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REVIEW

Apolipoprotein E Structure and Substrate and Receptor-Binding Activities of Triglyceride-Rich Human Plasma Lipoproteins in Normo- and Hypertriglyceridemia

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Abstract—Cysteine-arginine interchanges along the primary sequence of human plasma apolipoprotein E (apoE) play an important role in determining its biological functions due to a high mutation frequency of cytosine in CGX triplet that codes 33 of 34 apolipoprotein arginine residues. The contribution of apoE secondary structure to apolipoprotein–lipid interaction is described. The significance of apolipoprotein in triglyceride synthesis, lipoprotein lipolysis, and receptor-mediated clearance of lipolytic remnants of triglyceride-rich lipoproteins is discussed as well. The metabolic flow of lipoproteins in normo- and hypertriglyceridemia can be described by separate compartments that contribute to lipoprotein interaction with at least six different receptors: 1) low density lipoprotein (LDL) receptor; 2) LDL receptor-related protein (LRP); 3) $apoB_{48}$ macrophage receptor for hypertriglyceridemic very low density lipoproteins (VLDL); 4) scavenger receptors; 5) VLDL receptor; 6) lipolysis-stimulated receptor. The contribution of the exposure of apoE molecules on the surface of triglyceride-rich particles sensitive both to lipolysis and plasma triglyceride content to the interaction with LDL receptor and LRP is emphasized.

Key words: apolipoprotein E, apolipoprotein–receptor interactions, metabolism of triglyceride-rich lipoprotein particles, protein structure

Apolipoprotein E (apoE) contributes to the metabolism of triglyceride (TG)-rich lipoproteins in at least three ways: 1) by inducing TG synthesis; 2) by influencing lipoprotein lipolysis; 3) by controlling receptor-mediated clearance. The last two processes are thought to be impaired in hypertriglyceridemia. The basic problem remains whether apolipoprotein expression on a particular lipoprotein particle would inhibit or provoke atherogenesis [1] as apolipoprotein-induced accumulation of cholesterol within macrophages [2] and of atherogenic intermediate density lipoprotein (IDL) particles in fibroblast and HepG2 cell cultures [3] has been observed; on the contrary, atherosclerosis was diminished in rabbits with genetic defect in low density lipoprotein (LDL) receptor after sustained administration of apolipoprotein [4]. The incubation of very low density lipoprotein (VLDL)/intermediate density lipoprotein (IDL) fraction from apoE-knockout mice with J774 macrophage cell line [5] or with peritoneal macrophages [6] resulted in cellular cholesteryl ester accumulation. LDL particles competed with apoE-free VLDL/IDL for accumulation and degradation and a common catabolic pathway for the two lipoproteins in macrophages has been suggested. Saito et al. [7] observed increased cholesteryl ester synthesis in human macrophage culture after the addition of TG-rich particles from E3/2 heterozygotes with hypertriglyceridemia compared to E3/3 homozygotes with hypertriglyceridemia also and suggested the atherogeneity of these particles. However, apoE-independent internalization of

Abbreviations: apoE) apolipoprotein E; Ch) cholesterol; CM) chylomicrons; CMR) chylomicron remnants; HDL) high density lipoprotein; HDL_2) high density lipoprotein of the second class; HDL_3) high density lipoprotein of the third class; HL) hepatic lipase; HTG) hypertriglyceridemia; IDL) intermediate density lipoprotein; LCAT) lecithin:cholesterol acyltransferase; LDL) low density lipoprotein; LPL) lipoprotein lipase; LRP) LDL receptor-related protein; LSR) lipolysis-stimulated receptor; SR) scavenger receptor; TG) triglyceride; VLDL) very low density lipoprotein.

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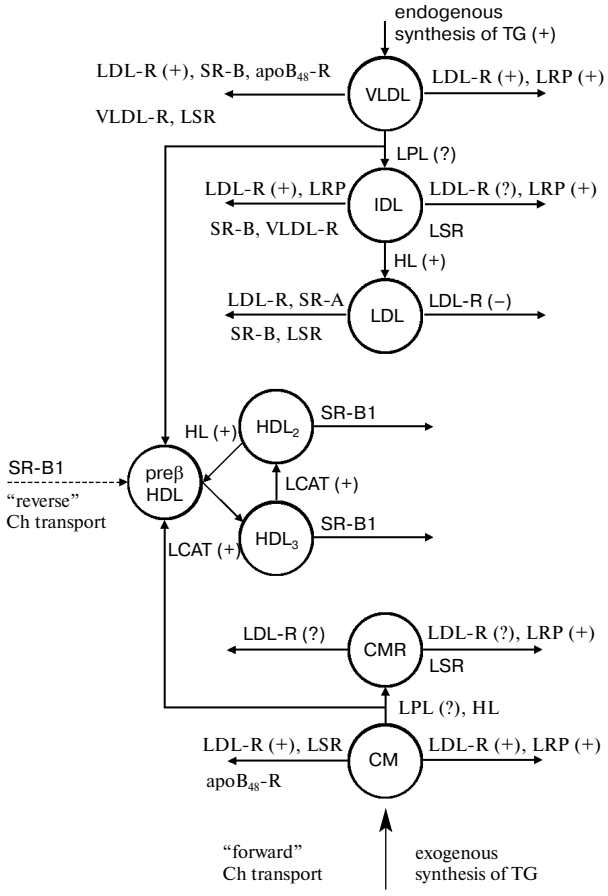
remnants by P388D$_1$ macrophage cell line has been suggested [8]; remnant particles, unlike nascent ones, originating as a result of self-assembly, are lipoproteins modified by hydrolase (lipase) activities and/or by apolipoprotein transfer to other lipoproteins. Kypreos et al. [9] found a decrease in plasma cholesterol in apoE-deficient mice on the expression of apoE4-202 containing the first 202 of the 299 residues; the triglyceride level was not changed, in contrast to a prominent increase in triglyceride synthesis in mice with the full-length apolipoprotein.

