliferate and synthesize matrix components such as collagen and proteoglycans.[42] As the development of lesions progresses, cholesteryl ester-laden monocytes/macrophages and smooth muscle cells in the plaque die. This leads to the creation of the necrotic core with extracellular cholesterol clefts, which characterize the advanced lesions.[49] The luminal face of the lesion often forms a fibrous cap consisting of smooth muscle cells, matrix components, and calcium deposits. This cap is produced by the smooth muscle cells and stabilizes the plaque. In humans, a clinical event, such as myocardial infarction or stroke, may occur as a result of rupture of unstable plaques that enriched with lipid-filled macrophages and have weak fibrous caps, or from intraplaque hemorrhage that leads to the generation of a thrombogenic event that will occlude the plaque.[50,51] It was shown recently that in late stages of necrosis or with thin caps, the region containing cholesterol clefts is enriched in glycophorin A and iron deposits. The findings suggest that erythrocytes contribute to cholesterol deposition, macrophage infiltration, enlargement of the necrotic core, and destabilization of the plaque.[52]

The involvement of lipoproteins and other factors in the cascade of events that leads to the initiation of the atherosclerotic lesion is shown in Figure 8.2[53,54] and discussed in detail in other chapters. It is believed that atherogenic lipoproteins, such as LDL and lipoprotein remnants, promote atherosclerosis, and

Table 8.6 Role of the chylomicron pathway in lipid homeostasis and in atherogenesis: lessons from animal models and human studies

Protein affected	*Susceptibility to atherosclerosis*	*Lipoprotein profile*	*Other pathologies*
ApoCI transgenic[394,395]	–	Mild hypertriglyceridemia	
ApoCII deficiency in humans[4]	None	Severe hypertriglyceridemia	Recurrent pancreatitis
ApoCII transgenic[396]	–	Severe hypertriglyceridemia	
ApoCIII transgenic[88,397]	Increased atherosclerosis compared to control	Severe hypertriglyceridemia	
ApoCIII-/-[398]		Reduced TG	
ApoCIII × apoA-I transgenic ApoCIII × CETP transgenic ApoCIII × apoA-I × CETP transgenic[4,399,400]	Reduced atherosclerosis compared to apoCIII transgenic	Severe hypertriglyceridemia Low HDL	
ApoCIV transgenic[389]	–	Two-fold increase in TG	
ApoE transgenic[84]	Low levels of expression are protective	Mice overexpressing apoE developed severe hypertriglyceridemia[86]	
ApoE knockout (ApoE-/-)[87,285]	Very susceptible mice develop spontaneous atherosclerosis at 8–10 weeks of age and have been used as models to study early and advanced atherosclerotic lesions	8–25-fold increase in plasma cholesterol Accumulation of lipoprotein remnants	
Lipoprotein lipase deficiency in humans[6]	None	Severe hypertriglyceridemia	Recurrent pancreatitis
MTP knockout mice[401]		In heterozygotes MTP+/- LDL↓ Lipoprotein secretion impaired	MTP-/- embryonic lethal Accumulation of cytosolic fat in the visceral endoderm of the yolk sac in MTP+/- and MTP-/-
ApoE2, apoE3, apoE4 knockin[286]	Susceptibility follows the order mouse E<h apoE3<h apoE4<h apoE2	Increase in VLDL and triglyceride follows the order apoE3<apoE4<apoE2	

Continued...

Table 8.6 Continued

Protein affected	*Susceptibility to atherosclerosis*	*Lipoprotein profile*	*Other pathologies*
Transplantation of apoE-/- mice with normal bone marrow[274,293]	Reduced atherosclerosis in young animals No change in the extent of atherosclerotic lesions in older animals	No change in plasma lipids and lipoproteins	
Transplantation of normal mice with apoE-/- bone marrow[292]	Induction of atherosclerosis	No change in lipid levels	
Adenovirus-mediated gene transfer of apoE3/apoE4 in apoE-/- normal or nude mice[287,288]	ApoE3 limited progression of early lesions and advanced regression of advanced lesions. ApoE4 was less effective	Reduction of plasma cholesterol, VLDL and remnant lipoproteins Increase of HDL cholesterol	
Adenovirus-mediated gene transfer of apoE in LDLr-/- mice[289]	Reduction of advanced lesions	No change in lipid levels	
Adenovirus-mediated gene transfer of C-terminal truncated apoE forms to apoE-/- mice[86]	–	Normalized plasma lipid and lipoprotein levels The truncated apoE had a dominant effect when coexpressed with full-length apoE	
Gene transfer of apoE by a helper-dependent adenovirus[296]	Low levels of apoE expression Prevented atherosclerosis	Low levels of apoE expression Normalization of plasma lipid levels	

Apo, apolipoprotein; TG, triglyceride; HDL, high-density lipoprotein; CETP, cholesteryl ester transfer protein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; LDLr, LDL receptor

antiatherogenic lipoproteins, such as HDL, protect from atherosclerosis. It has been proposed that when the concentration of LDL and other atherogenic lipoprotein particles is high, they enter the subendothelium. In atherosclerosis-prone mice, lipid and lipoprotein aggregates are found in the subendothelial space of the arteries.[55] The retention of LDL in the vessel wall depends on their interaction with extracellular proteoglycans. Transgenics of apoB-100 with defective binding to glycosaminoglycans have substantially less initial atheroma.[56]

The tendency of LDL to adhere to proteoglycans increases with treatment of LDL using phospholipase A_2. This generates small, dense LDL which is atherogenic.[57] Phospholipase A_2 transgenic mice on a high- or a low-fat diet have decreased HDL and paraoxonase levels, and slightly increased LDL levels, and develop more aortic lesions than do control mice.[58,59]

Modification of LDL is mediated by products of lipid peroxidation in plasma and in the subendothelial space. Oxidized LDL is taken up by the scavenger receptors SRAI, SRAII, and CD36, leading to the accumula-

Table 8.7 Role of the low-density lipoprotein (LDL) pathway in lipid homeostasis and in atherogenesis: lessons from animal models and human studies

Protein affected	*Susceptibility to atherosclerosis*	*Lipoprotein profile*	*Other pathologies*
ApoB transgenic[80]	Increased atherosclerosis in response to atherogenic diet	LDL↑	
ApoB-100 transgenics with mutations in the proteoglycan-binding domain of apoB[56]	Less atherosclerosis than normal apoB-100 transgenics		
LDLr-/- × apoB transgenic defective in apoB editing[71]	Increased atherosclerosis on chow diet	LDL ↑	
LDLr knockout[402]	Increased atherosclerosis in response to atherogenic diet	LDL↑	
ApoB transgenic × LDL receptor knockout[72]	Increased atherosclerosis in response to chow diet; used as models to study development of early and advanced atherosclerosis	LDL↑	
Hepatic lipase deficiency in humans[6] *See HL transgenic and deficient animal models in Table 8.8*	Susceptible, starts at the age of 40–50	VLDL remnants↑ TG-enriched LDL and HDL	
Human apo(a) transgenic[222,223]	Increased atherosclerosis of atherogenic diets	Apo(a) is found in d > 1.21 g/ml Lp(a) is formed after infusion of LDL	
ApoA-I × apo(a) transgenic[245]	Increased atherosclerosis; fewer lesions compared to apo(a) transgenics	Twofold increase in HDL	
ApoB x apo(a) transgenics[247]	Modest increase in atherosclerosis compared to either apoB or apo(a) transgenics	Fourfold increase in LDL Lipoprotein profile resembles that of humans	
ApoB transgenic × LDLr-/- × apo(a) transgenic[72]	Severe atherosclerosis on chow or atherogenic diet, but quantitatively similar to the levels observed in apoB transgenic × LDLr-/- mice	Increased LDL Decreased HDL	

Unless otherwise stated, 'transgenic' indicates transgenic mice expressing the human transgene.
Apo, apolipoprotein; LDLr, LDL receptor; VLDL, very low-density lipoprotein; TG, triglyceride; HDL, high-density lipoprotein; Lp(a), lipoprotein (a)

Table 8.8 Role of the high-density lipoprotein (HDL) pathway in lipid homeostasis and in atherogenesis: lessons from animal models and human studies

Protein affected	*Susceptibility to atherosclerosis*	*Lipoprotein profile*	*Other pathologies*
ApoA-I transgenic (Tg)[79]	Similar to WT	HDL↑	
ApoA-I Tg rabbits[403]	Reduced atherosclerosis by 50% in response to atherogenic diet	Twofold increase in HDL ApoB lipoproteins did not change	
ApoA-I Tg × apoE$^{-/-}$ mice[319]	Reduced atherosclerosis compared to apoE$^{-/-}$		
ApoA-I-/- Tg × LDLr$^{-/-}$ mice [325]	Reduced atherosclerosis compared to LDLr-/-	HDL↑	
ApoA-I-/- Tg × LDLr-/- rabbits[89]	Reduced atherosclerosis compared to LDLr-/-	HDL↑	
ApoA-I-/- × apoB Tg [321,322]	Increased atherosclerosis as compared to apoB transgenic	HDL↓	
ApoA-I-deficient in humans[4]	CHD at age 11–52 in different probands	Absence of HDL	
ApoA-I knockout mice[323]	No phenotype	HDL↓	
ApoA-II transgenic[82,83]	Increased atherosclerosis	Abnormal composition, HDL↑	
ApoA-II deficiency in humans[4]	None	Normal lipids	
ApoA-II-/- mice[404]	–	50% reduction in HDL due to increased catabolism	Decreased FFA insulin and glucose levels
ApoA-I × apoA-II Tg[405]	15-fold increase in atherosclerosis as compared to apoA-I transgenics in response to atherogenic diet	Cholesterol and HDL were similar in apoA-I and apoA-I × apoA-II transgenics	
Gene transfer of apoA-I to LDLr-/- or apoE-/-[324,325,327]	Reduced atherosclerosis of LDLr-/- or apoE-/-	Increased HDL	
ApoA$^{+/+}$ × apoE-/- transplanted with normal bone marrow[326]	Reduced atherosclerosis Reduced cholesterol content of atherosclerotic aorta compared to apoA-/- × apoE-/- transplanted with normal bone marrow	Cholesterol levels of apoA-/- × apoE-/- reduced compared to apoA-I$^{+/+}$ × apoE-/- mice	
Human apoA-IV Tg × apoE-/- mice[89]	Reduced atherosclerosis compared to apoE-/-	Cholesterol ↑ HDL not affected	
Mouse apoA-IV Tg[90]	Reduced atherosclerosis	Increased HDL	
ApoA-V-/-[390]	–	Fourfold increase in plasma TG	

Continued...

Table 8.8 Continued

Protein affected	*Susceptibility to atherosclerosis*	*Lipoprotein profile*	*Other pathologies*
ApoA-V Tg [390]	–	Decreased plasma TG No change in HDL	
ABCA1 mutations (Tangier disease in humans)[13]	Partially susceptible	Lack of HDL, reduced LDL	
ABCA1-/- mice[340]	None	Low cholesterol Absence of HDL, deficiency in fat-soluble vitamins A, E, K	Impaired growth and neuronal development Hemolysis, platelet abnormalities
ABCA1 Tg[339]	Protected from diet-induced atherosclerosis	Twofold HDL and apoA-I Decreased apoB and LDL	
ABCA Tg × apoE-/-[339]	Increased atherosclerosis compared to apoE-/- mice	Lipids and lipoproteins same as in apoE-/- mice	
Transplantation of ABCA1-/- bone marrow in LDLr-/- mice[342]	Increased atherosclerosis	Lipid levels same as in LDLr-/- mice	
Transplantation of ABCA1-/- bone marrow in apoE-/- mice[406]	Increased atherosclerosis	Lipid levels same as in LDLr-/- and apoE-/- mice	
Transplantation of normal marrow in ABCA1-/- mice[341]	–	Minimal effect on HDL levels	
SRBI Tg [353,360–362]	–	HDL↓ apoA-I↓ VLDL↓ LDL↓ Accelerated clearance of HDL Decreased cholesterol content of steroidogenic tissues and increased bile excretion	
SRBI-/- mice[356,407]	–	HDL↑ Abnormal composition	Impaired oocyte development and red blood cell maturation
SRBI+/- × LDLr-/- [363]	Increased atherosclerosis	Increased LDL cholesterol	
SRBI-/- × apoE-/- [355,356,364]	Occlusive coronary atherosclerosis	HDL↑	Cardiac dysfunction Die at 8 weeks of age
SRBI Tg × LDLr$^{+/-}$ [360]	Reduced atherosclerosis compared to LDLr+/- mice	VLDL↓ LDL↓ HDL↓	

Continued...

Table 8.8 Continued

Protein affected	*Susceptibility to atherosclerosis*	*Lipoprotein profile*	*Other pathologies*
SR-BI Tg × apoB transgenics[361]	Reduced atherosclerosis at high levels of SRBI expression, not at low levels	HDL↓	
LCAT deficiency in humans[12]	Susceptible	Total C↓ TG↑ HDL↓ Discoidal HDL Small, spherical HDL	Corneal opacity Renal failure
Fish eye disease[12]	Susceptible	Total C↓ TG↑ HDL↓ Increased HDL catabolism	Corneal opacity
LCAT-/- mice[408,409]	None	HDL apoA-I↓ Total C↓ TG↑ Discoidal HDL	Renal lesions
LCAT Tg rabbits[410–412]	Reduced diet-induced atherosclerosis	HDL and apoA-I ↑ Decreased HDL catabolism LDL and apoB↓ Increased LDL catabolism	Decrease in HDL catabolism
LCAT Tg mice or LCAT Tg × apoA-I Tg or LCAT Tg × apoA-II Tg[371,413]	Increased atherosclerosis	HDL apoA-I↑ LDL normal HDL dysfunctional	
LCAT Tg × CETP Tg mice[372]	Atherogenic profile of LCAT Transgenic reversed	HDL normal function	
LCAT Tg × HL-/- mice[414]	Atherogenic profile of LCAT Transgenic reversed	No change in HDL cholesterol and apoA-I levels	
LCAT × CETP Tg mice[372]	Reduced atherosclerosis compared to LCAT Tg	Increase in plasma cholesterol	
Gene transfer of human LCAT to non-human primates[415]	–	HDL and apoA-I↑ LDL and apoB↓	
HL Tg mice[384]	Same as WT mice	HDL apoA-I↓	
HL Tg rabbits[385,386]		HDL apoA-I↓	
HL-/- × apoE-/- mice[383]	Reduced atherosclerosis relative to apoE-/-		
HL-/- mice[382]	Reduced atherosclerosis in arteries	Large HDL enriched in phospholipids↑	
Endothelial lipase (EL) transgenic mice[29,30]	–	HDL↓	
EL knockout mice[30]	–	HDL↑	
Mouse CETP Tg[416]	Severe atherosclerosis	VLDL↑ LDL↑	
CETP × apoE-/- or CETP × LDLr-/- [369]	Increased atherosclerosis compared to apoE-/- or LDLr-/- mice	Same profile as apoE-/- and LDLr-/- mice HDL↓	

Continued...

Table 8.8 Continued

Protein affected	*Susceptibility to atherosclerosis*	*Lipoprotein profile*	*Other pathologies*
CETP deficiency in humans[4]	Variable susceptibility Some mutations, increased CHD, others do not	HDL↑ Abnormal composition	
CETP x apoA-I Tg mice[368]	–	HDL↑ apoA-I↑ Compared to CETP transgenics	
CETP Tg mice[367]	–	HDL and apoA-I↓ Increased HDL clearance	
CETP × apoCIII Tg mice[399]	–	HDL↓ TG↑	
PLTP Tg[374]	–	Small or no changes in HDL	
PLTP gene transfer[417]	–	HDL↓ Formation of preβ HDL	
PLTP-/-[375,376]	–	Decreased HDL and apoA-I due to hypercatabolism of HDL increase Increased VLDL and LDL on high-fat diet Increase in apoA-IV	
PLTP-/- × apoB Tg or PLTP-/- × apoE-/-[377]	Reduced atherosclerosis compared to apoB transgenic or apoE-/- mice	VLDL↓ LDL↓	
Paraoxanase-/- x apoE-/-[337]	Reduced atherosclerosis	No changes in lipid and lipoproteins Increased lipoprotein oxidation	

Apo, apolipoprotein; WT, wild-type; LDLr, low-density lipoprotein receptor; CHD, coronary heart disease; FFA, free fatty acid; VLDL, very low-density lipoprotein; SRBI, scavenger receptor type BI; LCAT, lecithin : cholesterol acyl transferase; CETP, cholesteryl ester transfer protein

tion of cholesteryl esters in these cells.[40,60] The atheroprotective function of HDL may involve inhibition of oxidation of LDL by HDL,[61] SRBI- and ABCA1-mediated efflux of cholesterol to HDL and apoA-I, respectively,[62] HDL-mediated increase in the activity of endothelial nitric oxide synthase (eNOS),[63,64] and possibly through other mechanisms. Mice are generally resistant to the development of atherosclerosis, and the genetic background of the mouse strain influences their susceptibility.[65–68] Mice can, however, become susceptible to atherosclerosis on normal diets or atherogenic western-type diets by mutations in the apoE or the LDL receptor genes, by overexpression of apoB-100, or by crosses with atherogenic mouse lines[69–72]. In addition, atherosclerosis-susceptible strains can become resistant by transfer into susceptible strains of an atherosclerosis resistance gene locus derived from a resistant strain.[68] Analysis of atherosclerotic lesions of normocholesterolemic children aged 1–13 who died of trauma or other causes indicated that maternal

Table 8.9 Other genes affecting atherogenesis

Protein affected	*Susceptibility to atherosclerosis*	*Lipoprotein profile*
P-selectin-/- x apoE-/- mice[92]	Less susceptible to atherosclerosis than apoE-/-	No significant change in plasma lipids and lipoproteins
P-selectin-/- x apoE selectin-/- x LDLr-/- mice[93]	Less atherosclerosis than the LDLr-/- mice	No significant change in plasma lipids and lipoproteins
Inducible NO synthase-/- x apoE-/-[92]	Modest reduction in atherosclerosis but little change in lesion composition	No significant change in plasma lipids and lipoproteins
12/15 lipoxygenase-/- x apoE-/-[105]	Reduced atherosclerosis Reduced levels of autoantibodies to oxLDL	No significant change in plasma lipids and lipoproteins
12/15 lipoxygenase Tg x LDLr-/-[106]	Increased atherosclerosis	No significant change in plasma lipids and lipoproteins
LPL-/- fetal liver cells or bone marrow transplantation in C57BL/6 mice[294,381]	Reduced susceptibility to atherosclerosis	No significant change in plasma lipids and lipoproteins
Transplantation of ACAT-/- bone marrow in apoE-/- or LDLr-/- mice[418]	Similar susceptibility to atherosclerosis as apoE-/- or LDLr-/- Reduced levels of macrophages and lipids in advanced lesions	Significant reduction in VLDL levels
Myeloperoxidase-/- x LDLr-/-[108,419]	Increased atherosclerosis	No change in plasma lipid levels
MCP-/- x apoE-/- MCP-1-/- x apoB transgenic[98–100]	Reduced atherosclerosis	No significant change in plasma lipids and lipoproteins
Transplantation of LDLr-/- mice with CXCR2-deficient bone marrow[101]	Reduced atherosclerosis	No significant change in plasma lipids and lipoproteins
M-CSF-/-apoE-/- mice[102,103]	Resistant to atherosclerosis	No significant change in plasma lipids and lipoproteins
ICAM-/- x apoE-/- mice[94]	Reduced atherosclerosis Reduced monocyte recruitment to lesions	No significant change in plasma lipids and lipoproteins
SRAI-/-xLDLr-/- or SRAI-/- x apoE-/-[199,200]	Reduced lesion size	
Transplantation of apoE-/- or LDLr-/- mice with SRAI overexpressing bone marrow[201,202]	No effect on atherosclerosis	Reduced VLDL remnants compared to apoE-/- mice
CD36-/-xapoE-/-[209]	80% reduction in aortic lesion than apoE-/- on western-type diet and reduced atherosclerosis with low-fat diet as compared with apoE-/- mice	Modest decreases in HDL and VLDL
CD36-/-[217]	–	Increased HDL cholesterol and VLDL triglycerides
CD36 muscle-specific Tg[210]	–	Decreased VLDL triglycerides, increased fatty acid oxidation in the muscle

Apo, apolipoprotein; LDL, low-density lipoprotein; LDLr, LDL receptor; ox, oxidized; VLDL, very low-density lipoprotein; LPL, lipoprotein lipase; MCP, monocyte chemoattractant protein; SR, scavenger receptor; ICAM, intercellular adhesive molecule

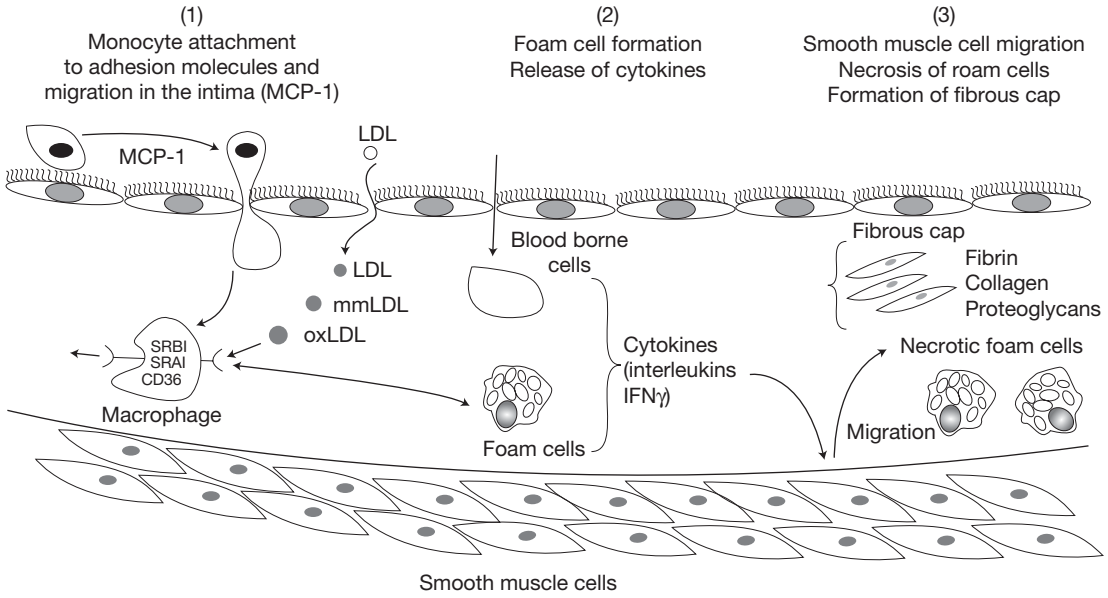


Figure 8.2 Schematic representation of the cascade of events which promote the initiation of atherosclerotic lesions and the formation of fatty streak and complex lesions. MCP, monocyte chemoattractant protein; LDL, low-density lipoprotein; mm, minimally modified; ox, oxidized; SR, scavenger receptor; IFN, interferon

hypercholesterolemia during pregnancy may influence the susceptibility to atherosclerosis of the offspring.[73] Similar observations were made in normocholesterolemic offspring of New Zealand White rabbits[74] and LDLr–/– mice.[75] Offspring of LDLr–/– mice showed that maternal hypercholesterolemia altered patterns of gene expression in the non-atherosclerotic descending aorta of the offspring. Although not rigorously proven, it is possible that hypercholesterolemia reprograms the expression of proatherogenic genes in the offspring, and that these changes may persist after birth and predispose to atherogenesis. In various mouse models the extent of atherosclerosis is assessed by measuring the area of aortic root lesions at the fatty streak stage in advanced lesions.[43,76]

The role of apolipoproteins in lipid homeostasis and in atherogenesis

Existing biochemical and genetic data suggest that increased plasma apoA-I and decreased plasma apoB levels can decrease the LDL/HDL ratio and thus protect humans against atherosclerosis.[10] Consistent with the human data, studies of transgenic mice showed that the plasma levels of apoB and apoA-I are correlated directly with plasma LDL and HDL levels[77,78] and, as predicted, apoA-I transgenics are protected from atherosclerosis, whereas the apoB transgenic mice develop atherosclerosis.[79,80] Overexpression of apoA-II leads to high triglyceride levels and abnormal composition of HDL and predisposes to atherosclerosis, particularly when plasma triglycerides are elevated.[81–83] ApoE expression may positively or negatively affect the catabolism of chylomicrons, depending on the plasma apoE levels,[84–86] whereas, as will be discussed in detail later, lack of apoE predisposes to atherosclerosis.[87] Overexpression of apoCIII, as well as apoCI, apoE, apoCII, and apoA-II, or diminished expression of apoCII, is associated with hypertriglyceridemia owing to inhibition of the hydrolysis of triglycerides of chylomicrons and VLDL.[81,88] Finally, overexpression of apoA-IV in mice increases HDL levels and protects from atherosclerosis, most probably by assuming some of the beneficial functions of HDL.[89,90] LDL levels may be increased by mutations in the LDL receptor, and these changes are associated with increased

atherosclerosis.[71] HDL levels may be affected by a variation in all the genes involved in the HDL pathway.[86] The involvement of apoE, the LDL receptor, proteins of the HDL pathway, some inflammatory mediators, scavenger receptors, and enzymes bound to HDL in atherogenesis are discussed in subsequent sections.

The role of oxidized lipoproteins, adhesion and immunoregulatory proteins, cytokines, chemokines and NO in atherogenesis

As discussed above, adhesion molecules, chemokines and their receptors, and inflammatory mediators have been implicated in the pathogenesis of atherosclerosis (Figure 8.2).[91] The contribution of these molecules to atherogenesis has been studied in atherosclerosis-prone apoE–/– or LDLr–/– mice (Table 8.9). Studies in P-selectin–/– × apoE–/– mice showed that at 4 months of age aortic root lesions were small, consisting primarily of fatty streaks, and had fewer smooth muscle cells than in apoE–/– mice. By 15 months of age the animals had developed fibrous plaques throughout the aorta, although the progression in lesion development had some delay compared to apoE–/– mice.[92] The P-selectin–/– and E-selectin–/– × LDLr–/– mice[93] reduced monocyte adhesion to endothelial cells, and had reduced atherosclerosis as compared to LDLr–/– mice. Similarly, ICAM–/– × apoE–/– mice had small but significant reductions in monocyte recruitment to atherosclerotic lesions compared to apoE–/– mice.[94] Minimally oxidized LDL in cell culture studies promoted the expression of MCP-1[95] and macrophage colony-stimulating factor (M-CSF),[96] and increased the adhesion of monocytes to endothelial cells.[97] Disruption of the mcp-1 or ccr2 genes markedly reduced the development of atherosclerosis in apoE–/– or apoB-overexpressing mice, respectively, indicating the importance of chemotaxis in atherogenesis.[98–100] Transplantation of LDLr–/– mice with bone marrow cells lacking CXCR2 (high-affinity receptors for IL-8) resulted in significantly less atherosclerosis than in mice reconstituted with wild-type bone marrow cells.[101] Furthermore, deficiency of the gene encoding M-CSF in an apoE–/– background promotes extreme resistance to the development of atherosclerosis, thereby indicating the importance of macrophage differentiation in atherogenesis.[102,103] Although atherosclerosis occurs in LDLr–/– mice, expression of the LDL receptor in macrophages promoted the formation of foam cell-rich lesions under conditions of moderate hypercholesterolemia.[104]

Lipoprotein oxidation is considered a key event in atherogenesis.[53] Two enzymes that contribute to lipoprotein oxidation are 12/15- and 15-lipoxygenases. Mice that were 12/15-lipoxygenase–/– × apoE–/– mice had decreased aortic atherosclerosis, and reduced levels of autoantibodies to oxidized LDL epitopes compared to apoE–/– mice.[105] In contrast, overexpression of 15-lipoxygenase in LDLr–/– mice resulted in larger aortic lesions.[106] In human patients, increased plasma myeloperoxidase level was a risk factor for myocardial infarction and other major cardiac events.[107] In a study of 470 healthy volunteers of 5-lipoxygenase (5-LO) promoter genotypes, a variant was statistically associated with increased intima-media thickness. Consistent with these findings, myeloperoxidase$^{+/-}$ × LDLr–/– mice have a dramatic decrease in atherosclerotic lesions (4% compared to the LDLr mice).[108] Other studies point to the importance of the 5-LO gene in atherogenesis. Mehrabian et al.[109] identified a major locus for atherosclerosis on mouse chromosome 6 in an atherosclerosis-resistant strain (CAST) which contained the 5-LO gene. A congenital strain containing the atherosclerosis-resistant locus had 20% of the normal 5-LO mRNA and protein levels compared to the control strain.[109] Dietary arachidonic acid, enhanced and *n*-3 fatty acid blunted the atherogenic effect of the variant genotype.[110] The involvement of macrophage-derived NO in atherogenesis was studied in double-deficient (inducible (i)) NOS–/– × apoE–/–) mice. In the C57BL/6 background, the double-deficient mice did not differ in early lesion formation as compared to apoE–/– mice,[111] however, iNOS–/– × apoE–/– mice of a mixed genetic background fed a western-type diet had a reduction in lesion area compared with apoE–/– mice, with little change in lesion composition.[111] Inhibition of iNOS also inhibited the progression of coronary atherosclerosis in cholesterol-fed hypercholesterolemic rabbits.[112]

The induction of neonatal tolerance to oxidized lipoproteins reduced atherosclerosis in apoE–/– mice, indicating that attenuation of the immune response has a beneficial effect on the progression of disease.[113] The involvement of immunoregulatory CD40 signaling was examined by injecting LDLr–/– mice fed an atherogenic diet with antibody to CD40 ligand. This

treatment limited the progression of aortic atherosclerosis, increased collagen and smooth muscle, decreased the macrophage cell content, and reduced the number of lesions.[114]

The biogenesis, functions, and catabolism of apoB-containing lipoproteins

Intracellular biogenesis of apoB-containing lipoproteins

Structure and biosynthesis of apoB-100 and apoB-48 forms

Apolipoprotein B is the main protein component of LDL and comprises 23.8% of the weight of the LDL particle.[1,5] The primary sequence of human apoB-100 was originally deduced by four independent research groups from the corresponding sequence of overlapping cDNA clones.[115] The mature apoB-100 protein contains 4536 amino acids.[115] Based on a <Mr> of 513 kDa, it can be calculated that there is one apoB-100 molecule per LDL particle.[115]

ApoB is synthesized by the liver and the intestine and has two protein forms designated apoB-100 and apoB-48. These forms are generated by a post-transcriptional modification that converts the CAA triplet of the apoB mRNA, which encodes for residue 2153, to a chain termination codon UAA.[116] The editing enzyme is a site-specific deaminase, apobec-1, which converts the C residue of the 2153 codon to U.[116]

The receptor and lipid-binding functions of apoB

ApoB-100 and apoB-48 are required for the assembly and secretion of VLDL and chylomicrons, respectively, and this process is prevented in abetalipoproteinemia and some forms of homozygous hypobetalipoproteinemia.[5] ApoB is the ligand that mediates the recognition of LDL by the LDL receptor. As discussed later, the LDL receptor–apoB interaction mediates the clearance of LDL from plasma and regulates cellular cholesterol biosynthesis.[117] Several independent studies showed that the carboxy-terminal domain of apoB-100 between residues 3000 and 3700 is probably involved in receptor binding. The sequence between residues 3359 and 3367 of apoB has 63% homology to the apoE sequence between residues 142 and 150, which has been implicated in receptor binding.[1,115] A naturally occurring R3500Q apoB point mutation is responsible for decreased receptor binding of the variant LDL. This condition was named familiar defective apoB-100.[118] Reduced receptor binding is also caused by an R3531C point mutation.[5,119] Finally, truncated apoB forms extending to apoB-75 bind LDL efficiently, whereas shorter apoB forms do not, and are associated with hypobetalipoproteinemia syndromes.[1,5]

Analysis of the lipid-binding properties of apoB peptides showed that the carboxy-terminal end (residues 4101–4536) and a domain in the middle of the molecule (residues 1701–3070) and some regions between residues 1001 and 1700, may represent the major lipid-binding domains of apoB.[1]

Based on the analysis of hydrophobicity and hydrophobic moment of the apoB sequence, it has been calculated that apoB consists of three regions enriched in amphipathic α-helices, separated by two regions of enriched amphipathic β-sheets. These domains were designated α, β, α2, β2, α3, and it has been proposed that they contribute to lipid binding. The approximate boundaries of these domains are α1 (aa 58–795), β1 (aa 827–2001), α2 (aa 2045–2587), β2 (aa 2571–4032), α3 (4017–4515).[16]

Intracellular assembly of VLDL and chylomicrons

VLDL assembly in the hepatocytes and chylomicron assembly in enterocytes involve a two-step process.[5,120] In the first step(s) of VLDL (chylomicron) assembly, apoB acquires small amounts of phospholipids cotranslationally in the rough endoplasmic reticulum (ER), and triglycerides are added. These lipids are interpreted in the lipovitellin-like lipid-binding domain that is found in the amino-terminal region of apoB by the action of MTP.[120] As apoB translation proceeds, more core lipids are added by the action of MTP and, eventually, spherical particles are formed and released from the ribosomes. In a parallel process that is catalyzed by MTP, a VLDL-sized particle free of apoB is formed in the smooth ER and migrates in the junctions between smooth and rough ER. In a second step of VLDL (chylomicron) assembly, the apoB-free/triglyceride-rich

particles fuse with the apoB-containing lipoprotein particles. VLDL (chylomicron) particles formed are transferred to the Golgi, where apoB is *N*-glycosylated and some additional phospholipids are added. Secretory vesicles containing VLDL (chylomicrons) migrate and fuse to the basolateral membranes of hepatocytes (enterocytes), and the lipoproteins are exocytosed.

MTP is a dimer consisting of a 55-kDa disulfide isomerase subunit, which is a ubiquitous protein, and a 97-kDa subunit found mainly in the liver and intestine.[5,121] MTP, which acts in the first step of VLDL chylomicron assembly, can transfer both triglycerides, cholesteryl esters, and, to some extent, phospholipids between lipid vesicles. Mutations in the 97-kDa subunit of MTP are associated with abetalipoproteinemia.[5,121] If apoB does not assemble with lipids, it is degraded.[1]

Small, dense LDL

Small, dense LDL represents a subpopulation of LDL particles that can be separated by ultracentrifugation in the range of 1.025–1.034 g/ml.[24] The presence of small, dense LDL in plasma can also be assayed by non-denaturing polyacrylamide gradient gel electrophoresis (GGE) or nuclear magnetic resonance (NMR).[24] The levels of small, dense LDL in plasma are affected by age, sex, pregnancy, diseases (such as insulin resistance, obesity, diabetes mellitus, hypertriglyceridemia, AIDS),[24] and by certain drugs (such as β-adrenergic receptor blockers).[24,122] Small, dense LDL is one of the features of the metabolic syndrome. This condition is also associated with abdominal obesity, dyslipidemia (elevated triglyceride), low HDL cholesterol, increased blood pressure, insulin resistance (with or without glucose intolerance), and prothrombotic and proinflammatory states. In subjects with elevated LDL levels, small, dense LDL may contribute to the risk for atherosclerosis.[24,122] The Physician's Health Study, which included 14 916 men, indicated that small LDL particles, when adjusted for plasma triglyceride levels, were not a good risk indicator for myocardial infarction. In contrast, plasma triglyceride levels were associated with increased relative risk.[123] Genetic studies of small kindreds showed that monogenic factors contributed to the presence of small, dense LDL in between 9 and 21% of the subjects at a young age, whereas at later ages monogenic factors accounted for the presence of small, dense LDL in 47–95% of the subjects in different studies.[124–127]

Small, dense LDL has a lower affinity for the LDL receptors, remains longer in the circulation (and thus has greater susceptibility to oxidation), is better retained by proteoglycans in the subendothelium, and is more cytotoxic than larger LDL.[24] Small, dense LDL may be generated by hydrolysis of triglycerides of IDL/LDL by hepatic lipase.[25] Load score-based linkage analysis of 19 families that included 142 members did not find a linkage of small, dense LDL with Mn superoxide dismutase, apoAII, apoCII, apoCIII, LPL, HL, MTP, insulin receptor, or LDL receptor.[126] However, other studies indicated an increase in small, dense LDL in subjects with LPL gene mutations, the apoE4 phenotype, and a CETP promoter polymorphism, increased plasma, PA-1 and fibrinogen levels.[124,128–130] Linkage analysis showed association between a HL promoter polymorphism and increased small, dense LDL levels in families with familial combined hyperlipidemia. However, this association was not detected by a genome scan of a subset of these families.[130] It is clear from the numerous linkage studies that several genes contribute to the size and heterogeneity of LDL in humans. It appears that exercise, which increases LPL and reduces HL levels, as well as a reduction in body mass,[131] a low-fat diet,[132] a polyunsaturated fat diet,[133] and lipid-lowering drugs (statins, niacin, fibrates) contribute to the reduction of the small, dense LDL levels.

The LDL receptor pathway, familial hypercholesterolemia and atherogenesis

Our current understanding of the molecular events involved in the receptor-mediated catabolism of lipoproteins has been shaped mainly by the pioneering work of Goldstein, Brown and colleagues, who first demonstrated the presence of a specific receptor on cell surfaces that recognizes, binds, and internalizes LDL.[1,11,117,134] Since then, several other members of the LDL receptor family have been described.[1,11,134–141] Ligands for the LDL receptor are the LDL, which contains apoB, and the apoE-containing lipoproteins, such as IDL, βVLDL, and HDL with apoE,[11,142] as well as apoE-containing proteoliposomes.[143] The affinity of HDL with apoE for the LDL receptor was more than 20 times greater than that for LDL itself. The structures of the LDL receptor

family members are shown in Figure 8.3, and their properties are summarized in Table 8.4.