ApoE polymorphism contributes to plasma cholesterol (Ch) level in the general population [10]. Primary, secondary, and supramolecular structure of apoE in solution and in lipoproteins is thought to be a key factor in lipoprotein metabolism in normo- and dyslipidemias. In particular, receptor-mediated catabolism of TG-rich lipoproteins with apoE as a ligand can be influenced by apolipoprotein conformation. ApoE synthesis, secretion, structure, and receptor- and non-receptor-directed clearance of TG-rich lipoproteins have been reviewed earlier [11-15].

ApoE can contribute to metabolism of TG-rich lipoproteins by influencing TG synthesis, enzyme lipolytic activities, and efficiency of the interaction of a particular lipoprotein particle with at least six receptors: 1) LDL receptor; 2) LDL receptor-related protein (LRP); 3) VLDL receptor; 4) scavenger receptor for oxidized lipoproteins and particles with increased negative charge; 5) macrophage receptor for hypertriglyceridemic VLDL; 6) lipolysis-stimulated receptor. The contribution of apoE to receptor-mediated clearance becomes prominent since TG-rich lipoproteins and the products of their lipolytic transformation, i.e., VLDL, chylomicrons, and remnants, rather than LDL, seems to be most atherogenic [16]. Besides apoE, a second apolipoprotein apoB$_{100}$ as physiologically important ligand may contribute to receptor binding of VLDL, IDL, and LDL; the ligand properties of chylomicron constituent apoB$_{48}$ (truncated by alternate splicing to the first 48% of the entire sequence) have been suggested only by one group [17]. A multi-compartment scheme of lipoprotein metabolism (figure) that includes a minimal number of lipoprotein pools with apoE-controlled size will be used throughout.



Multi-compartment scheme of lipoprotein metabolism with the minimal number of pools that are controlled by apoE. Enzymes and receptors involved are indicated (apoB$_{48}$-R, apoB$_{48}$ receptor; LRP, LDL receptor-related protein; LDL-R, low density lipoprotein receptor; VLDL-R, very low density lipoprotein receptor; LSR, lipolysis-stimulated receptor; SR, scavenger receptor) and activator (+), inhibitor (–), or inconclusive (?) modes of apoE action are mentioned as well. The solid to the right arrows correspond to receptor-mediated catabolism through liver, the dashed arrow indicates the "reverse" cholesterol (Ch) transport from cell to liver. The solid to the left arrows correspond to "forward" cholesterol transport to cells

METABOLIC PATHWAYS OF TG-RICH LIPOPROTEINS

Role of Lipoprotein Lipase

Lipoprotein lipase (LPL) hydrolyzes the acyl bonds in tri-, di-, and monoglycerides mainly in VLDL and chylomicrons. After synthesis by parenchymatous cells, i.e., adipocytes and muscle cells, the enzyme molecules bind to the intima surface of capillary endothelium via heparan sulfate proteoglycans [18]. Liberated free fatty acids are thought to be taken up by adipose tissue in TG resynthesis [19] or by myocytes on energy consumption [20]. ApoC-II is an activator of LPL activity; however, a prominent hypertriglyceridemia developed in mice on apolipoprotein overexpression [21]. Besides enzyme activity, the LPL molecule serves as a ligand or "bridge" in receptor- and heparansulfate proteoglycan-mediated lipoprotein degradation by cells [22, 23] and in selective uptake of lipids and lipid-soluble vitamins [24]. The involvement of LPL in high density lipoprotein (HDL) generation follows from the diminished HDL content in type I hyperlipidemia [25]. Within the HDL pool, preβ$_1$-HDL particles with two apoA-I molecules and up to 10%

of phospholipid that corresponds to a molecular mass of 67 kD [26] proved to accept cellular cholesterol, which initiates a reverse Ch transport from cell to liver [27]. Preβ$_1$-HDL content is positively correlated with plasma TG level [28]. Hepatic lipase activity is thought to generate preβ$_1$-HDL from HDL$_2$ [28], as the action of bezafibrate as a hepatic lipase activator resulted in increase in preβ$_1$-HDL and decrease in high density lipoprotein of the second class (HDL$_2$) through the "preβ-HDL" cycle. LPL activity seems to be involved in preβ-HDL generation also, as followed from the absence both of preβ-HDL and "mature" HDL particles in LPL-knockout mice [29] despite normal plasma apoA-I content.