Functional definition of the LDL receptor: its intracellular itinerary and the feedback regulatory mechanisms

Early biochemical and genetic studies established that the cell surface of cultured human fibroblasts and other cell types contains high-affinity receptors for LDL. The presence of these receptors was initially assayed by binding of [^{125}I]-LDL to cell cultures (Figure 8.4a). Based on these assays, it was found that patients with familial hypercholesterolemia (FH) either lack or have defective receptors.[11,117,134]

Binding of LDL to the LDL receptor can be detected at 4°C, but the receptor is not internalized.[11,117,134] When cell cultures are incubated at 37°C, the coated pits containing the LDL receptor complex invaginate into the cell and pinch off to form endocytic vesicles called endosomes, which carry LDL to lysosomes (Figure 8.5a).[11,117,134,144] Following dissociation of the clathrin, several endocytic vesicles fuse to form endosomes.[144] The endosome develops an acidic pH by the action of an ATP-driven protein pump,[145] which mediates the dissociation of the lipoprotein receptor complex.[146] The free receptor in recycling vesicles returns to the cell surface prior to the fusion of endosomes with primary lysosomes.[146] Fusion of endosomes with primary lysosomes results in the hydrolytic degradation of apoB to amino acids, and hydrolysis of cholesteryl esters by the lysosomal enzyme acid lipase.[1,11,117,134] Liberated cholesterol is used by cells for membrane synthesis. The kinetics of receptor internalization hydrolysis of apoB and cholesterol esterification are saturable processes, as indicated in Figure 8.4b.

Regulatory mechanisms that follow the interaction of LDL with the LDL receptor

Hydrolysis of cholesteryl esters by acid lipase triggers three regulatory responses that contribute to cellular cholesterol homeostasis: inhibition of cholesterol synthesis by reduction of the activity of HMG-CoA reductase gene, the rate-limiting enzyme of cholesterol biosynthesis (Figure 8.4b),[147] a decrease in the number of surface LDL receptors, which prevents additional cholesterol influx into the cell (Figure 8.5a),[11,117,134] and activation of acyl-CoA:cholesterol acyltransferase (ACAT), which re-esterifies excess cholesterol preferentially with oleic acid, resulting in cytoplasmic storage of cholesteryl ester droplets (Figure 8.5a).[148] As will be discussed later, the decrease of the LDL receptor number is regulated at the level of transcription.[1,11] Furthermore, HMG-CoA reductase, located in the ER, contains a cholesterol-sensing domain, and the increase in the membrane cholesterol content triggers the rapid ubiquitin-dependent proteosomal degradation of the enzyme and, ultimately, inhibition of cholesterol biosynthesis.[11]

Purification of the LDL receptor cloning cDNA and the gene encoding for the LDL receptor

Following the initial cell culture-based assays for the LDL receptor activity and the cellular regulatory responses, an assay was developed for LDL receptor activity using cell membrane preparations.[149] This development allowed the purification of the LDL receptor from bovine adrenal cortex.[150] The mature receptor is an acetic glycoprotein of apparent molecular mass of 160 kDa and isoelectric point 4.3.[11,151] Sequencing of peptides obtained from the purified bovine LDL receptor allowed the synthesis of degenerated oligonucleotides, which were used as probes for cloning of cDNA encoding for the bovine LDL receptor,[152] and subsequently for the cDNA and the gene encoding the human LDL receptor.[153] This represents a basic approach that was also used for the isolation of cDNA and genes that encode several proteins of the lipoprotein system (Tables 8.2–8.5).

The LDL receptor mRNA is 5.3 kb long, including a 2.6 kb long 3′ untranslated region. The LDL receptor gene is 45 kb long and contains 17 exons and 18 introns;[153] it maps to the short arm of chromosome 19 (p13.1–p13.3).[11]

The human LDL receptor cDNA and gene encode a protein of 860 amino acids, including a 21-residue long signal peptide.[152,153] Computer analysis of the receptor sequence showing that it consists of five domains is illustrated in Figure 8.3a. It is important to note that the cytoplasmic domain contains a conserved NPVY sequence between residues 804 and 807, which is required for movement of the receptor to the coated pits. Computer analysis of the structure of the LDL receptor showed the presence of seven imperfect amino terminal repeats, designated 1–7 (Figure 8.3a). Two

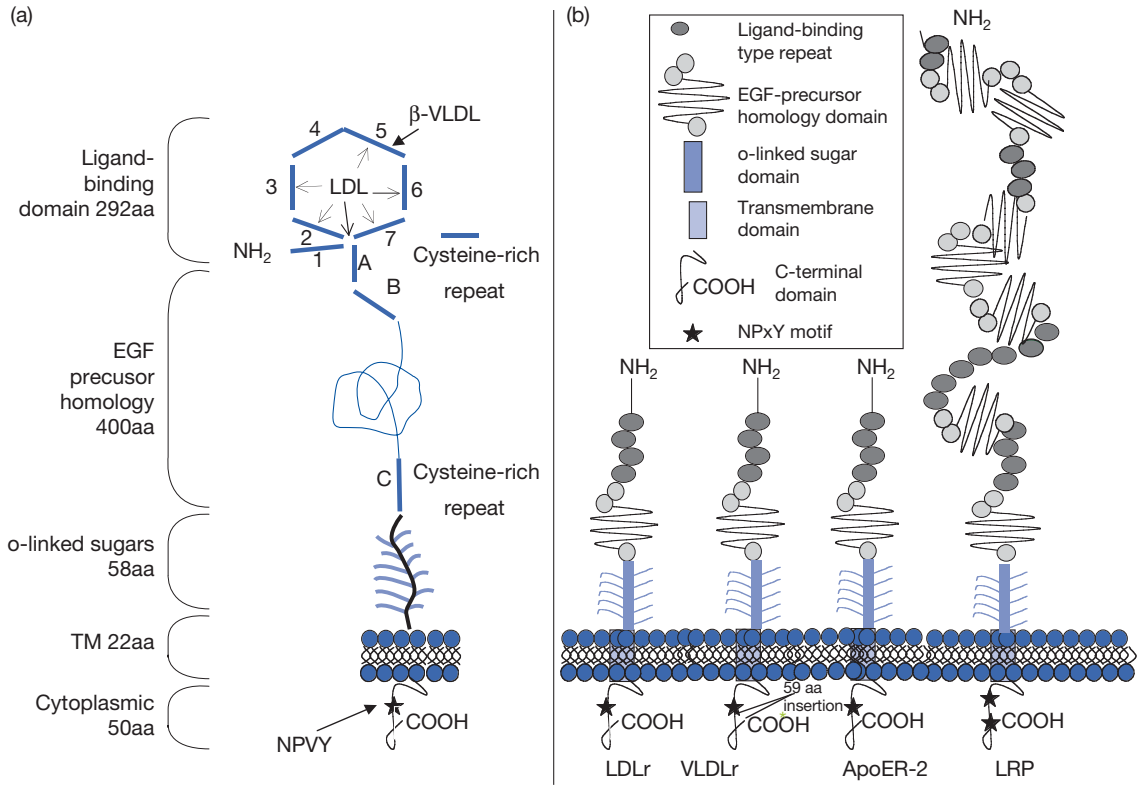


Figure 8.3 (a) Schematic representation depicting the low-density lipoprotein receptor (LDLr) and the five different structural domains of the receptor. (b) Schematic representation of the LDLr and some members of the LDLr family. The symbols used in the diagram are defined in the box. (Adapted from reference 136, with permission). EGF, epidermal growth factor; TM, transmembrane; LPR, LDLr-related protein; apo, apolipoprotein

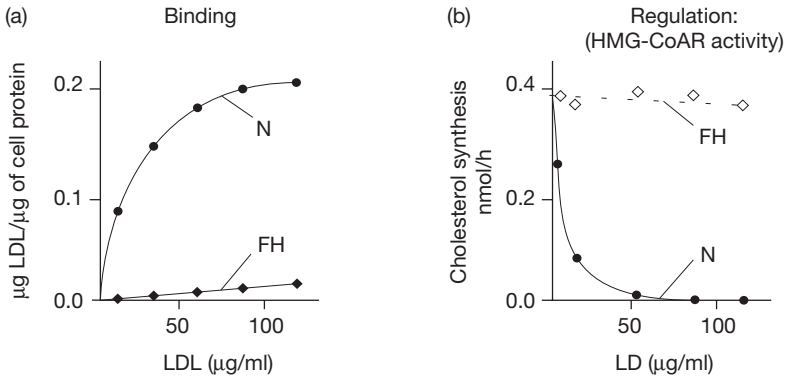
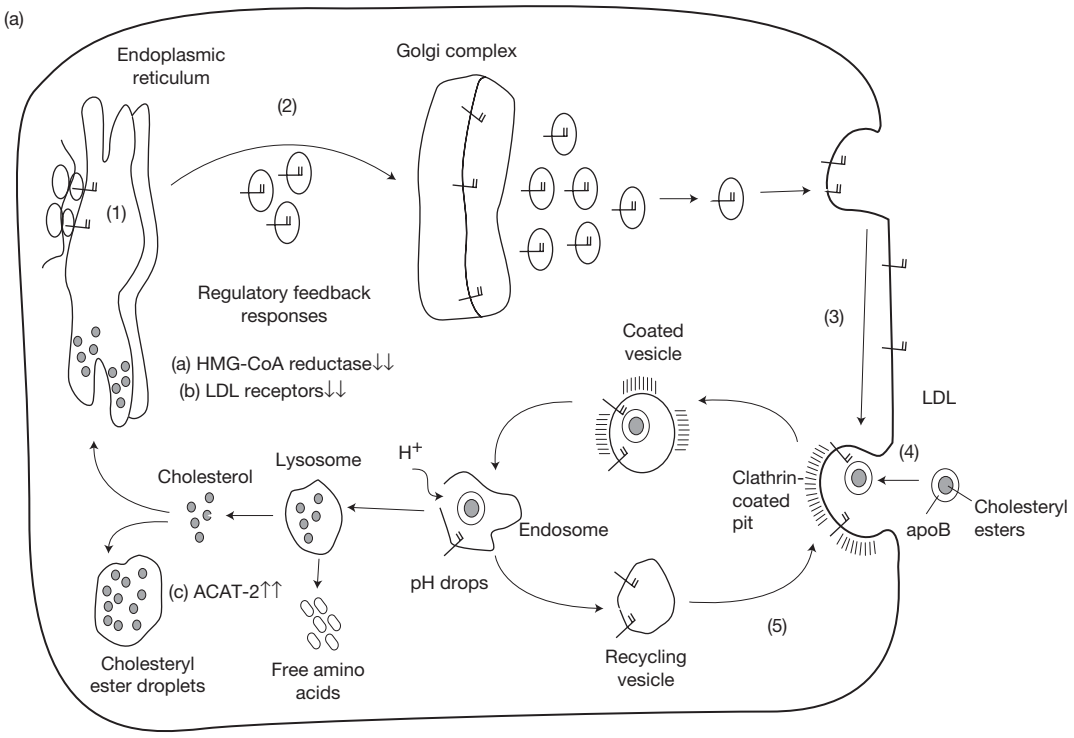


Figure 8.4 A representative experiment of binding of [^{125}I]-LDL (low-density lipoprotein) to cultured skin fibroblasts containing normal and deficient LDL receptors and the regulatory feedback mechanism that ensues following the entry of LDL into the cell. (a) Receptor binding. (b) A representative experiment of cholesterol synthesis (HMG-CoAR activity) following uptake of LDL by the cells. The kinetics of receptor internalization hydrolysis of apolipoprotein (apoB) and cholesterol esterification (reflecting the ACAT-activity) are similar. (Adapted from reference 11, with permission). N, normal; FH, familial hypercholesterolemia

(a)



(b)

Class of mutation	Lack of synthesis	Defective transport from ER to Golgi	Defective binding of LDL	Defective clustering in coated pits	Defective recycling	Response to statins
1	X					–
2	→	X				–
3	→	→	X			–
4	→	→	→	X		+
5	→	→	→	→	X	–
Heterozygosity for classes 1–5						+

(c)

Principles of cholesterol lowering

Statins: HMG-CoA reductase↓ Cellular C↓ LDLr↑↑ Plasma C↓↓
Bile acid resins: BA↓ Cellular C→BA LDLr↑ Plasma C↓
Combinations of statins and bile acid resins: HMG-CoA reductase↓, BA↓ } Cellular C↓↓ LDLr↑↑↑ Plasma C↓↓↓

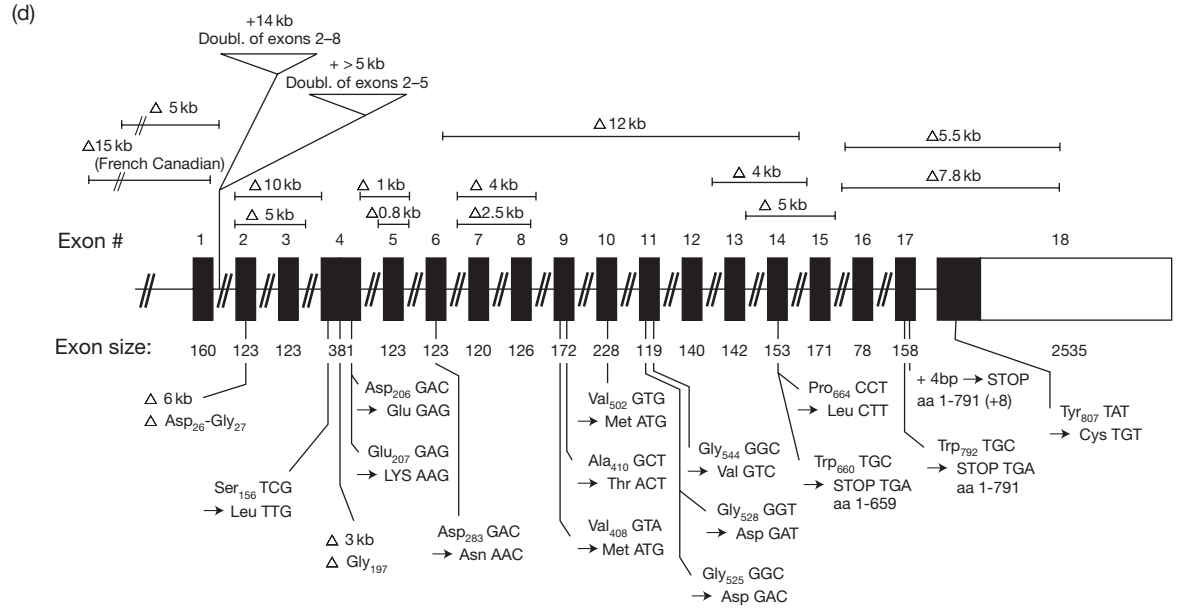


Figure 8.5 (a) Schematic representation of the biosynthesis and the itinerary of the low-density lipoprotein (LDL) receptor and the feedback regulatory mechanisms that follow the entry of cholesterol into the cell. Numbers 1–5 represent different steps of biosynthesis, modifications, positioning into the coated pits, intracellular transfer and recycling of the receptor. (b) Classes of the LDL receptor based on different steps of the receptor itinerary, and pharmacogenetic predictions on how patients with mutations will respond to HMG-CoA reductase inhibitors (statins). (c) Principles of cholesterol lowering using statins and bile acid (BA)-binding resins. The response to BA-binding resins is smaller, owing to feedback cellular response to increase cholesterol biosynthesis. (d) Schematic representation of the human LDL receptor gene and the various mutations associated with familial hypercholesterolemia. The mutations are described in the text.[1,135] ER, endoplasmic reticulum; C, cholesterol

adjacent repeats, designated A and B, are located in the epidermal growth factor precursor homology region.[153] Deletion or oligonucleotide-directed mutagenesis within each of the repeated sequences, and functional analysis of the mutant receptors following expression in COS cells, confirmed the importance of these regions for ligand binding. This analysis also showed that repeats 2, 3, 6, 7, and A are required for maximum binding of LDL (via apoB) but not βVLDL (via apoE), whereas repeat 5 is required for maximum binding of both LDL and bVLDL (Figure 8.3a).[154,155]

Biosynthesis and post-translational modifications and recycling of the LDL receptor

Following synthesis, the LDL receptor protein is modified post-translationally in the ER and Golgi by *N*- and *O*-linked glycosylation.[151,156] The modified receptors reach the cell surface, are initially incorporated in various areas of the plasma membrane, and subsequently cluster in the clathrin-coated pits (Figure 8.5a).

Mutations in the LDL receptor gene are associated with familial hypercholesterolemia

Familial hypercholesterolemia (FH) is an autosomal dominant disease characterized clinically by increased LDL cholesterol, xanthomas in tendons and skin, and premature coronary atherosclerosis. Homozygotes are more severely affected than heterozygotes. The frequency of heterozygotes is approximately 1 in 500 persons, and these individuals have plasma cholesterol 350–550 mg/dl from birth. Tendon xanthomas and coronary atherosclerosis appear after ages 20 and 30, respectively. Homozygotes have a frequency of 1 in 1 000 000 and cholesterol levels 650–1000 mg/dl; they develop cutaneous xanthomas by the fourth year of

life, and without treatment die from myocardial infarction by the age of 20.[11]

The functional, biochemical and genetic analysis of the LDL receptor facilitated enormously the delineation of the molecular defects that underlie familial hypercholesterolemia. The LDL receptor deficiency prevents the uptake of IDL and LDL, and increases the conversion of IDL to LDL.[157] Based on the new molecular information, a variety of defects in the LDL receptor identified previously with binding studies could be assigned to one of five classes (Figure 8.5b). The class 1 mutants are characterized by the absence of receptor protein, owing to either gross alterations (insertions, deletions) in the receptor gene or nonsense mutations leading to premature chain termination.[1,11,135] The class 2 mutants are characterized by defective modification of the precursor *N*- and *O*-linked carbohydrates.[158,159] These mutations consist mostly of amino acid deletions or substitutions, and have been localized in exons 2, 4, 6, 11, and 14,[1,11,135] and cause the entrapment of a precursor form of <Mr>=120 kDa in the ER.[159] The Watanabe heritable hyperlipidemic (WHHL) rabbit mutation belongs in this category. The class 3 mutants are characterized by receptors of either normal or aberrant apparent <Mr> that are modified, normally reach the cell surface, but have reduced affinity for LDL. Such mutants result from deletions/insertions or amino acid substitutions in exons 2–8[1,11,135] in the cysteine-rich domain of the receptor. The class 4 mutants are characterized by normal synthesis, modification, and transport to the cell surface, but inability to cluster into the coated pits. As a result, these mutant receptors bind LDL normally but do not internalize the complex.[1,11,135] All these mutants have alterations within the first 21 residues of the cytoplasmic tail. Functional analysis of either naturally occurring mutants or mutants generated by in vitro mutagenesis showed that substitutions of Tyr to Cys or other non-aromatic amino acids were sufficient to create an internalization defect.[160] The class 5 mutations are characterized by receptors that are synthesized, secreted, and internalized normally, but cannot be released from the endosomes, and hence cannot recycle to the cell surface. These mutations are found in the epidermal growth factor (EGF) precursor homology domain and may involve residues located on or in the vicinity of repeats A, B, and C (Figure 8.3a).[11,154,161] The EGF precursor homology domain may mediate the dissociation of the receptor–ligand complex in the ER that is the result of the lowering of the pH in this organelle.[11,162] Figure 8.5b shows how patients with different types of mutation may respond to cholesterol-lowering therapies. Figure 8.5c shows the principle of cholesterol lowering using 3-hydroxyl-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors (statins) and bile acid-binding resins. The mutations described for the LDL receptor have been reviewed extensively;[135] several known mutations are shown in Figure 8.5d.