Connection between Metabolism of HDL and TG-Rich Lipoproteins

Preβ-HDL accepts phospholipid and Ch molecules from the cell membrane by the "efflux"-mechanism that includes ATP-binding cassette transporter 1 (ABC1) [30]. The subsequent preβ-HDL transformation into "mature" high density lipoprotein of the third class (HDL$_3$) and HDL$_2$ seems to include lecithin:cholesterol acyltransferase (LCAT)-dependent cholesteryl ester formation [31], the acquisition of surface remnants formed on lipolysis of TG-rich lipoproteins [32, 33], and fusion of HDL particles. The last two processes are controlled by cholesteryl ester transfer protein [34]. It thus appears that LPL activity deficiency results in the impairment of generation and "maturation" of HDL. This contributes to the direct HDL-lowering effect in hypertriglyceridemia, while an indirect effect may originate from increased cholesteryl ester transfer to VLDL and concomitant decrease in HDL-Ch content. Then VLDL particles may be considered as indirect cholesteryl ester acceptors; the direct accepting by chylomicrons and VLDL has been suggested by Chung et al. [35]. Besides, these authors have also suggested that chylomicrons rather than HDL could be major acceptors of cellular cholesterol and that HDL particles provoke, by an unclear mechanism, more efficient chylomicron clearance. This paradoxical point of view does not contradict, first, the absence of correlation between HDL-Ch content and centripetal Ch flow to the liver in transgenic mice with cholesteryl ester transfer protein [36]; apoE may contribute to HDL-controlled clearance of remnants of TG-rich particles by a still unresolved mechanism. Second, the rate of chylomicron remnant clearance after fat load positively correlated with HDL content [37]. However, the lack of contribution of cholesteryl ester transfer from HDL to VLDL as TG hydrolysis proceeded within the latter particles [38] is thought to contradict the acceptor role of TG-rich lipoproteins in reverse Ch transport. Somehow or other, the possible connection between HDL and TG-rich lipoproteins becomes the hot point in studying of reverse Ch transport [39], especially in hypertriglyceridemia. It has to be pointed out that we recently suggested [40, 41] the existence of transitory complexes between preβ-HDL and TG-rich lipoproteins that could provoke LDL receptor-mediated clearance of the latter particles.

Chylomicrons that contain apoA-I, apoA-IV, and apoB$_{48}$ are synthesized in the intestine after fat absorption. The efficiency of chylomicron metabolism is determined by apolipoprotein composition and the rate of particle synthesis per se is controlled by apoB$_{48}$ production rate, which in turn depends of mRNA maturation: if apoB gene expression in liver results in a full-length protein copy as apoB$_{100}$, then a truncated apoB$_{48}$ is synthesized in intestine due to mRNA deamination and stop-codon appearance [42]. This mechanism is thought to abort the lipoprotein binding to LDL receptor and to induce alternate catabolic pathways [43]. After secretion into plasma, chylomicrons acquire apoE and apoC from HDL and are transformed by LPL into remnants with shuttling of low-molecular-weight apolipoproteins and phospholipids back to HDL. Remnants are bound and degraded by hepatocytes via apoE-directed interaction with LRP [44]. The impairment of metabolism of TG-rich lipoproteins in patients with coronary heart disease is likely to be related to HDL and LDL metabolism [43]. Endogenous TG are synthesized by liver and secreted already with apoB, apoE, and apoC. Large VLDL (S_f 60-400) is believed not to interact with LDL receptor, while smaller VLDL particles (S_f 20-60) from normolipidemics do, however, without any appreciable lipid accumulation in macrophages [16]. Conversely, chylomicrons and large hypertriglyceridemic VLDL bound to monocyte-macrophage receptors [45] with subsequent lipid accumulation contribute to the "direct" pro-atherogenic action of TG-rich lipoproteins [43]. Only LPL activity is thought to be responsible for a first step in the lipolytic cascade VLDL $\rightarrow$ IDL $\rightarrow$ HDL, while both LPL and hepatic lipase activities are involved in a second step [46]; apoE may be necessary for the efficient transformation of VLDL remnants (IDL) to LDL particles [47, 48]. Besides, hepatic lipase participates in transformation of HDL$_2$, generated from HDL$_3$ by LCAT activity, into HDL$_3$ [46]. The involvement of hepatic lipase in IDL and HDL metabolism is supported by the accumulation of large TG-rich HDL particles on enzyme activity deficiency [46] due to TG transfer from VLDL to HDL by cholesteryl ester transfer protein that performs equimolar TG/cholesteryl ester exchange [49]. HDL$_2$ with high cholesteryl ester content are accumulated in TG-rich lipoprotein deficiency (abetalipoproteinemia) [50] or low cholesteryl ester transfer protein activity [51], but the causal relationship remains to be solved [49]. In contrast, increased cholesteryl ester transfer protein activity in hypertriglyceridemia [52] resulted in cholesteryl ester accumulation in VLDL (β-VLDL) with subsequent lipid accumulation in macrophages; HDL content decreased

simultaneously due to the increased clearance that occurred with increased TG hydrolysis. This contributes to "indirect" pro-atherogenic action of TG-rich lipoproteins [43]. Chung et al. [53] have observed cholesteryl ester formation on incubation of hypertriglyceridemic plasma *in vitro* due to endogenous LCAT activity together with cholesteryl ester increase and TG decrease in VLDL and LDL enrichment by TG. After LPL addition, the formation of small dense LDL occurred concomitantly with a small shift of VLDL particles to IDL/LDL fraction. In post-lipolysis plasma VLDL remnants with β- and preβ-mobility contained both of $apoB_{100}$ and apoE but not of apoC. Inhibition of LCAT prevented these changes. Also, the changes were smaller for normolipidemic plasma samples and became more evident on VLDL addition or in post-prandial samples. β-VLDLs are thought to originate on heparin-induced lipolysis of plasma of patients with hypertriglyceridemia type IV or V, though they are absent before lipolysis [54], or on hepatic lipase deficiency [55].