A rare form of familiar hypercholesterolemia with an autosomal recessive mode of inheritance has been described.[163,164] Cultured lymphocytes of the patients have normal or increased receptor binding but defective internalization. The disease is caused by mutations in the phosphotyrosine-binding domain of a protein designated autosomal recessive hypercholesterolemia (ARH).[165] ARH binds to the NPVY domain of the LDL receptor (Figure 8.3a), and may function to chaperone the LDL receptor to the coated pits and anchor it to the clathrin and AP-2.[164] Alternatively, ARH may act as an adaptor molecule by binding to the LDL receptor after it reaches the coated pits and anchoring it to the clathrin and AP-2.[164]

HMG-CoA reductase (statins) increases the LDL receptor numbers on the cell surface and hence the clearance of plasma cholesterol. As shown in Figure 8.5b, statins can only be used for the treatment of LDL heterozygotes and homozygotes of the fourth class of mutations, which may bind LDL with reduced affinity. Combination of statins with bile acid-binding resins that bind and remove bile acids from the intestine, or new drugs that affect intestinal sterol absorption, have an even greater cholesterol-lowering effect.[166,167]

How a cell senses fluctuations in cholesterol levels, and the effect of receptor mutations

As shown in Figure 8.5a, when the cell senses excess intracellular cholesterol it inhibits the endogenous cholesterol biosynthesis and decreases the LDL receptor number, decreases HMG-CoA reductase activity by proteosome-mediated degradation of this enzyme, and increases the esterification of excess cholesterol in order to store limited amounts of cholesteryl esters in the form of lipid droplets. In the opposite situation, when

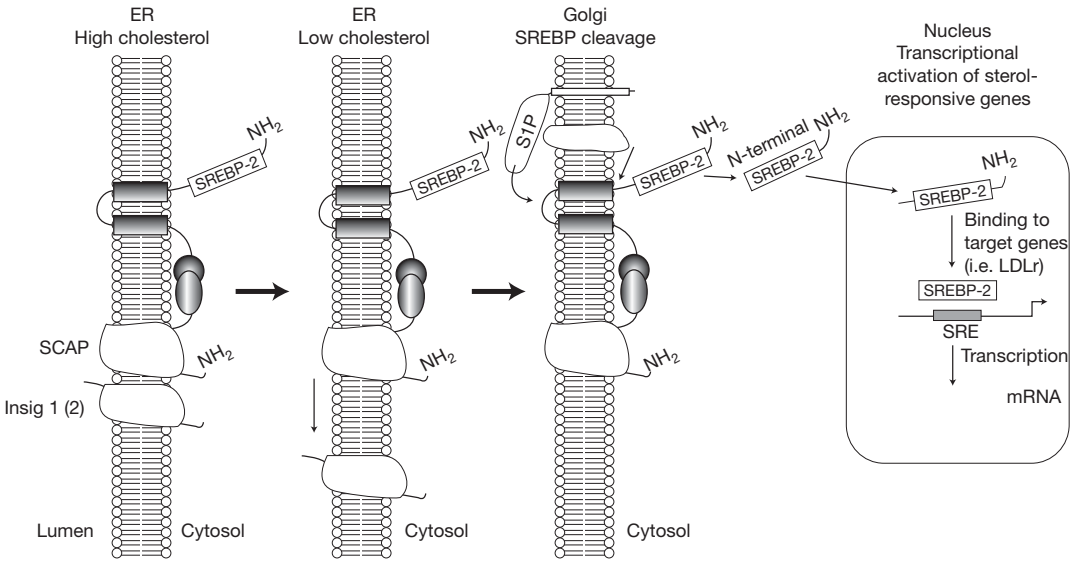


Figure 8.6 Schematic representation of proteolytic activation of sterol regulatory element-binding protein (SREBP) through a cholesterol sensing mechanism which leads to the release of the N-terminal segment of SREBP, which translocates to the nucleus and activates target genes. (Adapted from reference 168, with permission). ER, endoplasmic reticulum; LDLr, low-density lipoprotein receptor; SCAP, SREBP cleavage activating protein

the cell is deprived of cholesterol it increases the LDL receptor numbers to import more cholesterol.

The mechanism through which a cell senses cholesterol levels has recently been clarified and involves a membrane-bound transcription factor named sterol regulatory element-binding protein-2 (SREBP-2) (Figure 8.6).[11,168] This membrane-bound basic helix–loop–helix transcription factor controls genes involved in cellular cholesterol homeostasis.[169] Another major member of the family, SREBP-1, controls genes involved in fatty acid biosynthesis.[170] All members of the family have a highly acidic amino-terminal activation domain that is recognized by the transcriptional coactivator CREB-binding protein (CBP).[171] When cholesterol is in excess, membrane protein SREBP cleavage activating protein (SCAP), present in the ER, interacts with SREBP as well as another cholesterol-sensing protein, insig1(2), and all three proteins remain anchored in the ER membrane. Under conditions of cholesterol depletion, insig1(2) dissociates from the complex and allows SREBP and SCAP to move via vesicular transport to the ER, where SREBP are cleaved by two proteases designated site 1 (S1P) and site 2 (S2P); SP1 cleaves the loop connecting the two transmembrane regions in the Golgi lumen, and SP2 cleaves within the first transmembrane domain.[168,172–174] The cleaved aminoterminal fragment containing ~480 amino acids of SREBP containing the bHLHZip motif, and the activation domain, translocates to the nucleus and induces transcription of several genes involved in cholesterol or fatty acid biosynthesis and transport.[11]

Animal models with LDL receptor deficiency or abundance

Transgenic mice overexpressing the human LDL receptor catabolize LDL effectively, leading to very low levels of plasma LDL,[175] and are protected from atherosclerosis in response to atherogenic diets.[176] In contrast, LDLr–/– mice, which have mild hypercholesterolemia but eightfold elevated IDL and LDL cholesterol, develop atherosclerosis in response to atherogenic diets.[177] Atherosclerosis is exacerbated in LDLr–/– × apoE–/– mice[178] and in LDLr–/– × apoB transgenic mice.[72] The use of LDL-deficient mouse models to test the atherogenicity of the other genes is discussed in later sections.

Other members of the LDL receptor family that recognize apoE-containing lipoproteins

In mammalian species, the LDL receptor gene family contains four additional structurally and evolutionarily-related members:[11,136–141] the LDL receptor-related protein (LRP), the megalin/gp330, the VLDL receptor (VLDLr), and the apoE receptor-2 (apoER2) (Figure 8.3).

LRP In 1988, a protein of 4525 amino acids was cloned from a human lymphocyte cDNA library with homology to the LDL receptor and was named LRR.[137] LRP, which is identical to protease-activated α_2-macroglobulin,[179] is synthesized as a precursor of Mr 600 kDa and is cleaved in the *trans* Golgi to two subunits of Mr 515 and 85 kDa, respectively, which associate non-covalently on the cell surface.[180] LRP has regions which are homologous to the cysteine-rich domain, the GGF precursor domain, and the cytoplasmic tail of the LDL receptor (Figure 8.3b). LRP mRNA and protein are present in various tissues, including liver, brain, and lung.[137] Studies with LDL receptor-negative skin fibroblasts showed that LRP-mediated binding and the uptake of βVLDL by the cells are stimulated by apoE and inhibited by apoCI and, to a lesser extent, by apoCII.[23,181] LRP also binds with high affinity and mediates the uptake of activated α_2-macroglobulin–protease complexes,[179] plasminogen activator–inhibitor complexes,[182,183] lactopherin,[182] and lipoprotein lipase, hormones and carrier proteins for vitamins.[136,138] Inactivation of the LRP gene in mice leads to embryonic lethality at around the implantation stage.[183]

Megalin Megalin, also known as gp330, is a large protein similar in size and domain structure to LRP.[184] It resides in coated pits on the apical surface of epithelial cells in the renal glomerulus and proximal tubule. Megalin is associated with Heymann-type autoimmune nephritis in rats.[185] The function of megalin/gp330 is unknown. It binds apoE-containing lipoproteins and most of the ligands that are recognized by LRP.[136,182]

VLDLr VLDLr was initially isolated from a rabbit and human cDNA libraries.[139,140] VLDLr is an evolutionarily conserved protein that has a striking homology to the LDL receptor. The domain structures of cysteine repeats, the EGF homology, the serine–threonine-rich, the transmembrane spanning, and the cytoplasmic regions are highly preserved between the two proteins. The intron–exon organization of the human VLDL and LDLr genes is the same, except for the presence of an additional repeat in the ligand-binding domain of VLDLr.[140] The VLDLr is expressed abundantly in heart, muscle, and adipose tissue, and is barely detectable in the liver.[139] The findings suggest that VLDLr may be involved in the catabolism of triglyceride-rich VLDL by muscle and the adipose tissue. Inactivation of the VLDLr gene in mice did not affect the lifespan or the plasma lipid and lipoprotein levels.[186]

ApoER2 ApoER2 is highly homologous and has a similar domain structure to LDLr and VLDLr, and has three different forms resulting from alternate splicing.[187] ApoER2 binds with high affinity apoE-rich β-migrating VLDL and apoE proteoliposomes, but does not bind to LDL.[141,188] The difference in ligand specificity has been attributed to structural differences between the two receptors in the linker sequence, which connects with cysteine-rich repeats 4 and 5.[154] ApoER2 is expressed abundantly in the brain, and to a lesser extent in the testes and ovaries.[141] The tissue distribution indicates that apoER2 may contribute to lipid homeostasis in the brain.[188] Studies in double-deficient VLDLr–/– × apoER2–/– mice indicated that these receptors may also play an important role in brain development and functions, mediated via its interaction with Reelin and activation of intracellular signaling pathways.[189]

The scavenger receptors SRAI and SRAII, and CD36

Functional definition of the scavenger receptor class A types I and II (SRAI and SRAII)

It has been shown that the protein and lipid moieties of LDL can be modified in vivo by malonyldialdehyde (MDA) and other short-chain aldehydes that are released by platelets or produced during lipid peroxidation or by oxidation of LDL in the intima.[190,191] These changes render the modified LDL substrate for a new class of receptors, called scavenger receptors. It has been estimated that in normal humans two-thirds of LDL is catabolized through the LDL receptor pathway and one-third by a receptor-independent pathway that may reside in scavenger cells.[117] It was initially

shown that mouse peritoneal macrophages and human monocyte-derived macrophages contain receptors that bind specifically and with high affinity to modified LDL.[192] The bound, modified LDL is internalized, the protein moiety degraded to amino acids, and the cholesteryl esters of the modified LDL hydrolyzed, presumably by a non-lysosomal cholesteryl ester hydrolase.[192,193] When the cells are grown in medium containing serum, half of the free cholesterol is secreted and the remainder is re-esterified by ACAT-1 and stored in the cytoplasm as cholesteryl ester droplets.[192] In the continuous presence of modified LDL in the culture medium the macrophages apparently fail to downregulate their receptor activity, and this results in a dramatic increase in cellular content and cholesteryl esters.[194] The accumulation of cholesteryl esters in the monocyte–macrophages may lead to the formation of foam cells characteristic of atherosclerotic lesions.[192]

Structure and functions of the scavenger receptors SRAI and SRAII

Krieger and colleagues purified the receptor for modified lipoprotein to near homogeneity from bovine lung,[195] and cloned the corresponding cDNA.[196,197] cDNA encoding the human receptor has also been obtained from a monocyte-derived cell line (THP1).[198] This analysis showed two forms of the human and the bovine receptor, type I (SRAI) (453 aa) and type II (SRAII) (349 aa).[195–197] Computer analysis of the predicted primary protein sequence showed that SRAI contains a 50-amino acid long amino-terminal cytoplasmic domain, a 26-amino acid long membrane-spanning domain, a 32-amino acid long membrane spacer region with two potential *N*-glycosylation sites, a rod-like structure which contains 163 residues that form an α-helical coiled coil and contains five potential *N*-glycosylation sites, 72 residues that form a collagen-like domain, and an 110 amino acid cysteine-rich carboxy-terminal domain. The SRAII is identical to the SRAI receptor, except that it contains only a six-residue carboxy-terminal domain. A schematic representation of the different domains of the SRAI and SRAII is shown in Figure 8.7. The rod-like structure is generated in the trimeric receptor by the merger of a triple-stranded left-handed superhelix formed by the α-helical coiled coil and a right-handed collagen-like triple helix.[196] The binding of modified LDL to both types of the receptor is similar,[196] suggesting that the carboxy-terminal cysteine-rich domain is not involved in receptor binding. Functional studies showed that the same receptor recognizes both acetyl LDL and oxidized LDL with different specificities, and that receptor binding is diminished by deletion of residues –320 to –342 of the collagen-like domain.[1]

The contribution of SRAI in atherogenesis has been studied by crossing SRAI-deficient mice with atherosclerosis-prone mice. SRAI–/–with different specificities LDL–/– or SRAI–/–with different specificities apoE–/– mice have smaller lesions.[199,200] Transplantation of bone marrow cells overexpressing SRAI in apoE–/– or LDLr–/– mice did not affect atherosclerosis, although it reduced VLDL remnants in the apoE–/– mice.[201,202] SRA–/–with different specificities $apoE_3$ Leiden-transgenic mice develop more complex lesions, with calcification, necrosis, cholesterol clefts, and fibrosis than do apoE3 Leiden-transgenic mice.[203]

CD36

CD36 is a membrane protein with two short cytoplasmic amino- and carboxy-terminal membrane domains and belongs to the scavenger receptor B family (Figure 8.7).[60,204,205] CD36 is expressed in a variety of cells and tissues, including monocyte-derived macrophages, microvascular endothelial cells, adipocytes, platelets, and striated muscle and heart (Table 8.5).[60,205] CD36 is a multiligand receptor that binds modified lipoprotein and apoptotic cells and may contribute to foam cell formation.[206,207] It also binds bacteria parasites and viruses.[207,208] The expression of CD36 in macrophages is induced by oxidized LDL, which is one of its ligands.[205] LDL modified by monocyte-reactive nitrogen species generated by myeloperoxidase is a CD36 ligand.[209] CD36 functions as a long-chain fatty acid translocase that transports fatty acids across the membrane, and contributes to energy metabolism.[210,211] CD36 is also involved in platelet adhesion and angiogenesis,[212] and modulates TGF-β activation[213] and the inflammatory response.[214] Owing to these properties, CD36 is thought to play an important role in atherosclerosis and other complex diseases, such as diabetes and cardiomyopathies.[60,205] Monocyte-derived macrophages from humans with CD36 deficiency have reduced binding and uptake of oxidized LDL compared to normal controls.[209]

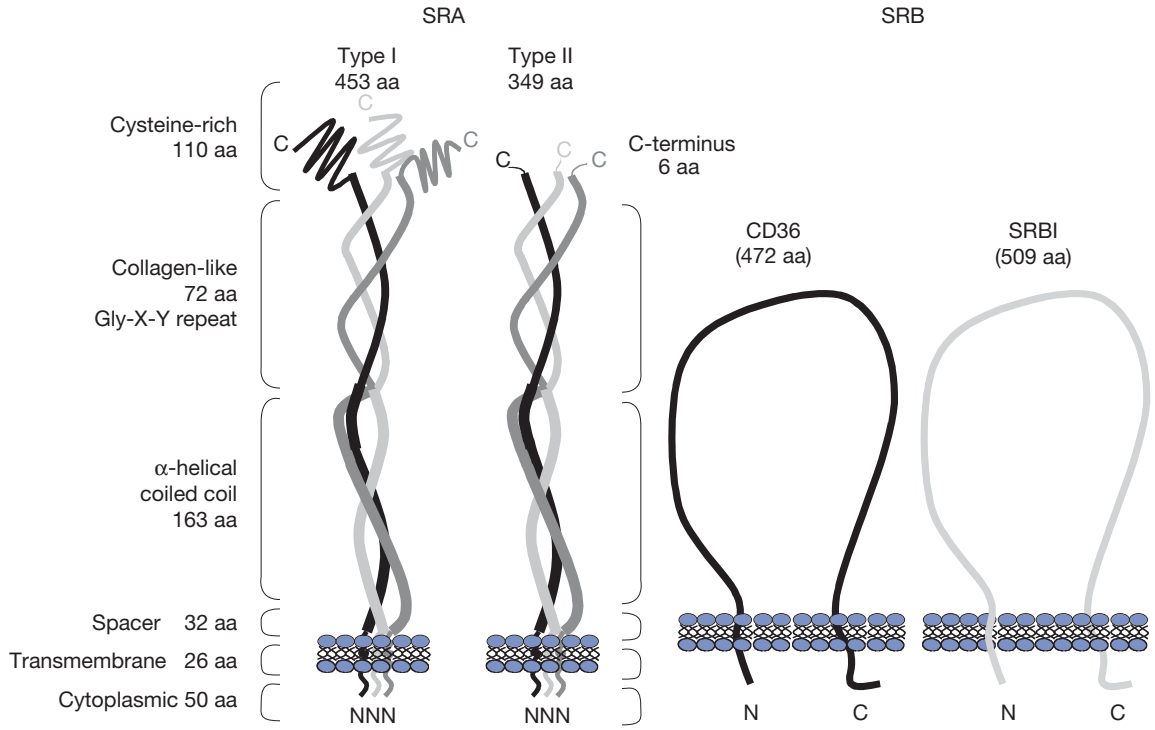


Figure 8.7 Schematic representation of class A and class B members of the scavenger receptor (SR) family[195,196,420]

Transgenic mice overexpressing CD36 in muscle had decreased circulating TG, fewer TG in VLDL, and increased fatty acid oxidation in muscle.[210] Replacement of the mutant CD36 locus by a wild-type locus ameliorated insulin resistance and lowered serum fatty acids in spontaneous hypertensive rats.[215] CD36–/– mice had reduced fatty acid uptake in heart, skeletal muscle, and adipose tissue, and increased HDL cholesterol and VLDL triglycerides.[216] Compared to apoE–/– mice, CD36–/– × apoE–/– mice had an 80% reduction in aortic lesions on an atherogenic diet, and a significant reduction in lesions on a normal diet.[217]

Lp(a)

Structure, biosynthesis, and catabolism

Lp(a) represents a distinct class of lipoprotein particles found mostly in the LDL density region but in other lipoprotein classes as well, including triglyceride-rich fractions.[3,218] The lipid moieties of these particles are similar to that of LDL (Table 8.1).[3,218] Apo(a) is a glycoprotein of 4529 amino acids synthesized by the liver, and contains approximately 30% of carbohydrate moieties.[3,219] The protein moiety of Lp(a) consists of one molecule of apoB-100 and one of apo(a). These proteins are linked by a single intradisulfide bridge between Cys4326 of apoB and Cys4057 (located on Kringle IV-9) of apo(a).[3]

Transgenic mice expressing human apo(a) secrete lipid-free apo(a), which could be converted to Lp(a) by intravenous injection of human LDL. Furthermore, human apo(a) × apoB-100 transgenics secrete Lp(a).[220,221] These findings support the extracellular assembly of LDL with Lp(a) and indicate that Lp(a) assembly does not require any enzymes or other protein in vivo.[222,223] The assembly of Lp(a) appears to be a two-step process. The first step is the docking of apo(a) to the LDL; the second is the formation of the disulfide bridge between Cys 4057 of apo(a) and Cys 4326 of apoB.[3]

The concentration of Lp(a) in plasma is determined by the rate of secretion of apo(a)/Lp(a) by liver cells, and is genetically determined.[224] Turnover studies have shown that the catabolism of Lp(a) in vitro is lower than that of LDL.[3] The mechanism and sites of Lp(a) catabolism remain unknown; it may involve the kidney, and to a lesser extent the liver and members of the LDL receptor family.