RELATIONSHIP BETWEEN apoE STRUCTURE AND LIPOPROTEIN METABOLISM

Both gene and protein structure will be considered. The apoE gene is localized within chromosome 19 [56] in close proximity to apoC-I and apoC-II and more distantly to LDL receptor genes [57]. The apoE gene consists of multiple repeats of 66 bp, each coding a 22-mer α-helix domain. The similarity of intron-exon structure together with amplified elements points to a common origin of genes of different apolipoproteins except for the apoB gene [56, 58]. ApoE is synthesized by many tissues and organs (liver, brain, adrenals, kidney, macrophages) and its expression is regulated on transcriptional [59], translational, and post-translational [60] levels. In liver where apolipoprotein synthesis is maximal, hepatic control regions that are located 15 and 27 kb downstream from the apoE gene determine the specificity of the expression [61]. Taylor and colleagues [62] identified two enhancers from the human apoE gene, termed multienhancer 1 and multienhancer 2 that direct macrophage- and adipose-specific expression in transgenic mice. In non-synthesizing cells, the apolipoprotein expression is inhibited by several tissue-specific and nonspecific repeats [59]. Cholesterol induced apoE mRNA and gene transcription in mice [63] and humans [64]; however, LDL did not change the apoE mRNA level in HepG2 cells [65]. The expression of apolipoprotein mRNA was suggested to depend on differentiation state of human macrophages [66]. The apoE gene is negatively regulated by cytokines through the protein kinase C pathway [67] and positively by peroxisome-proliferator-activated γ-receptor [68] and by activated liver X receptor in murine and human macrophages [69].

After cleavage of an 18-mer hydrophobic signal peptide, the complete apoE sequence consists of 299 residues with 34 kD molecular mass [70]. Two polymorphism types are thought to belong to apoE. First, three alleles ε2, ε3, and ε4 exist inside one gene locus that correspond on a protein level to three major, i.e., E2, E3, E4, and several minor isoforms [71, 72]. Three homo- and three heterozygote phenotypes exist, ε3 allele and E3/3 phenotype being the most frequent. Second, a variable number of sialic acid residues interacting with Thr194 contribute to post-translational modification [73] and to the appearance of minor apoE isoforms that could be disrupted by neuraminidase treatment [72]. Glycosylation seemed not to interfere with apoE secretion by cells [73]. ApoE acetylation and sulfation may occur also, the latter being correlated with prominent increase in apoE synthesis in rats after periphery denervating [74]. ApoE is able to interact with proteoglycans, in particular with heparin. Two heparin-binding regions have been identified and one of them (142-147) coincided with the receptor-binding domain [75, 76]. However, in contrast to receptor binding, apoE interacts with heparin through this region in a lipid-independent manner [76]. The common property of heparin-binding proteins is thought to be a high surface density of positive charges, specifically lysine [77] and arginine [78] residues within helical regions. The involvement of heparin-binding region in the development of type III dyslipidemia, in particular with dominant expression, has been suggested [78]. Two independent N- and C-domains were proved to exist in the apoE molecule and apoE in solution associated via C-domain as tetramer [79, 80] with concomitant increase in the stability of apolipoprotein structure [81]. We suggested the existence of various apoE structures in solution: oligomer (in aged preparations) $\leftrightarrows$ "closed" tetramer $\leftrightarrows$ "open" tetramer ("molten globule" state) $\leftrightarrows$ native or partially denatured monomer $\leftrightarrows$ fully denatured monomer [82]. Five amphipathic helical regions 60-78, 130-150, 203-221, 226-243, and 245-266 are thought to be responsible for this association [70]; two regions, 129-169 [83] and 263-286 [84], have been experimentally observed. The number and structure of these domains determine both the lipid-binding affinity and self-association potency of apoE and other amphipathic apolipoproteins A and C [13, 70, 85-87].

The region of 140-160 residues responsible for LDL receptor binding has been localized [11]. Only lipid-bound apoE could interact with receptor [88]; the affinity constant values for interaction of apoE3–phospholipid complexes with LDL receptor varied with complex dimension as $(0.6\text{-}3.9)\cdot 10^8$ M^{-1} [40]. Various defects in amino acid sequence, in particular, arginine–cysteine interchanges within and close to the receptor-binding region, can result in type III dyslipidemia [89]. ApoE3 differs from E4 and apoE2 from apoE3 by arginine-for-cysteine substitutions in positions 112 and 158, respectively, the latter being associated with prominent (≈1%

relative to E3) affinity decrease to LDL receptor [90]. ApoE–LDL receptor interactions are thought to be stabilized by the 171-183 region [91]. The development of type III dyslipidemia is connected with replacement of positively charged arginine residues by negatively charged cysteine residues in the receptor-binding region in E2/2 homozygotes or in E3 heterozygote with one normal ε3 allele and double mutation in another, Cys112→Arg and Arg142→Cys [92]. In contrast, Arg228→Cys change with charge modification and E2 isoform appearance did not interfere with the receptor-binding activity [93].

A suggestion about the possible discovery in the future of additional apoE isoforms with reciprocal Arg–Cys substitutions seems to be quite reasonable based on an unusually high frequency coding of arginine residues in humans by the CGX sequence [94], the cytosine of which easily transforms to thymine with subsequent change for cysteine coding. Arg→Cys substitutions for three of five arginine residues in the 140-160 region have been described [11]. With E2/2 phenotype frequency of 1% in the general population, type III dyslipidemic pattern contributes not more than 2-10% to this phenotype [10, 95], i.e., this disorder may be attributed to multifactorial disease [95]. In the population, cholesterol content in relation to apolipoprotein isoform increased in the order E2 < E3 < E4, i.e., apoE2 was associated with the lowest Ch level in the absence of disease [10]. The remnants of TG-rich particles are thought to accumulate, together with LDL content reduction, in E2/2 normolipidemics; if some metabolic disturbances have been added then type III dyslipidemia developed and, in contrast, it disappeared if these disturbances are corrected, the remnants still having persisted [10, 96]. Moreover, apoE from normo-, hypo-, and hypercholesterolemics with E2/2 phenotype has been characterized by equally diminished affinity to LDL receptor [97].

Three questions remain to be answered. First, if definite location of mutation in amino acid sequence can determine the rate of lipoprotein catabolism. Markedly increased content of mutant isoform compared to the normal one has been observed [98, 99]; however, the content of both isoforms did differ in the majority of E3/2 heterozygotes [100] and in patients with double substitution Cys112→Arg, Arg142→Cys [92]. Moreover, the latter observation has been revised in favor of increased content of mutant protein in VLDL [78]. Second, if only a homozygous state is a characteristic feature of type III hyperlipidemia. Besides E2/2 patients [11], some heterozygotes with different mutations have been described as well [92, 101]. Dominant expression of type III dyslipidemia both in the case of double substitution Cys112→Arg, Arg142→Cys and apoE3-Leiden (with Arg112 and insertion of seven additional residues) is thought to be associated with the accumulation of mutant protein in VLDL particles [78]. Third, if apoE isoform and additional serum factors are responsible for impaired conversion of VLDL remnants to LDL by hepatic lipase in type III dyslipidemia. ApoE3 but not apoE2 is efficient in the transformation of β-VLDL to LDL by concerted action of LPL and hepatic lipase [47]. However, apoE2 activates the hydrolysis of phospholipid monolayers by hepatic lipase more efficiently compared to apoE4 [102]. The suggested apoE–hepatic lipase interaction [102] seems not to be sufficient for efficient remnant lipolysis.

ApoE CONTRIBUTION TO RECEPTOR-MEDIATED CLEARANCE OF TG-RICH LIPOPROTEINS

Structural Heterogeneity of Lipoprotein Receptors

Four families of lipoprotein receptors can be identified based on cDNA cloning of some representatives: 1) LDL receptor family [103]; 2) scavenger receptor (SR) family [104, 105]; 3) receptor for hypertriglyceridemic VLDL and β-VLDL ($apoB_{48}$-R) [17]; 4) lipolysis-stimulated receptor [106].

LDL receptor family. The LDL receptor family is composed of a class of single transmembrane glycoproteins that bind and internalize extracellular ligands for degradation by lysosomes and includes: LDL receptor (LDL-R), LDL receptor-related protein (LRP), VLDL receptor (VLDL-R), apoE receptor 2 (apoE-R2), vitellogenin receptor, *C. elegans* receptor, gp330/megalin. The LRP subfamily consists of LRP/α-macroglobulin receptor (LRP1) [107], megalin as LRP2 [108], LR3 [109], LRP4 [110], LRP5 [111], LRP6 [112], VLDL receptor/LR8 [113], LRP9 [114], LRP11 [115], and LRP12 [116]. Multidomain structure is a common feature of the LDL receptor family (Table 1). Inside the family, the receptors differ by repeat number in different domains, by C-ended sequence, and by number and localization of cysteine residues in spacers between domains [119]. In the LDL receptor molecule there are five domains that can be deleted without altering the function of the other domains: 1) an NH_2-terminal ligand-binding domain composed of seven imperfect repeats each approximately 40 amino acids long and containing three disulfide bonds (LDL-A module [103]). Each module is thought to fold independently, and different modules or combinations of modules interact with various ligands to determine receptor specificity [103]. The carboxyl terminus of each LDL-A module contains a cluster of negatively charged amino acids required for receptor function and postulated to bind to positively charged residues in lipoprotein at least in part via ionic interactions [103]. The repeats are shared with proteins of the complement system; 2) a domain of 400 amino acids that is shared with the precursor for epidermal growth factor (EGF) and that functions to modulate LDL binding and to permit receptor recycling; 3) a