Genetics

The structure of apo(a) has been derived from the cDNA sequence of overlapping cDNA clones.[219] Human apo(a) consists of a protease domain which has 94% homology to that of plasminogen, one domain which has homology to kringle V of plasminogen, and a variable number of domains (ranging from 15 to 40) with homology to kringle IV of plasminogen.[219] Lp(a) has proteolytic activity that is different from that of plasmin.[219,225] An extensive polymorphism for apo(a) has been observed in humans. The different apo(a) isoforms differ in molecular mass, ranging from 400 to 800 kDa, and their transmission follows Mendelian inheritance.[3] The difference in Lp(a) size arises from the different number of kringle IV repeats, which ranges from 2 to 40.[3,226] The Lp(a) concentration in subjects with the same phenotype is determined primarily by differences in the production rates.[3] Epidemiological studies have shown that the Lp(a) size is inversely related to the plasma Lp(a) concentration.[226]. The plasma Lp(a) levels are controlled by the apo(a) locus as well as by other genetic factors, such as LDL receptor defects, and environmental factors such as drugs and hormones.[3]

Functions

It has been suggested that Lp(a) may contribute to the pathogenesis of atherosclerosis, and possibly thrombosis, by interfering with the metabolism of LDL and plasminogen, respectively. It is believed that Lp(a) modulates the balance between fibrinolysis and clotting by binding to a forming fibrin thrombus. Existing evidence suggests that Lp(a) may inhibit fibrinolysis by interfering with the binding of plasminogen to fibrinogen.[3] Lp(a) also competes for the binding of plasminogen to fibrin.[227,228] Lp(a) inhibits the streptokinase-mediated activation of plasminogen,[229] inhibits the urokinase or tissue plasminogen activator (tPA)-mediated activation of plasminogen,[228,230,231] affects the synthesis of plasminogen activator inhibitor-1 (PAI-1),[232] and inhibits the binding of plasminogen to tetranectin, an interaction that enhances plasminogen activation by tPA.[231] All these functions of Lp(a) can interfere with fibrinolysis.

Lp(a) has been found in atherosclerotic lesions,[233] and there are several in vitro and in vivo studies indicating that Lp(a)/apo(a) has proatherogenic properties. Lp(a) is a substrate for factor XIIIa,[234] which may crosslink Lp(a) to fibrin, fibrinogen, and fibronectin in atherosclerotic lesions in vivo. Lp(a) also binds proteoglycans.[235] Peptide fragments generated by the proteolytic activity of Lp(a) can be taken up by macrophages and might contribute further to atherogenesis.[3] Apo(a)/Lp(a) interacts with the extracellular matrix, including fibrin,[236] fibronectin,[225] tetranectin,[231] proteoglycans (e.g. decorin),[235] and macrophage receptors,[237] and thus may contribute to atherogenesis.

Lp(a) has chemoattractant activity for monocytes,[238] and induces the release of monocyte chemotactic activity (MCA) from endothelial cells.[239] Through its inhibition of the conversion of plasminogen to plasmin, apo(a) /Lp(a) also inhibits the plasmin-catalyzed activation of transforming growth factor-β (TGF-β).[240] Decreased TGF-β levels promote cell proliferation and migration of smooth muscle cells in vitro[241] and in vivo in apo(a) transgenic mice.[242] In addition, Lp(a) stimulates the expression of ICAM-1, VCAM-1, and E-selectin at the surface of endothelial cells,[243,244] all of which participate in the recruitment of leukocytes to the vessel wall. These properties may explain the atherogenicity of Lp(a).

The role of Lp(a) in atherogenesis is supported by studies in transgenic mice. The apo(a) transgenics developed plaques when fed a high-cholesterol atherogenic diet.[223] Atherosclerotic lesions were shown to be significantly reduced in human apo(a) × apoA-I transgenic mice.[245] Transgenics for a mutant human apo(a) in which the lysine-binding sites of apo(a) were altered failed to develop lesions.[246] A modest increase in atherosclerosis was observed in human apo(a) × apoB–/– double transgenic mice.[247] In contrast, the severity of atherosclerosis was similar to that in apo(a) × apoB–/– transgenic x LDLr–/– mice and apoB–/– transgenic × LDLr–/– mice (Table 8.7).[72]

Lp(a) and atherothrombotic vascular disease

In studies in different ethnic groups, in patients with familial hypercholesterolemia,[3,248] or in subjects with

high LDL levels, it was shown that high plasma Lp(a) levels are associated with coronary heart disease (CHD) and early myocardial infarction.[3,249] Numerous prospective studies have shown that Lp(a) is an independent risk factor for CHD, and in 14 out of 18 studies high levels of Lp(a) were associated with CHD.[3] High Lp(a) is also associated with peripheral vascular disease and stroke.[3,250] Lp(a) levels were significantly reduced in a group of hypertriglyceridemic patients by treatment with nicotinic acid;[251] however, currently there are no well-established pharmacological treatments for high Lp(a) levels in the general population.[3]

In vivo antifibrinolytic activity of Lp(a) was demonstrated only in the transgenic mice.[252] Most studies did not find an association between fibrinolytic parameters and Lp(a) levels.[3,253] Moreover, several studies failed to find a strong connection between plasma Lp(a) levels and thrombogenicity.[3] There is, however, a strong association between increased Lp(a) levels and the occurrence of thrombotic events in patients with immune-mediated diseases. It is possible that Lp(a) β_2-glycoprotein I interactions may be a link between thrombosis and autoimmune disease.[254] The affinity of Lp(a) for fibrin is increased by homocysteine,[255] and this may explain the atherosclerosis and thromboembolic phenomena associated with hyperhomocysteinemia.

The contribution of lipid and bile acid transporters in whole-body cholesterol homeostasis

Lipid and bile acid transporters in the liver and the intestine

To understand overall lipid homeostasis in humans, we must also consider the absorption of dietary lipids in the intestine, the biosynthesis of bile acids in the liver, the uptake and efflux of sterols, phospholipids, and bile acids by the liver and the intestine, and the excretion of bile in the feces. Liver contains the enzymes that synthesize bile acids from precursor cholesterol molecules.[256–258] Bile acids are exported into the bile by the ABCB11 transporter, also known as bile salt export pump (BSEP) or sister of *p*-glycoprotein (SPGP), and possibly by other transporters. Phospholipids are also exported from the liver by the initial action of ABCB4 flippase, and cholesterol is exported into the bile by the ABCG5/ABCG8 transporter (Figure 8.8; Table 8.10).[259,260]

The role of ABCB4 and ABCB11 in bile acid metabolism has been supported by studies in human subjects and animal models. Thus, transgenic mice overexpressing murine ABCB11 in the liver have increased hepatobiliary secretion but normal fecal bile acid secretion.[261] ABCB11 deficiency in mice fed a cholic acid-supplemented diet led to the development of severe cholestasis.[262]. Mutation of ABCB4 in humans is associated with cholethiasis.[263]

Exported phospholipid, cholesterol, and bile acids form micelles and, through ducts, concentrate in the gallbladder. In response to dietary fats, bile is released into the lumen of the small intestine to promote their solubilization and catabolism.[256]

Dietary sterols are taken through an unknown carrier in the intestine. Ninety-nine per cent of the plant and shellfish sterols are resecreted into the intestinal lumen by the ABCG5/ABCG8 transporters and removed in the feces. Mutations in either ABCG5 or ABCG8 cause sitosterolemia in humans.[259] Bile acids are transported into the enterocytes by the ileal bile acid transporter (IBAT), a Na^+-linked symporter. Bile acids are carried in the cytosol by the cytosolic ileal bile acid-binding protein (IBABP), and are released in the bloodstream by the action of an unknown transporter. The bile acids that reach the liver via the enterohepatic circulation are imported into the liver by aNa^+-linked symporter, the sodium taurocholate cotransporting polypeptide (NTCP).[168,256,260]

Normally, 5% of bile acids are excreted into the feces. As will be discussed further, bile acid sequestrants, such as cholestyramine, which bind and remove bile acids via the feces, are used as hypocholesterolemic drugs, usually in combination with statins (Figure 8.5c).[167]

Dietary fats absorbed into the intestine assemble intracellularly into chylomicrons and are secreted into the lymph and reach the circulation. Similarly, fatty acids taken up by the liver (via albumin and other mechanisms) are incorporated into VLDL. ApoA-I secreted by the hepatocytes and enterocytes accepts cellular phospholipids and cholesterol from ABCA1 to form precursor HDL particles, which are converted to mature spherical HDL (Figure 8.8a).

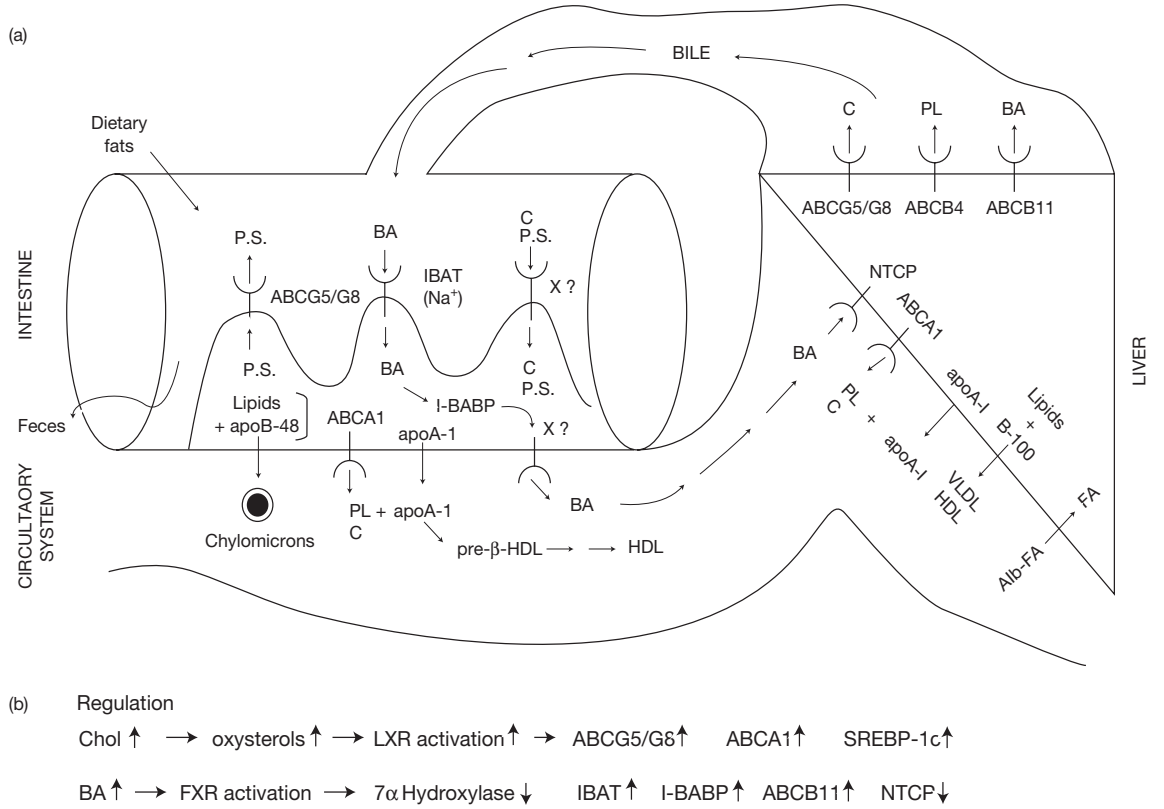


Figure 8.8 (a) Schematic representation of whole-body sterol homeostasis that takes into account the dietary sterol uptake, the biosynthesis and transport of bile acids, cholesterol and phospholipids, and the excretion of bile. (b) Nuclear receptor-mediated regulation of intestinal and hepatic transporters of bile acid (BA) sterols and phospholipids. PS,; ABC, ATP-binding casette; C, cholesterol; IBAT, ileal bile acid transporter; IBABP, ileal bile acid binding protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo, apolipoprotein; NTCP,; PL,; FA,; Alb,; VLDL, very low-density lipoprotein; FXR,; LAR,; SREBP, sterol regulatory element-binding protein

Bile acids activate hormone nuclear receptors and affect lipid homeostasis

The liver contains 70% of the body's LDL receptors. Nuclear hormone receptors represent a superfamily of transcriptional factors that are activated by hormones, such as retinoids, steroids, thyroid hormone, prostaglandins, products of lipid metabolism, vitamin D oxysterols, and bile acids, and regulate cell differentiation, development, homeostasis, and reproduction.[264] The nuclear receptor superfamily also includes numerous orphan receptors. Liver X receptors (LXRα and LXRβ) and farnesoid X receptors (FXRα and FXRβ) are nuclear hormone receptors that are activated by heterodimerization with RXR in the presence of their permissive ligands, as well as by 9-*cis*-retinoic acid.[265] Ligands for LXRα are 22-*R*-hydroxycholesterol and other oxysterols.[265,266] Ligands for FXRα are bile acids such as chenodeoxycholic acid, lithocholic acid and deoxycholic acid.[267–269] When bile acids levels increase, FXR, which is expressed in hepatocytes and intestinal epithelial cells, is activated and represses the transcription of 7α hydroxylase, which is the rate-limiting enzyme in the synthesis of bile acids (Figure 8.8b).

When cellular sterols increase in the liver the level of oxysterols also increases, leading to the activation of

Table 8.10 Phospholipid, sterol, and bile acid transporters

Name/site	*Function*	*Other pathologies*
ABCA1 hepatic and intestinal, basolateral, membrane bound	ATP-dependent efflux of cellular cholesterol and phospholipid to apoA-I and other acceptors	Tangier disease
NTCP (sodium taurocholate cotransporting polypeptide) hepatic, basolateral, membrane-bound	Binds and imports into the hepatocyte 1 molecule of bile acid along with 2 molecules of Na^+	
I-BABP (ileal bile acid binding protein) Cytosolic	Binds intracellularly and promotes the transport of bile acid to the basolateral membrane of the enterocytes. Bile acids are subsequently exported via an unknown membrane bound transporter	
ABCB4 hepatic, apical, membrane bound	Flips phospholipids from the inner to the outer membrane leaflet which is then transferred to bile	
ABCB11 hepatic apical membrane bound	ATP dependent, exports phospholipid into bile	
ABCG5/ABCG8 intestinal, apical and hepatic membrane bound	In the intestine, exports into the intestinal lumen plant sterols (PS), shellfish sterols and cholesterol. These sterols have been absorbed from the intestinal lumen by an unknown apical transporter. In the liver, promotes the apical transport of cholesterol into the bile	Sitosterolemia characterized by high concentrations of plant and shellfish sterols in the bloodstream
I-BAT (ileal bile acid transporter protein) intestinal apical membrane bound	Binds and imports into the enterocyte 1 molecule of bile acid and 2 molecules of Na^+	
Unknown transporter(s), intestinal, apical membrane bound	Binds and imports into the enterocyte cholesterol and plant sterols (PS) and shellfish sterols	
Unknown transporter(s) intestinal, basolateral membrane bound`	Binds and exports from the enterocyte bile acid	

LXR and the transcription of target genes such as ABCG5/ABCG8 and ABCA1, as well as SREBP-1c, which upregulates genes involved in fatty acid biosynthesis. The activation of these genes provides cholesteryl esters and phospholipids to balance the increased levels of free cholesterol.[168] FXR also controls the enterohepatic circulation of bile acids by increasing the transcription of IBAT in the intestine, and increasing the expression of ABCB11 and decreasing the expression of NTCP in the liver.

Role of apoE in cholesterol and triglyceride homeostasis and in atherogenesis:molecular causes of type III hyperlipoproteinemia

Apolipoprotein E

Apolipoprotein E (apoE) is a component of VLDL, IDL, HDL, chylomicrons, and chylomicron remnants,

and is required for the clearance of lipoprotein remnants from the circulation (Figure 8.1b). Lipoprotein-bound apoE is the ligand for the LDL receptor, as well as for other receptors.[1,139,141,188,270] In vitro and in vivo studies have shown that mutations in apoE that prevent binding of apoE-containing lipoproteins to the LDL receptor are associated with high plasma cholesterol levels and cause premature atherosclerosis in humans and experimental animals.[87,271] ApoE is also involved in cholesterol efflux processes, and is atheroprotective.[272–274] ApoE may also modulate the macrophage- and T lymphocyte-mediated immune response in atheroma.[47]

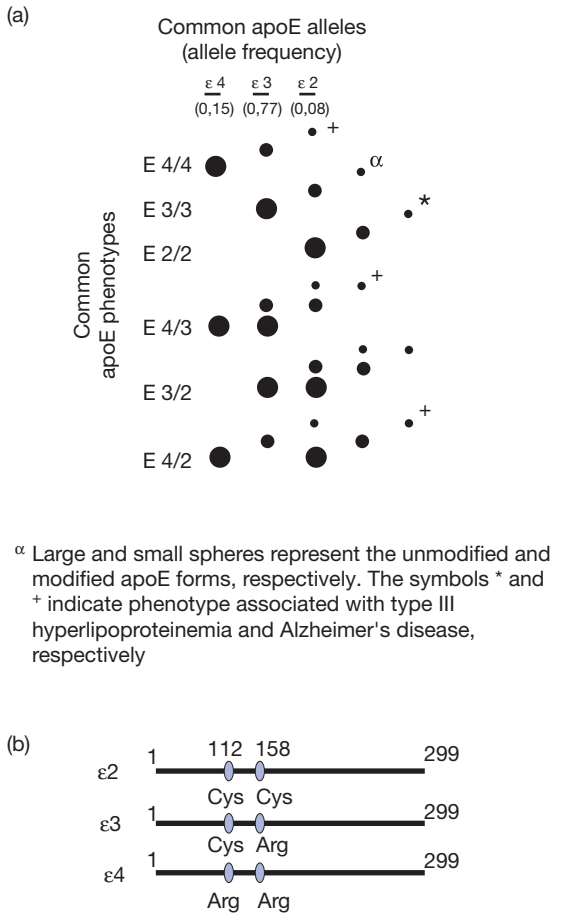
In humans, three common alleles at a single genetic locus exist, which give rise to three homozygous and three heterozygous apoE phenotypes.[275] The phenotype E2/2 is associated with cardiovascular disease,[276] and the phenotype E4/4 is a risk factor for Alzheimer's disease (Figure 8.9).[277] Similar to apoA-I and apoA-IV, apoE contains 17–22 amino acid repeats and 11 amino acid repeats which, based on X-ray crystallography and computer modeling, are organized in amphipathic α-helices.[278,279] These a helices contribute to the ability of apoE to bind to lipids and form lipoproteins. X-ray crystallography of the amino-terminal 22 kDa fragment of apoE showed that this region forms a four-helix bundle that is stabilized by hydrophobic interactions and salt bridges.[279]

It is possible that disturbances in lipid homeostasis,[188] as well as interactions of apoE with other brain proteins, may contribute to the neurodegeneration observed in Alzheimer's disease (Figure 8.10).[280]

ApoE structure and functions

Certain apoE phenotypes and genotypes are associated with type III hyperlipoproteinemia (type III HLP)

Familial type III HLP, also called familial dysbetalipoproteinemia, or broad β, or floating β disease, is characterized by xanthomas, elevated plasma cholesterol and triglyceride levels, cholesterol-enriched βVLDL and IDL particles, increased plasma apoE levels, and premature coronary and peripheral atherosclerosis.[7] The frequency of the disease was estimated to be 0.01–0.1% in the population. The great majority of the patients with type III HLP have the E2/2 phenotype,[276] which results from the substitution of Cys for Arg-158.



α Large and small spheres represent the unmodified and modified apoE forms, respectively. The symbols * and + indicate phenotype associated with type III hyperlipoproteinemia and Alzheimer's disease, respectively

Figure 8.9 (a) Schematic representation of the common and rare apolipoprotein E (apoE) alleles and apoE phenotypes. Large and small spheres represent the unmodified and the o-glycosylated sialylated forms of apoE, respectively. The symbols * and + indicate phenotype associated with type III hyperlipoproteinemia and Alzheimer's disease, respectively. (b) Amino acid differences between the ε2 ε3 and ε4 alleles

This mutation, combined with other genetic or environmental factors, affects the catabolism of apoE-containing lipoproteins and causes type III HLP.[7] Another feature of type III HLP is that it results in the accumulation in plasma of remnants of lipoprotein metabolism enriched in cholesteryl esters and apoE.[281]

Dominant and recessive forms of type III hyperlipoproteinemia

The Arg-158→Cys mutation in the homozygote state, depending on other genetic or environmental factors,

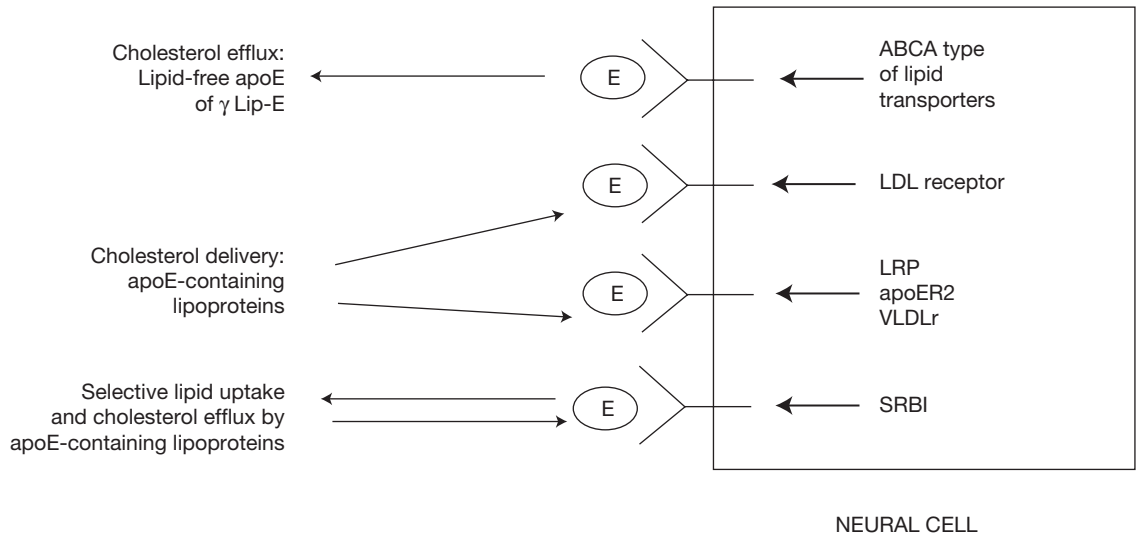


Figure 8.10 Contribution of apolipoprotein E (apoE), apoE-containing lipoproteins, low-density lipoprotein receptor (LDLr) family members, ATP-binding cassette-A1 (ABCA1) and scavenger receptor type BA (SRBI) in cellular cholesterol homeostasis. VLDL, very low-density lipoprotein

may result in type III hyperlipoproteinemia. This form of type III HLP is inherited in an autosomal recessive fashion.[7] A variety of rare apoE mutations have also been described (Figure 8.11),[7] some of which are associated with a dominant mode of inheritance of type III HLP, which is expressed at an early age. These include substitutions Arg-136→Glu, Arg-142→Cys, Arg-145→Cys, Lys-146→Gln, Lys-146→Glu, and an insertion of seven amino acids (duplication of residues 121–27).[1] These apoE mutations which are associated with dominant forms of type III HLP are between residues 136 and 152. The importance of the 136–152 region of apoE for receptor binding was also assessed by in vitro mutagenesis.[1] Mutations within this region reduced the receptor-binding activity 10–50% of control.[282]

In vitro and in vivo analysis of the molecular basis of a human disease associated with apoE deficiency

A rare form of type III HLP associated with familial apoE deficiency was first described in a family in 1981.[283] Familial apoE deficiency in humans is associated with increased plasma cholesterol, the accumulation of remnants in the VLDL and IDL region, and premature atherosclerosis.[271] The biochemical and clinical features of patients with apoE deficiency were lack of plasma apoE; Chol 529 ± 74, TG 221 ± 62, VLDL Chol 243 ± 9, IDL Chol 230 ± 41; cholesterol-rich VLDL and IDL remnants which could be lowered by diets; the clinical picture of type III hyperlipoproteinemia, i.e. the appearance of tuboeruptive and palmar xanthomas; and the development of premature atherosclerosis.[271,283]

Initial studies using cultures of peripheral blood human monocyte–macrophages obtained from an apoE-deficient patient and normal controls showed that the apoE-deficient cultures synthesize low amounts of two aberrant forms of apoE mRNA[284] (Figure 8.11) and do not produce any immunoprecipitable forms of apoE[284] that resulted from aberrant splicing. Both aberrant mRNA contained termination codons within the intronic sequences of the mRNA, and were predicted to encode short peptides that could not be detected in the culture medium (Figure 8.11).

Generation of animal models of apoE deficiency and type III hyperlipoproteinemia

The importance of apoE for the clearance of lipoprotein remnants has been established by the generation of

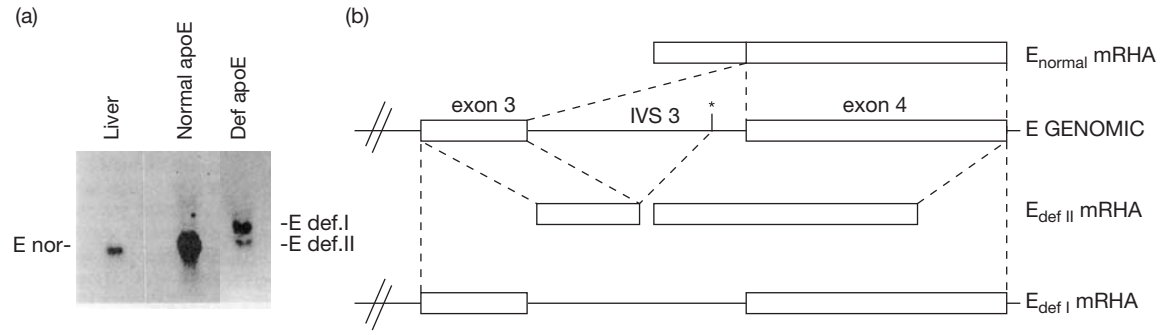


Figure 8.11 (a) Autoradiograph of blotting analysis of RNA isolated from human liver and transformed mouse C127 cells expressing either the normal or apolipoprotein E (apoE)-deficient gene. First lane: mRNA (5 μg) obtained from fetal human liver. Second and third lanes: RNA isolated from one 50 mm diameter Petri dish of clones of C127 cells expressing the normal or deficient apoE gene, respectively. (b) Schematic representation of the mRNA species that are generated by aberrant splicing in the apoE-deficient (E_{def}) gene. The symbol * indicates the position of the activated cryptic splice site, as determined by S1 nuclease mapping and sequence analysis of the deficient apoE gene[197]

mice deficient in apoE (Table 8.6). Comparison of the lipid and lipoprotein profiles of the deficient and control mice on a normal chow diet showed that there is a dramatic increase in total cholesterol in the deficient mice, from approximately 60 mg/dl to 480 mg/dl. All of this increase could be accounted for by increases in the VLDL and LDL cholesterol.[87] More dramatic increases in total VLDL and IDL cholesterol occur when the apoE-deficient mice are fed western-type diets rich in cholesterol and saturated fats (Figure 8.12a). A vitamin A fat tolerance test performed on the apoE-deficient and normal mice showed that the administration of an intragastric bolus of retinol in corn oil impaired the clearance of retinol palmitate esters (which accumulate in the chylomicron remnant fractions) in the deficient but not in the control mice. These observations establish that apoE is required for the clearance of the remnants of lipoprotein metabolism which are rich in cholesteryl esters. Patient information as well as genetic mouse studies has established that the formation of these remnants occurs independently of apoE synthesis; however, clearance of these remnants, which float in the VLDL and IDL region, is critically dependent on the presence of apoE. Deficient mice develop coronary and pulmonary atherosclerotic lesions within 10 weeks on a chow diet, which is accelerated further when the animals are placed on western-type diets (Figure 8.12b). Thus, the biochemical and clinical features of apoE deficiency in mice resembles closely the picture observed previously in apoE-deficient humans.

The lesions generated in apoE–/– mice resemble those seen in humans.[285] In mice expressing the human apoE isoforms, aortic root atherosclerosis increased in the following order: murine apoE<apoE3-<apoE4<apoE2 (Table 8.6).[286] The extent of atherosclerosis was highly correlated with VLDL clearance.[286] ApoE3- and apoE4 expression in the liver by adenovirus-mediated gene transfer in apoE–/– and apoE–/– nude mice showed that apoE3 limited progression and induced regression of early and advanced lesions, whereas apoE4 had only limited lesion progression but little or no effect on regression. Lesions in apoE3- and apoE4-expressing mice had fewer foam cells, less lipid, and an increased fibrous cap.[287,288]

Adenovirus-mediated gene transfer of apoE in LDLr–/– mice caused regression of advanced lesions and reduced the content of isoprostanes without a change in lipid or lipoprotein levels, which is a specific marker of lipid peroxidation.[289] Similar results were obtained in LDL–/– × apoE transgenic mice.[290] The lesions had reduced macrophage content and increased extracellular matrix component. In most (but not all cases),[291] low levels of apoE expression by bone marrow transplantation or retroviral gene transfer protected from atherosclerosis.[274,292,293] A reduction in lesions following bone marrow transplantation was found in young animals, but not in older animals with established lesions.[293]

Macrophages derived from apoE3 Leiden and apoE2 transgenics did not protect apoE–/– mice from

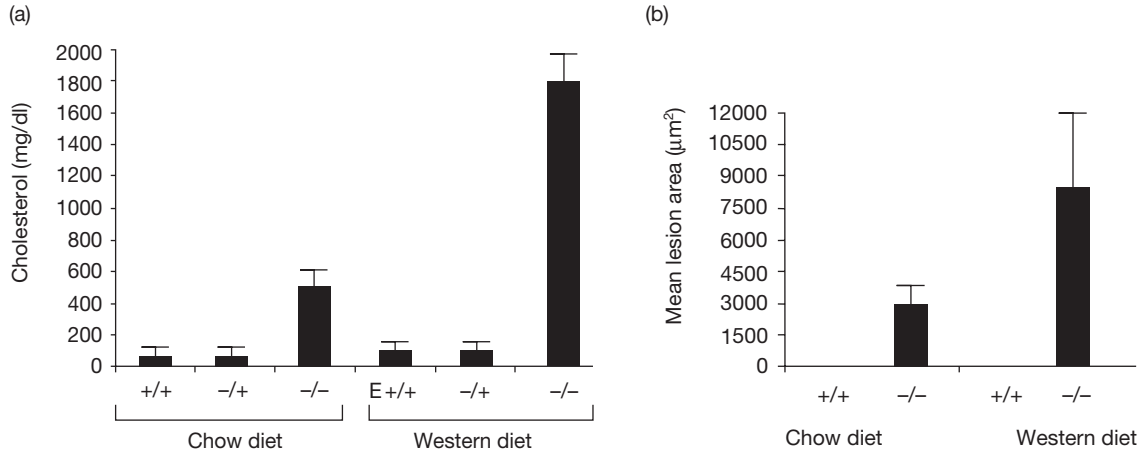


Figure 8.12 (a) Plasma cholesterol levels of apolipoprotein E (apoE)-deficient and control mice maintained on two different diets. Left side, normal chow diet. Right side, western-type diet, consisting of modified chow containing 21% (w/w) fats P/S 0.07, 19.5% (w/w) casein, 0.15% cholesterol, and lacking sodium cholate. (b) Quantitative analysis of atherosclerosis determined in the proximal aorta in apoE-deficient and control mice fed both the mouse chow and western-type diets. Values summarized in the figure represent mean ± standard deviation; +/+, control mice; -/-, homozygous apoE-deficient mice; +/-, heterozygous mice for apoE deficiency. (Adapted from reference 87, with permission)

atherosclerosis, although high levels of expression of apoE3 Leiden in apoE–/– × apoE3 Leiden transgenics reduced atherosclerosis.[294] Low expression of apoE in the adrenals protected apoE–/– and LDLr–/– mice from atherosclerosis without a change in plasma lipid levels.[295]

Recently a helper-dependent apoE-expressing adenovirus was used to correct the high-cholesterol profile of apoE–/– mice.[296] It was found that lifelong correction of the high cholesterol profile was achieved in two mice using the appropriate helper virus serotype with one initial injection and reinjection of the virus after 18 months. It is remarkable that, despite the fact that the plasma apoE levels of the cured mice ranged from 1 to 7 mg/dl, the plasma cholesterol was, for most of their lifespan, below 100 mg/dl and the mice were protected from atherosclerosis.[296]

New insights on the in vivo functions of apoE in cholesterol and triglyceride homeostasis using adenovirus-mediated gene transfer

Specific effect of the carboxy-terminal domain of apoE in the induction of hypertriglyceridemia In humans, apoE levels correlate with plasma triglyceride levels.[281] Similar observations have been reported for experimental animals.[85,87,271,276,297] A series of recent studies used adenoviruses expressing full-length and truncated genomic apoE sequences to correct the high cholesterol profile of the apoE-deficient (apoE–/–) mice. It was shown that overexpression of full-length apoE (by infection of mice with $1–2 \times 10^9$ pfu) did not correct the high cholesterol levels of the apoE–/– mice: in contrast, it induced high triglyceride levels; however, the high cholesterol profile of apoE–/– mice was corrected by infection with truncated apoE forms (Figure 8.13a).[298–301] These studies also showed that infection of C57BL/6 mice with adenoviruses expressing truncated apoE forms that lack their carboxy-terminal region did not change their plasma lipid and lipoprotein profile, whereas overexpression of full-length apoE induced combined hyperlipidemia, characterized by high cholesterol and high triglyceride levels.[298,301] The greatest concentration of the cholesterol and triglycerides induced by apoE was in the VLDL and IDL region.[298–301] The data suggested either increased production of VLDL or defect(s) in lipolysis and/or remnant clearance.

Other experiments showed that the increase in triglycerides was caused by increased VLDL secretion and decreased lipolysis of the hypertriglyceridemic VLDL. When mice were coinfected with adenoviruses

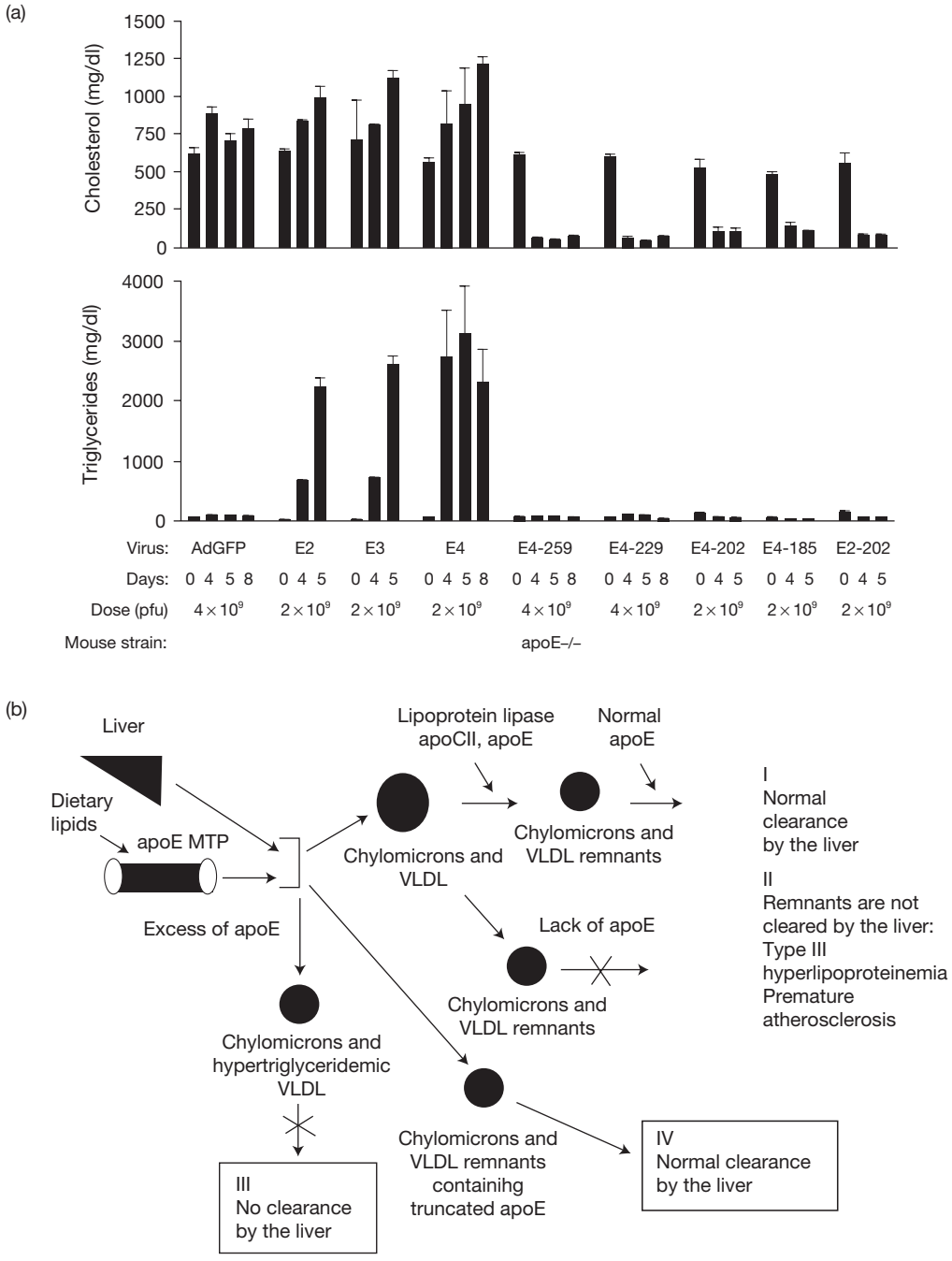


Figure 8.13 (a) Cholesterol and triglyceride levels of apolipoprotein E (apoE) -/- mice infected with the control adenovirus AdGFP, or recombinant adenoviruses expressing apoE2, apoE3, apoE4 or apoE4 carboxy-terminal deletion mutants. (b) Schematic representation of the pathway of biosynthesis and catabolism of chylomicrons. I Normal catabolism. II Defective catabolism of chylomicron remnants due to deficiency in apoE. III Defective catabolism of chylomicrons due to overexpression of apoE. IV Normal clearance of chylomicron remnants by the truncated apoE forms

expressing full-length apoE and lipoprotein lipase, hypertriglyceridemia was corrected, indicating that under conditions of apoE overexpression the endogenous lipoprotein lipase activity may be rate limiting for the lipolysis and/or clearance of VLDL.