Table 1. LDL receptor family

Receptor	Domain structure	Homology	Tissue expression	Function
Human LDL receptor	7A-2B.2-S-1B.2-1O-1T-1C [117-119]	13 of 18 exons to C9 component of complement; repeats of EGF precursor, factors IX and X, protein C; non-repeats of EGF precursor [120]	wide variety of tissues [117]	provide mammalian cells with cholesterol [117]
Human LRP	2A-2B.2-S-1B.2-S-1B.2-8A-2B.2-S-1B.2-S-1B.2-S-1B.2-S-1B.2-10A-2B.2-S-1B.2-11A-2B.2-S-1B.2-6B.1-1T-1C (no O-domain) [107]	to LDL receptor and EGF precursor [107]	liver, lung, brain [107]	endocytosis of chylomicron remnants and protease–inhibitor complexes [107]
Human VLDL receptor	8A-2B.2-S-1B.2-1O-1T-1C [121]	53-56% to human LDL receptor [121]	blood vessels, heart, skeletal muscle, adipose tissue [122]	delivery of fatty acids to peripheral tissues [122]
Human apoE receptor 2	7A-2B.2-S-1B.2-1O-1T-1C with insertion [123]	27-62% to human LDL receptor [123]	humans (brain and placenta); rabbit (brain and testis); rat (brain, cerebellar cortex) [123]	uptake of apoE-HDL in the central nervous system [123]
Drosophila vitellogenin R	5A-2B.2-S-1B.2-S-1B.2-8A-2B.2-S-1B.2-1T-1C (no NPXY) [124]	38% to 8A domain of chicken vitellogenin receptor [124]	rat oocyte [124]	uptake of vitellogenin [124]
Chicken vitellogenin R	8A-2B-S-1B-1T-1C (no O-domain) [125]	84% to ligand-binding domain of rabbit VLDL receptor [125]	ovary [125]	deposition of the yolk mass components of chicken oocytes—VLDL and vitellogenin [125]
C. elegans R	6A-2B.2-S-1B.2-S-1B.2-8A-2B.2-S-1B.2-S-1B.2-S-1B.2-10A-2B.2-S-1B.2-11A-2B.2-S-1B.2-1B.1-1T-1C [118]	26% to human LRP over long stretches [118]	ovarian cells [118]	cell migration, outgrowth of axons, development of organs that involve reorganization of cells, programmed cell death [118]
Rat gp330/megalin	7A-2B.2-S-1B.2-S-1B.2-8A-2B.2-S-1B.2-S-1B.2-S-1B.2-S-1B.2-10A-2B.2-S-1B.2-11A-2B.2-S-1B.2-1B.1-1T-1C [119]	30-50% to *C. elegans* protein and 35-51% to human LRP [119]	epithelial cells of the kidney glomerulus and proximal tubule, lung, epididymis and yolk sack [119]	binding of multiple ligands, including β-VLDL, and RAP, major antigen for rat Heymann nephritis [119]

Note: A, a motif in N-terminal, imperfect, cysteine-rich ligand-binding domain; B.1 and B.2, different six-cysteine motifs in epidermal growth factor (EGF) precursor homology domain [107]; C, cytoplasmic domain with FDNPVY sequence required for internalization of the receptor [126]; O, O-linked sugar domain; S, "spacer" with YWXD sequence; T, transmembrane domain. The number of repeats in each domain is indicated. The LRP, *Drosophila* vitellogenin receptor, *C. elegans* receptor, and gp330/megalin are fragmented to illustrate the similar modular arrangement of motifs among the eight proteins. RAP, receptor-associated protein.

domain of 48 amino acids which is serine- and threonine-rich and contains multiple O-linked sugar chains; 4) a membrane-spanning domain of 22 amino acids; and 5) a 50 amino acid cytoplasmic domain.

LRP is a multidomain receptor with a multitude of functions that is present in liver, placenta, lung, and brain [107, 127]. LRP is known to function as an endocytic receptor for more than 25 known different ligands, including α_2M–proteinase complexes, lipoprotein-containing particles, and complexes between plasminogen activators and plasminogen activator inhibitor-1. Initially a high molecular mass protein (600 kD) has been suggested to be involved in remnant clearance [107]. Then the existence of LRP functioning as a specific apoE receptor has been suggested [44, 128-130]. The apoE-dependent interaction of β-VLDL with LRP is controlled by a receptor-associated protein with molecular mass of 39 kD [131] that interacted with LRP with high affinity [132]: the β-VLDL binding to fibroblasts decreased prominently on receptor-associated protein (RAP) hyperexpression [133]. RAP probably acts as chaperon to LRP [134].