The hypertriglyceridemic effect of the full-length apoE was dose dependent. In transgenic mice expressing human apoE2, dyslipidemia could be corrected by low doses ($2–5 \times 10^8$ pfu) of recombinant adenoviruses expressing full-length apoE, or by high doses of truncated apoE; however, dyslipidemia was aggravated by high doses of full-length apoE.[299,302]

The role of the carboxy-terminal segment of apoE in hypertriglyceridemia is also supported by one additional set of experiments that involved remnant clearance in apoE and LDL receptor double-deficient (apoE–/– × LDLr–/–) mice by truncated apoE forms. This analysis showed that although overexpression of truncated apoE2-202 or apoE4-202 can normalize the high cholesterol and triglyceride profiles of the apoE–/– mice (Figure 8.13a), similar doses of apoE2-202 or apoE4-202 did not correct the high cholesterol profiles of the apoE–/– × LDLr–/– double-deficient mice but did not induce hypertriglyceridemia. On the other hand, infection of the double-deficient mice with the full-length apoE2 or apoE4 induced hypertriglyceridemia in these mice.[301]

Based on in vivo and in vitro studies, it appears that the truncated apoE forms extending from residue 1 to residues 185 or 202 or 229 or 259 maintain their ability to associate with pre-existing lipoproteins.[298–301] Once apoE is lipoprotein bound, it may be taken up by the LDL receptor and possibly other apoE-recognizing receptors.[298–301] Hypertriglyceridemia may result from a distorted conformation of full-length apoE on the surface of triglyceride-rich lipoprotein particles that mask their receptor-binding domain.

Mechanism of type III hyperlipoproteinemia caused by the E2/E2 phenotype The E2/E2 phenotype represents an interesting case because it is generally believed that the R158 for C substitution reduces the affinity of apoE for the LDL receptor and results in type III hyperlipoproteinemia.[276,303] Adenovirus-mediated gene transfer of full-length and truncated apoE forms showed that overexpression of apoE2 in apoE–/– mice is associated with high cholesterol and triglyceride levels, whereas overexpression of the truncated apoE2-202 normalizes cholesterol levels of apoE–/– mice and does not trigger hypertriglyceridemia (Figure 8.13a). Thus, full-length apoE2 behaved in vivo like the apoE3 and apoE4 isoforms,[298–301] except that it exacerbated the high cholesterol levels of apoE–/– mice (Figure 8.13a). Unexpectedly, however, the truncated apoE2-202 form behaves in vivo like the truncated apoE4-202 form, despite the fact that apoE2-202 retains the R158 for C substitution (Figure 8.13a). Full-length apoE2 induces combined hyperlipidemia in C57BL/6 mice, characterized by high plasma cholesterol and triglyceride levels.

Receptor-binding experiments showed that the removal of the carboxy-terminal 203–299 amino acids of apoE2 increased the affinity of apoE-containing proteoliposomes for the LDLr.[301] A coinfection experiment with full-length apoE2 and lipoprotein lipase suggested that the activity of lipoprotein lipase, rather than apoCII, becomes rate limiting for the clearance of VLDL triglycerides.

Hypertriglyceridemia occurs in a subfraction of individuals who have the apoE2/2 phenotype,[276,304] and it is possible that these dyslipidemic subjects may present the same remnant-clearance defect as the mice overexpressing full-length apoE2, apoE3, and apoE4 isoforms.[276,298–301,304] It is possible that binding of apoE2 to triglyceride-rich VLDL may distort or mask the receptor-binding site of apoE, thus preventing receptor-mediated clearance of triglyceride-rich VLDL.

The LDL receptor may be the predominant receptor for remnant clearance Experiments using apoE–/– × LDLr–/– double-deficient mice showed that neither the full-length apoE2 or apoE4 nor the truncated apoE2-202 or apoE4-202 corrected the high cholesterol profiles of the apoE–/– × LDLr–/– double-deficient mice. The data indicate that lipoprotein clearance by the truncated apoE forms is mediated mostly by the LDL receptor.

Therapeutic potential of truncated apoE forms Expression of apoE within a physiological range clears lipoprotein remnants,[302] whereas overexpression of full-length apoE results in hypertriglyceridemia.[298–301] The undesirable side-effect of apoE overexpression significantly diminishes its therapeutic potential. The ability of the truncated apoE forms that lack the carboxy-terminal helices from residues 185 to 299 to clear cholesterol without induction of hypertriglyceridemia[298–301] makes them attractive therapeutic targets in future

gene therapy applications to correct remnant removal disorders. Overall, the adenovirus-mediated gene transfer studies reviewed provide the following new information on apoE (Figure 8.13b).

The amino-terminal 1–185 domain of apoE is sufficient to direct receptor-mediated lipoprotein clearance in vivo; clearance is mediated mainly by the LDL receptor; the carboxy-terminal 261–299 domain of apoE induces hypertriglyceridemia. Hypertriglyceridemia results partially from increased VLDL secretion, diminished lipolysis, and inefficient VLDL clearance. The dyslipidemia induced by E2 in mice (and possibly in humans) may not be only the result of R158 for C substitution, and can be partially attributed to the carboxy-terminal segment of apoE. Truncated apoE forms have a dominant effect in remnant clearance, and may have future therapeutic applications for the correction of remnant removal disorders (Figure 8.13b).

The HDL pathway: the roles of apoA-I, the ABCA1 lipid transporter and the HDL receptor (SRBI) in the biogenesis and the atheroprotective functions of HDL

Apolipoprotein A-I

ApoA-I is the major protein component of HDL and plays a key role in the biogenesis and function of HDL. In the absence of apoA-I HDL is not formed.[4,13,18,305–308] ApoA-I has a unique structure that may underlie its functions. ApoA-I contains 22- and 11-amino acid repeats[15] which, based on X-ray crystallography[309] and physicochemical studies,[15] are organized predominantly in amphipathic α-helices (Figure 8.14a). Based on the crystal structure and several structural studies, detailed belt- as well as hairpin-shaped models have been proposed that describe the binding of apoA-I in discoidal and spherical HDL particles.[310,311]

In the belt model, two apolipoprotein A-I molecules are wrapped beltwise around a small discoidal patch of bilayer containing 160 lipid molecules. Each apoA-I monomer forms a curved, planar, amphipathic α-helical ring. The apoA-I amphipathic helices have an average of approximately 3.67 residues per helical turn (instead of 3.60 residues for the conventional α-helices) and with the hydrophobic surface facing inward toward the fatty acyl chains of the disc. This apoA-I helix makes three full turns every 11 residues, and provides a physiological meaning to the 11-residue repeats of apoA-I. When the cholesterol of the discoidal particle is esterified, the discs are converted to spheres and apoA-I structure has to readjust on the helical surface. This structure of apoA-I helices contributes to its lipid-binding properties (Figure 8.14b).[311]

Lipid-bound apoA-I activates the enzyme LCAT.[312] Lipid-free apoA-I interacts functionally with ABCA1 to promote lipid efflux from cells.[18] Lipid-bound apoA-I also interacts functionally with SRBI.[17,313,314] On binding to HDL, SRBI mediates selective uptake of both cholesteryl esters and other lipids from HDL to cells, and net efflux of excess cholesterol (Figure 8.15a).[314]

Lipid-binding and LCAT activation properties of apoA-I in vitro and in vivo

Analysis of the ability of point mutants in a different region of apoA-I to solubilize multilamellar phospholipid vesicles showed that substitution of a series of charged amino acids between residues 191 and 239 in apoA-I did not substantially affect the ability of the mutant proteins to bind to HDL. In contrast, substitution of the positively charged lysine for specific hydrophobic residues in helix 10 (Leu222, Phe225, Phe229), or substitution of valine residues for the more bulky leucine residues in helix 9 (Leu 211, Leu214, Leu218, Leu219), dramatically altered the ability of the mutant protein to solubilize multilamellar 1,2-dimyristoyl-sn-glycero-3 (phosphorac (1-glycerol))-sodium salt (DMPC) vesicles and to bind to HDL.[315]

Adenovirus-mediated gene transfer in apoA-I-deficient (apoA-I–/–) mice was used to assess the role of the carboxy-terminal amino acids of apoA-I in the biogenesis of HDL in mice.[305] These analyses showed that mutations that prevent binding of apoA-I to phospholipid and HDL in vitro result in low apoA-I and HDL levels in vivo following gene transfer of mutant apoA-I forms in apoA-I–/–. The low apoA-I and HDL levels

in these animal models are the result of defective maturation of HDL in vivo that traps HDL at the stage of discoidal particles.[305]

Atheroprotective functions of apoA-I and HDL

ApoA-I and HDL have been implicated in the inhibition or regression of atherosclerosis in humans and experimental animals.[316–318] Initial studies showed that overexpression of the apoA-I gene in the atherosclerosis-susceptible C57BL/6 mouse protected the transgenic mice from atherosclerosis in response to a high-fat diet, compared to non-transgenic controls placed on the same diet (Table 8.8).[79] The same effect was observed in atherosclerosis-prone apoE-deficient mice, in which the expression of apoA-I gene significantly reduced lesion formation.[319] Expression of human apoA-I in apoE–/– mice does not influence the expression of VCAM-1 and the early stages of lipid deposition in the subendothelial matrix and monocyte adhesion to the endothelium, indicating that apoA-I exerts its atheroprotective functions through other mechanisms.[320]

ApoB transgenic × apoA-I–/– mice had a 1.8–3.0-fold increase in fatty streak lesions on an atherogenic diet compared to apoB transgenics.[321,322] In addition, when human apoA-I transgenic rabbits were crossed with WHHL rabbits (lacking the LDL receptor), the apoA-I × LDL–/– rabbits had increased HDL cholesterol levels and were protected from atherosclerosis, compared to the control LDL–/– WHHL rabbits.[89] It remains a paradox that apoA-I–/– mice that lack HDL did not develop atherosclerosis on normal or atherogenic diets.[323] It is possible that apoE and apoA-IV associated with HDL may assume some of the antiatherogenic functions of apoA-I and HDL. Human patients with specific defects in apoA-I develop atherosclerosis.[4]

Gene transfer of apoA-I in apoE–/– × apoA-I transgenic, apoE–/– or LDLr–/– mice fed a western diet limited the progression of fatty streak lesions and led to lesion regression.[324,325] The expression of apoA-I was necessary to reduce atherosclerosis in apoE–/– mice which express apoE only in macrophages.[326]

Stable expression of apoA-I in LDLr–/– mice using a helper-dependent apoA-I-expressing adenovirus did not alter significantly the plasma lipid profile of the mice.[327] However, the treatment reduced by more than 50% the development of atherosclerosis in response to an atherogenic diet over the 24-month period. It also altered the composition of the atherosclerotic lesions.[327] Most recently, intravenous administration in five doses at weekly intervals of 15 mg/kg of apoA-I Milano/phospholipid complexes in patients with acute coronary syndrome caused a small but statistically significant reduction of coronary atherosclerosis, as determined by intravascular ultrasound.[318] However, the small number of patients studied, and limitations in the methodologies used, necessitates that these suggestive findings be confirmed with larger studies.

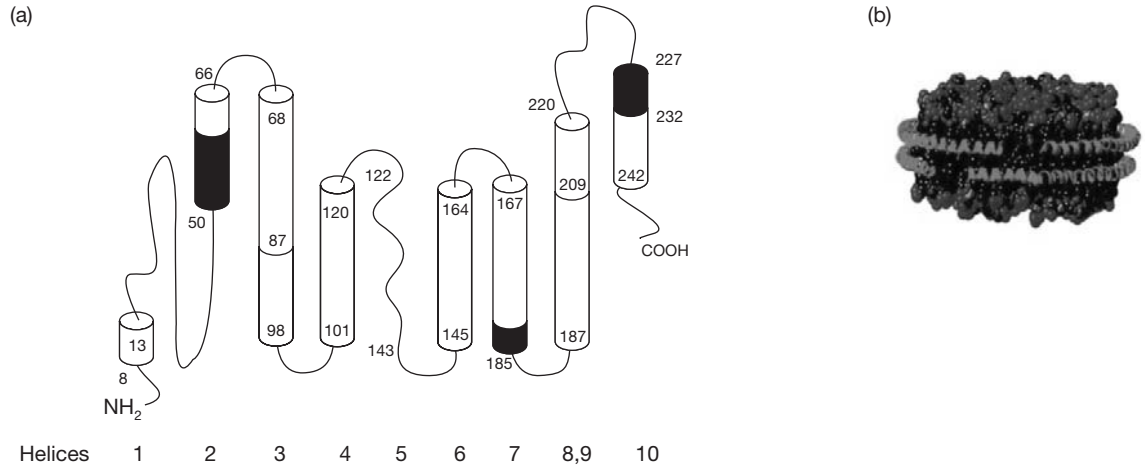
HDL and apoA-I have been reported to have antioxidant and anti-inflammatory properties, can alter prostacyclin levels and platelet function, and modulate NO release following interaction of HDL with SRBI.[63,64,328] All these properties may contribute to atheroprotection by HDL.[329]

ApoA-I may directly or indirectly protect against oxidation of LDL. In vitro, apoA-I renders LDL resistant to lipoxygenase-mediated oxidation.[61,330,331] An indirect effect rests on the presence on HDL of the antioxidant enzymes paraoxonase and platelet-activating factor-acetyl hydrolase (PAFAH), which prevents the formation of oxidized LDL in vitro.[332–334] ApoA-I transgenics both in C57BL/6 and in apoE–/– background or apoA-I gene transfer in apoE–/– mice, resulted in increased levels of both of these antioxidant enzymes.[335] In mice with advanced lesions,[336] the overexpression of human apoA-I or PAFAH reduced macrophage adhesion to the vessel wall. Paraoxonase –/– × apoE–/– mice had increased lipoprotein oxidation and atherosclerosis compared to control mice.[337]

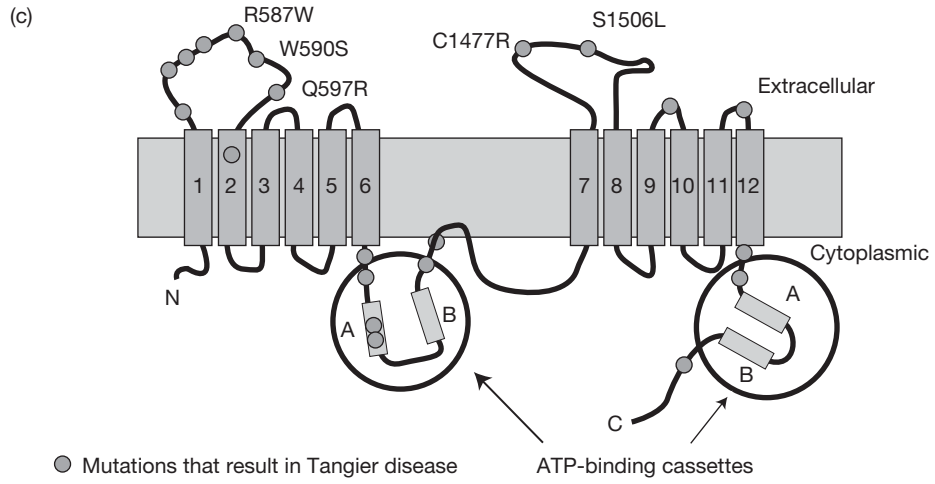
It has been proposed that oxidized LDL binds to CD36 receptor and disrupts endothelial nitric oxide (eNOS) activation, and that this is reversed by binding of HDL to SRBI.[328] The beneficial effect of HDL on the arterial wall was also demonstrated in subjects with heterozygote deficiency of ABCA1. Low HDL levels in these subjects are associated with impairment in basal and stimulated NO bioactivity, and this defect could be corrected by infusion of discoidal phosphatidylcholine/apoA-I particles.[338]

Functional interactions between apoA-I and ABCA1 in vitro and in vivo

Functional interactions between apoA-I and ABCA1 promote the efflux of cellular cholesterol and



Structure: ApoA-I consists of repeated units that form ten amphipathic α-helices
Functions: ApoA-I is essential for the biogenesis of HDL and has atheroprotective functions
ApoA-I activates LCAT, interacts functionally with ABCA1 and promotes efflux of cholesterol and phospholipids and interacts functionally with the HDL receptor (SRBI) and promotes selective lipid uptake and cholesterol efflux



- ABCA1 is involved in the efflux of cellular cholesterol and phospholipids
- Tangier patients and ABCA1 knockout mice have very low apoA-I and HDL levels
- ABCA1 transgenic mice have decreased plasma cholesterol and apoB levels, have 2–3-fold increase in HDL and apoA-I levels and are protected from diet-induced atherosclerosis
- The ABCA1 deficiency in mice did not affect the lipid content and the secretion rates of bile