Scavenger receptor family. The structures of two types of bovine macrophage scavenger receptor, SR-AI and SR-AII, have been deduced from cDNA sequences [104, 105]. The sequence of the type I cDNA predicts a 453-amino acid protein with the following domains: I, N-terminal cytoplasmic, 1-50; II, transmembrane, 51-76; III, spacer, 77-150; IV, α-helical coiled-coil (subdomain IVa, 151-210; subdomain IVb, 211-271); V, collagenous, 272-343; and VI, C-terminal (hinge subdomain VIa, 344-351; SR cysteine-rich (SRCR) subdomain VIb, 352-453). SR-AII is identical to SR-AI except that the 110-amino acid C-terminal hinge/SRCR domain is replaced by a 6-amino acid C-terminus [135]. Three glycosylated monomers of 453 (SR-AI) or 349 (SR-AII) amino acid residues each associate as trimers, the extracellular part of which formed by left-handed triple helix is connected to right-handed triple collagen-like helix [135]. Type A scavenger receptors bind acetyl-LDL. Type B scavenger receptors include BI, BII, CD36, and croquemort [136]; c-DNA-predicted protein sequences of hamster [137] and human [138] SR-BI are 509- and 409-amino acid, respectively, and show 20-30% homology with that of CD36 family of membrane proteins. Both sequences include two internal transmembrane domains and a series of conserved cysteines and putative N-linked glycosylation sites. SR-B is characterized by broad ligand specificity, i.e., HDL, LDL, VLDL, negatively charged phospholipids, and apoptotic cells [136]. Of note, native LDL, which does not bind to either class A receptors or CD36, binds with high affinity to SR-BI [137]. This receptor is probably involved both in HDL interaction with hepatocytes, the late phase of reverse cholesterol transport [139], and in cholesterol elimination from macrophages [140].

The expression of SR-BI in liver, adrenals, macrophages, and adipocytes has been observed [140]. CD36 is expressed in monocyte/macrophages, thrombocytes, adipocytes, epithelial cells of mammary glands, and endothelial and liver sinusoidal cells [141]. CD36 and SR-BI expression is regulated during cell development and differentiation [137, 138]. Several types of scavenger receptor that are involved in atherogenesis have been described: MARCO [142], macrosialin [143], FcgRII-B2 [144], SRB-I [137], CD36 [145], CD68 (SR-D class) [146], LOX-1 (SR-E class) [147], SREC (SR-F class) [148], and PSOX (SR-G class) [146].

$ApoB_{48}$ receptor. The human $apoB_{48}$-R cDNA (3,744 bp) encodes a protein with no known homologs [17]. Its mRNA is expressed primarily by reticuloendothelial cells: monocytes, macrophages, and endothelial cells. Functionally significant conserved domains include an N-terminal hydrophobic domain, a glycosaminoglycan attachment site, an N-glycosylation site, and an ExxxLL internalization motif C-terminal to the putative internal transmembrane domain. Two conserved coiled-coil domains are probably involved in the spontaneous homodimerization that generates the active dimeric ligand binding species (mouse, approximately 190 kD; human, approximately 200 kD) [149]. Cellular sterol levels and state of differentiation do not affect $apoB_{48}$-R expression that distinguish the $apoB_{48}$-R from the LDL-R and the scavenger receptor families; $apoB_{48}$, rather than apoE being a ligand in an interaction of TG-rich particles with this receptor [17].

Lipolysis-stimulated receptor. The mRNA of lipolysis-stimulated receptor originates from a single gene and codes for α and β subunits due to alternate splicing [106]. The cDNA-predicted protein sequence showed no homology with that of LDL receptor or any of its related proteins. The different putative domains of the cDNA are compatible with a function as plasma membrane receptor: a single cluster of hydrophobic residues of a length sufficient to constitute a potential transmembrane-spanning domain and several putative intracellular routing motifs are present. Among those are N-terminal phosphorylation site that corresponds to a clathrin binding site and a dileucine-lysosomal targeting signal. A single copy of cysteine-rich motif is found near the transmembrane-spanning domain. Distal to this motif is a cluster of alternatively positively and negatively charged residues that provide a potential apolipoprotein-binding site. The receptor is a heterotrimer or tetramer consisting of one 68 kD (α) and two-three 56 kD (β) subunits associated through disulfide bridges; the β subunit lacks the putative lysosomal targeting signals, the transmembrane-spanning domain, and the cysteine-rich domain, but contains the putative free fatty acid- and ligand binding domains. The lipolysis-stimulated receptor affinity is highest for the TG-rich lipoproteins and low for β-VLDL, apoE being as a ligand. In contrast to LRP, the LRP-associated protein,

Table 2. Ligand properties of apoE and apoB in the binding of atherogenic lipoproteins to cell receptors

Lipoprotein	Apolipoprotein as a ligand to specific receptor					
	LDL-R	LRP	VLDL-R	SR	$apoB_{48}$-R	LSR
Chylomicron remnants	apoE	apoE	n.d.	n.b.	$apoB_{48}$	apoE
Large n-VLDL	n.b.	n.b.	apoE	n.b.	n.b.	apoE
Large HTG-VLDL	apoE	apoE	n.d.	n.b.	$apoB_{100}$, $apoB_{48}$	n.d.
β-VLDL	apoE	apoE	apoE	n.b.	$apoB_{100}$, $apoB_{48}$	n.b.
LDL	$apoB_{100}$	n.b.	n.b.	$apoB^{*}_{100}$	n.b.	$apoB_{100}$

Note: $apoB^{*}_{100}$, $apoB_{100}$ with increased negative charge; $apoB_{48}$-R, $apoB_{48}$ receptor; HTG-VLDL, VLDL from hypertriglyceridemics; LDL-R, LDL receptor; LRP, LDL receptor-related protein; LSR, lipolysis-stimulated receptor; n.b., negligible binding; n.d., not determined; n-VLDL, VLDL from normolipidemics; SR, scavenger receptor; VLDL-R, VLDL receptor.