Figure 8.14 (a) Schematic representation of the boundaries of the α-helical regions of apolipoprotein A (apoA)-I based on computer modeling,[15] X-ray crystallography, and physicochemical studies.[262] Cylinders represent amphipathic α-helices. Predicted amphipathic α-helices are shown in white; additional α-helical regions that were observed by X-ray crystallography are shown in black. (b) Belt model of apolipoprotein (apoA)-I conformation on discoidal high-density lipoprotein (HDL) particles. The figure is adapted from Segrest et al[311], with permission. (c) Schematic representation of the structure of the ATP-binding cassette A1 (ABCA1) transporter and summary of its functions.[346] Some representative mutations that result in Tangier disease are indicated in the figure. LCAT, lecithin: cholesterol acyltransferase

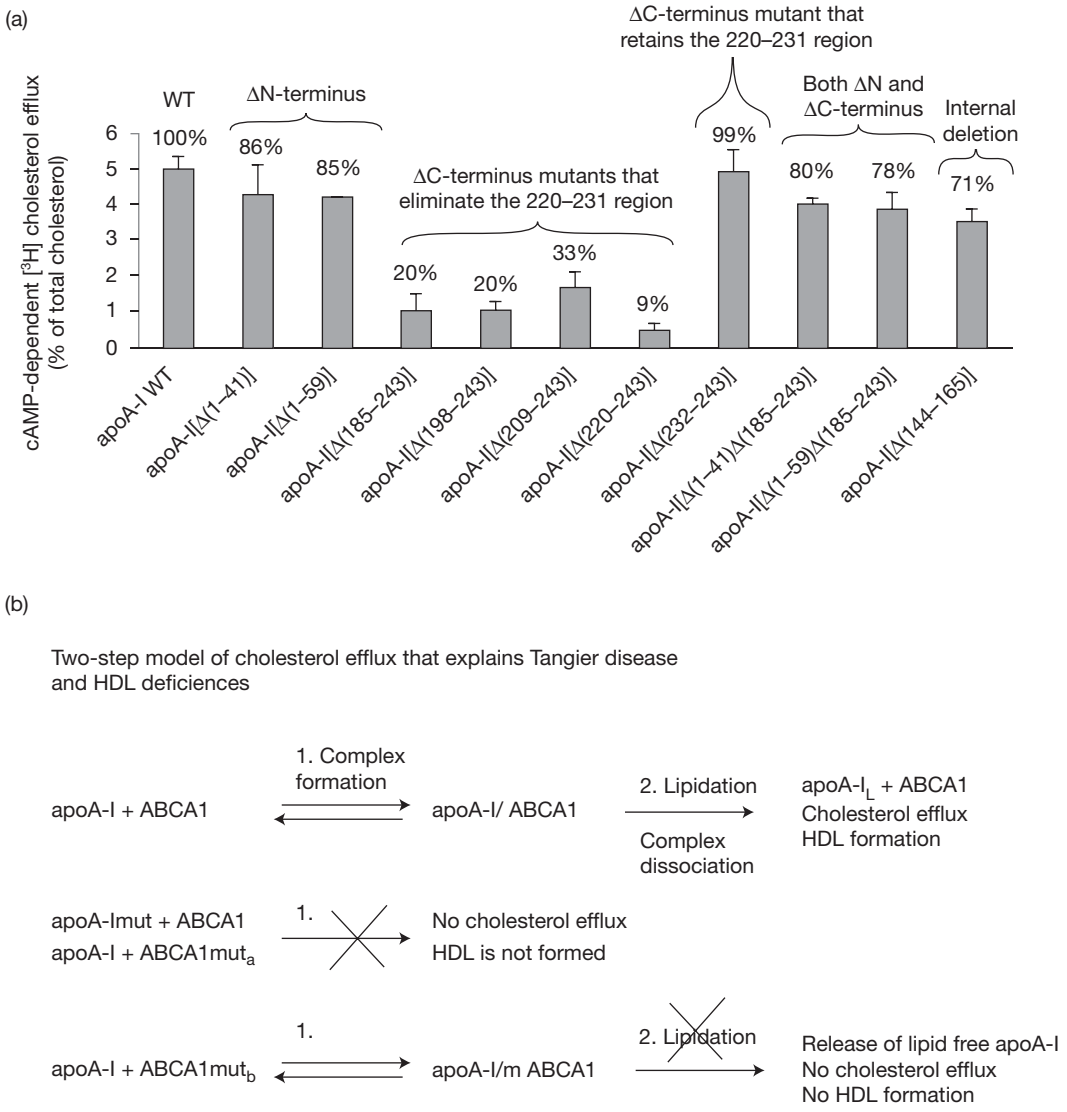


Figure 8.15 (a) cAMP-dependent (ATP-binding cassette-A1 (ABCA1)-mediated) cholesterol efflux in J774 mouse macrophages. Cholesterol efflux studies were performed as described.[18] (b) Two-step model of cholesterol efflux that explains Tangier disease and high-density lipoprotein (HDL) deficiencies. The first step involves complex formation between apolipoprotein (apoA)-I and ABCA1, and the second step involves lipidation of apoA-I and release of the complex. Defects in the two steps may result from either lack of association due to the mutations in apoA-I or ABCA1, or lack of lipidation of the apoA-I acceptor. Both types of defect will inhibit HDL biogenesis

phospholipids and, through a series of intermediate steps, lead to the formation of HDL in the circulation. Human patients or animal models that lack or have defective forms of apoA-I or ABCA1 fail to form HDL. Recent studies established that ABCA1, a member of the ABC family of transporters, is responsible for Tangier disease.[13] ABCA1 is a ubiquitous membrane protein containing 12 membrane-spanning regions and two ATP-binding cassette motifs (Figure 8.14c). ABCA1 is expressed abundantly in the liver, macrophages, lung, adrenal gland, intestine, brain, and

fetal tissues, and at lower levels in the stomach, testis, and other tissues.[13]

Transgenic mice overexpressing human ABCA1 had decreased plasma cholesterol and apoB levels, but a more than two fold increase in HDL-cholesterol and apoA-I levels, and were better protected from atherosclerosis than C57BL/6 mice in response to atherogenic diets (Table 8.8).[339] Unexpectedly, ABCA1 overexpression in apoE–/– mice resulted in a 2–2.6-fold increase in aortic atherosclerosis despite showing little effect on their plasma lipid profile.[339] These findings suggested the potential involvement of ABCA1 in the atheroprotective functions of apoE.

ABCA1–/– mice have lower total serum cholesterol and lipid deposition in various tissues and impaired growth and neuronal development, thus mimicking the phenotype of human Tangier disease patients.[340] ABCA1–/– mice had moderately increased cholesterol absorption in response to a high-cholesterol diet compared to WT mice.[307] Transplantation of normal bone marrow in ABCA1–/– mice indicated that expression of ABCA1 in monocyte/macrophages contributes minimally to HDL formation.[341]

Bone marrow transplantation of ABCA1–/– leukocytes to LDL–/– did not affect HDL levels, but it increased the amount of macrophages in peripheral blood leukocytes in spleen and liver and also increased atherosclerosis.[342] Similar bone marrow transplantation of ABCA1–/– macrophages in apoE–/– or LDLr–/– mice increased foam cell accumulation and accelerated atherosclerosis in apoE–/– mice.[307]

The role of ABCA1 on the lipid content of bile salts and on bile secretion is not clear. One study found that in ABCA1–/– mice the bile acid content and the secretion rates of biliary cholesterol, bile salts, and phospholipid were not impaired.[343] Another study showed that cholesterol and phospholipid concentrations in the bile of human ABCA1-transgenic mice were increased 1.8-fold, indicating that overexpression of ABCA1 increases biliary lipid secretion.[344]

Domains of apoA-I required for cholesterol efflux, and mode of interaction between apoA-I and ABCA1

Although it is clear that functions of both apoA-I and ABCA1 are critical for the formation of HDL, the question that persisted is, what is the nature of the functional interactions between these two very important proteins.

Systematic in vitro studies using a variety of apoA-I mutants showed that:

- The amino-terminal deletions do not affect the ABCA1-mediated lipid efflux.
- The carboxy-terminal deletions that remove the 220–231 region diminished the ABCA1-mediated lipid efflux.
- The carboxy-terminal (232–243) deletion that retains the 220–231 region does not affect the ABCA1-mediated lipid efflux.
- Double amino-terminal and carboxy-terminal deletions which contain only the central helices 3–7 of apoA-I restored the ABCA1-mediated lipid efflux.
- Point mutations and deletions in the central helices 3–7 of apoA-I do not affect ABCA1-mediated lipid efflux (Figure 8.15a).[18,345]

Other studies using direct binding and competition crosslinking experiments showed that apoA-I mutants that fail to promote cholesterol efflux crosslink inefficiently with ABCA1, and those that promote cholesterol efflux crosslink efficiently.[345]

The end-product of the functional interactions between apoA-I and ABCA1 is the biogenesis of HDL. Thus a fundamental question, based on the in vitro analysis, is how cholesterol efflux is related to the biogenesis of HDL. This question was addressed by a combination of in vitro experiments and adenovirus-mediated gene transfer of apoA-I mutants to apoA-I-deficient mice.[345,346] Overall, these studies showed that:

- ApoA-I mutants that promote cholesterol efflux crosslink efficiently to ABCA1 and vice versa.
- ApoA-I mutants that lack the 220–231 domain are defective in the efflux of cellular cholesterol and phospholipids, and crosslink poorly to ABCA1.
- The carboxy-terminal mutants fail to form discoidal or spherical HDL particles.
- The central region of apoA-I alone, which contains helices 3–7, has the capacity to promote ABCA1-mediated lipid efflux and to form discoidal HDL particles in vivo.

Several models have been advanced to explain the ABCA1/apoA-I interactions that lead to lipid efflux. Some proposed that there is no direct association between ABCA1 and apoA-I; rather, they suggested that ABCA1 generates an unstable membrane domain by flipping phosphatidylserine to the outer leaflet of the plasma membrane, which allows docking and subsequent lipidation of the amphipathic helices of apoA-I.[347,348] One of the models, designated hybrid model, proposed that an initial tethering of apoA-I to membranes occurs through the carboxy-terminal apoA-I region, followed by association of apoA-I with ABCA1.[349]

A third intuitive model is the direct association model, which assumes physical interactions between apoA-I and ABCA1,[346] and by in vitro and in vivo studies by us and others.[345,346] The information obtained from the in vitro and in vivo studies was used to explain the mode of interaction between apoA-I and ABCA1.

A two-step model in the ABCA1-mediated cholesterol efflux that explains Tangier disease and HDL deficiencies

Previous studies showed that an ABCA1 mutant, ABCA1[W590S], crosslinks more efficiently to apoA-I than WT ABCA1 at 37°C; however, the W590S mutant has defective lipid efflux and is associated with Tangier disease.[4,13,346] Analysis of the dissociation of the complexes formed between apoA-I and ABCA1 showed that the rate of dissociation was similar for the WT ABCA1 and the ABCA1[W590S] mutants;[350] however, the apoA-I released from WT ABCA1 was bound to lipids, whereas the apoA-I released from ABCA1[W590S] was lipid free.[350] Taking together all the available in vitro and in vivo data, we suggest a two-step model of cholesterol efflux that can explain the functional interactions of ABCA1 with apoA-I and other cholesterol acceptors (Figure 8.15b). The first step is the formation of a tight complex between ABCA1 and its ligands. The second step is lipidation of HDL and the dissociation of the complex. The first step is strongly supported by the crosslinking data between WT and mutant forms of ABCA1 and apoA-I forms.[18,345,346] Lack of association between ABCA1 and apoA-I variants, or the formation of a weak complex, may prevent lipidation of apoA-I and cholesterol efflux. Formation of a weak complex may, for instance, occur in the case of the carboxy-terminal mutants of apoA-I which were shown to have reduced affinity for ABCA1, as well as in the case of the various ABCA1 mutants that are associated with Tangier disease and fail to crosslink to ABCA1.[345,346]

The second step in cholesterol efflux is supported by the binding and dissociation studies between WT and mutant forms of apoA-I and ABCA1. It has been shown that WT and mutant forms of apoA-I crosslink with different efficiencies to WT ABCA1 and the W590S mutant.[345] In addition, WT apoA-I forms a complex with ABCA1[W590S] but is released lipid-free.[350] Overall, in vitro and in vivo analysis of several apoA-I mutants showed that the central helices, along with the 220–231 region, of apoA-I are required for cholesterol efflux in vitro and HDL biogenesis in vivo. The studies favor direct binding of apoA-I to ABCA1 and provide a molecular explanation for Tangier disease and HDL deficiencies (Figure 8.15b).

Functional interactions between lipid-bound apoA-I and the HDL receptor/scavenger receptor class B type I

SRBI is a membrane protein with two transmembrane regions and two short amino- and carboxy-terminal cytosolic regions (see Figure 8.7). Initial studies involving direct binding and competition experiments established that SRBI is a multiligand receptor that binds HDL, LDL, and modified lipoproteins. SRBI binds to HDL and reconstituted HDL, at least in part by apoA-I.[17,351] On binding to HDL, SRBI mediates selective uptake of both cholesteryl esters and other lipids from HDL to cells, bidirectional movement of unesterified cholesterol, and net efflux of excess cholesterol.[314] Binding of HDL and selective lipid uptake is preserved when purified SRBI is reconstituted into phospholipid/cholesterol liposomes.[351] SRBI has been purified from lysates of cells expressing epitope-tagged SRBI, and was reconstituted into phospholipid/cholesterol liposomes. The reconstituted receptors displayed high-affinity binding and selective lipid uptake.[351] It has recently been shown that interaction of HDL with SRBI activates eNOS.[64] It was proposed that binding of HDL to SRBI initiates tyrosine kinase (Src)-mediated signaling, which leads to parallel activation of protein kinase B (PKB) and mitogen-activated protein (MAP) kinases via phosphatidylinositol 3 (PI3) kinases. These signaling cascades activated the phosphorylation of eNOS and

promoted the release of NO.[64] Another study suggested that eNOS activation following binding of HDL to SRBI is not mediated by an increase in the intracellular calcium concentration or by activation of PKB, but rather results from a reversible increase in the intracellular ceramide levels.[352]

Overexpression of SRBI protects mice from atherosclerosis despite the reduction of plasma HDL levels

The physiological importance of SRBI interaction with HDL (apoA-I) has been established by a variety of in vivo studies in mice. SRBI expression in the liver of mice decreased HDL cholesterol and apoA-I in a dose-dependent manner, irrespective of diet, owing to the accelerated clearance of the HDL. It also decreased plasma VLDL and LDL levels.[353,354]

A deficiency of SRBI in SRBI–/– mice resulted in increased total plasma cholesterol, decreased stores of cholesteryl ester in steroidogenic tissues, and the generation of apoE-enriched HDL-like particles.[355] It also decreased cholesterol secretion without alterations in bile acid secretion, bile acid pool size, or fecal bile excretion.[355–357] The findings suggested that SRBI appears to mediate the transfer of cholesterol from plasma HDL to the bile for excretion, and the delivery of cholesterol to steroidogenic tissues for synthesis of steroid hormones.

Adenovirus-mediated gene transfer of SRBI in LDLr–/– mice with early or advanced lesions reduced plasma apoA-I and HDL levels, had small effects on non-HDL cholesterol levels, and protected the mice from diet-induced atherosclerosis.[358,359] Atherosclerosis was also reduced in SRBI transgenics heterozygous for LDLr background.[360] Using apoB-transgenic × SRBI-transgenic mice, atherosclerosis was reduced in mice that expressed low, but not high, levels of SRBI, suggesting that SRBI exerts a beneficial effect within a certain range.[361] The inactivation of the SRBI gene in mice dramatically accelerated the onset of atherosclerosis in the background of apoE–/– or LDLr–/– mice.[356,362,363]

SRBI–/– × apoE–/– mice develop premature occlusive coronary atherosclerosis, spontaneous myocardial infarction, have cardiac hypertrophy and other severe cardiac dysfunctions, and die within 8 weeks of birth. These defects resemble those found in human patients with coronary heart disease.[364] Remarkably, treatment of the SRBI–/– × apoE–/– mice with probucol extended their life by up to 60 weeks, and reversed most of their cardiac and red blood cell pathologies, as well as their lipid and lipoprotein profiles.[365]

Overall, the combination of in vivo and in vitro studies established that interactions of HDL with SRBI appear to control the structure and composition of plasma HDL,[314,353–355,359] the cholesterol contents of HDL, the adrenal gland, the ovaries and the bile,[355,357,362] and to protect mice from atherosclerosis.[356,360–363]

Effect of other proteins on HDL levels, composition, and functions

HDL is remodeled in the circulation by LCAT, CETP, PLTP, LPL, HL, and EL.[366] CETP transgenic mice had a significant decrease in apoA-I and HDL levels.[367] The HDL and apoA-I levels were restored in human CETP × apoA-I double-transgenic mice.[368] In addition, plasma from these mice expressed in the background of apoE or LDL-receptor deficiency had a proatherogenic effect.[369]

Lecithin cholesterol acyltransferase-transgenic mice have increased atherosclerosis, possibly owing to the generation of an abnormal HDL species. Mice transgenic for human LCAT, or double transgenics for LCAT × apoA-I or LCAT × apoA-II, had increased apoA-I and HDL cholesterol levels.[370] Despite this increase, these transgenic animals display marked increases in atherosclerosis compared to controls.[371] Atherosclerosis was reduced by coexpression of LCAT and CETP in transgenic mice.[372] In contrast, the rabbits overexpressing the human LCAT gene had decreased levels of atherosclerotic lesions,[373] indicating the importance of species differences in the development of atherosclerosis.

Mice transgenic for human PLTP did not have significant changes in plasma lipids and lipoprotein. However, human PLTP × apoA-I transgenic mice increased apoA-I in pre-b HDL levels.[374] In contrast, PLTP–/– mice had a marked decrease in HDL and apoA-I.[375] Furthermore, LPL–/– × apoB-transgenic and LPL–/– × E–/– mice had markedly decreased atherosclerosis compared to apoB transgenic or apoE–/– mice.[376,377]

LPL–/– mice had severe hypertriglyceridemia and very low HDL levels, and survived up to 18 hours after birth.[378] Mice expressing LPL in cardiac muscle had normal plasma triglycerides and HDL.[379] The overexpression of human LPL drastically lowered VLDL and increased HDL,[380] confirming the inverse relationship between hypertriglyceridemia and plasma HDL levels that was observed previously in clinical studies. Transplantation of LPL-negative fetal liver cells [381] or bone marrow cells[294] into C57BL/6 mice resulted in a reduction in lesions in the proximal aorta, and had no effect on serum lipid levels.[381]

HL-deficient mice had elevated levels of large HDL particles enriched in phospholipid and apoE.[382] HL × apoE–/– mice have reduced atherosclerosis compared to apoE–/– mice.[383] Rabbits and mice transgenic for human HL [384-386] have decreased levels of HDL-cholesterol in plasma and decreased HDL particle size. However, rabbits overexpressing HL do not have increased susceptibility to atherosclerosis.[385,386] Reduction in aortic cholesterol has been reported in mice that express human HL.[384]

Mice deficient in EL have increased HDL levels,[29,30] whereas the EL knockout mice have decreased HDL levels.[30] The impact of EL on the development of atherosclerosis has not yet been determined.

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