RAP, which inhibits LRP, had no effect on the lipolysis-stimulated receptor, and binding of lipoproteins to lipolysis-stimulated receptor was independent of Ca^{2+}. Lipolysis-stimulated receptor is expressed mainly in liver [106]. Ligand properties of apolipoproteins on lipoprotein binding to receptors of different families are summarized in Table 2.

Interaction of TG-Rich Particles with LDL Receptor

Lipid accumulation in macrophages is likely to be a key factor in atherogenesis, but modulation of the lipoprotein interaction with hepatocyte and extrahepatic cells results in the change in stationary concentration of lipoprotein particle and its structure [150]. The uptake of cholesterol within apoB- and/or apoE-containing particle by the cell is controlled through a feedback mechanism via the expression of LDL receptor molecules sensitive to intracellular Ch content [150]. Unusual LDL receptor with low and high affinities to apoB and apoE, respectively, and insensitive to cellular Ch content is thought to be contained in mouse peritoneal macrophages [151]. LDL receptor as suggested by Demant et al. [96] is involved in metabolism of smaller VLDL (S_f 20-60), IDL, and LDL. The positive feedback response of the LDL receptor expression with the concomitant decrease in LDL production rate thought to be responsible for the decreased LDL content at E2 phenotype and, vice versa, the increased LDL production may result in the decreased LDL receptor expression on the cell surface and increased LDL content at E4 as an example of negative feedback response [10]. However, increase in the rate of LDL catabolism has not been observed in normolipidemics with E2 phenotype and the decrease in LDL content has been postulated to originate from increased IDL direct catabolism [96]. Lipoprotein–receptor interaction is Ca^{2+}-sensitive [150]. The regions of LDL receptor molecule responsible for the interaction with apoE and apoB may not coincide as some mutations in LDL receptor gene destroyed LDL binding, β-VLDL binding still being unaffected [152].

Seventy five percent of normolipidemic VLDL are small particles that resemble IDLs in structure and receptor binding via apoB [153]; they are catabolized directly by liver without conversion into LDL [154]. The majority of hypertriglyceridemic VLDL are larger particles that are catabolized via LDL receptor [154]; more apoE, $apoB_{48}$, and $apoB_{100}$ are accumulated in these particles compared to normolipidemic ones [153]. These additional apoE molecules are thought to possess an exogenous origin due to the increased residence time of hypertriglyceridemic VLDL in plasma and concomitant increased apolipoprotein transfer from other lipoproteins [16]. However, apoE in normolipidemic VLDL resides from particle synthesis and exists in conformation different from that in hypertriglyceridemic VLDL [16]. The latter is thought to induce apolipoprotein interaction with LDL receptor and availability of apoE to proteolytic attack by thrombin [153].

Which factors determine so different apolipoprotein behavior in the binding of normo- and hypertriglyceridemic VLDL to LDL receptor? One of the key factors is thought to be different masking from aqueous phase (shielding) of apoE by apoC molecules in these particles. This shielding has been observed experimentally [45, 155]; moreover, the cluster organization of amphipathic apolipoprotein molecules on the VLDL surface has been suggested by us [156]. Two steps on binding of apoB-containing LDL to LDL receptor have been assumed [157]: 1) a fast apoB interaction with receptor molecule; 2) a slow isomerization of apolipoprotein–receptor complex that resulted in higher affinity. This model well described both the absence of equilibrium conditions even at high incubation times and heterogeneous complex dissociation. The second step was absent in the interaction of B,E-containing lipoproteins with the receptor, and apoE–receptor complex was thought not to isomerize.

However, heterogeneous dissociation observed for this complex [157] that cannot be explained by the isomerization model led us to suggest the equilibrium between monomer and self-associated forms of apoE on the surface of TG-rich particles. These apolipoprotein forms probably interact differently with receptor molecules. This suggestion seems to reasonably explain heterogeneous dissociation of apoE–receptor complex [157], lipolysis-induced "activation" of apoE molecules at receptor binding [158] as a result of cluster solubilization by free fatty acids, and cholesterol influence on chylomicron binding to LDL receptor [159] due to the shift of monomer–cluster equilibrium. In turn, this suggestion is related indirectly with the "multi-center" interaction of one particle with several receptor molecules suggested earlier for HDL [160] and VLDL [45, 161, 162]. Self-association of apoE within VLDL particles that increased in hypertriglyceridemia has been shown by us recently [163]; apoE as monomer is thought to interact with phospholipid [164]; however, secondary self-association in lipid may occur [82]. Heterogeneous dissociation of apoA-I, apoA-II, and apoC-III-1 from the surface of model lipoprotein particle [87], the existence of pools of apoC-III [165] and apoE [166] that began to be involved in VLDL/HDL exchange only on lipolysis of TG-rich lipoproteins is likely to originate also from the self-association of amphipathic apolipoproteins. Besides apoE self-association, the concentration-dependent influence of apoC molecules associated with VLDL and IDL fractions on apoE topography and/or conformation may result in the inhibition of binding of these lipoproteins to fibroblast LDL receptor [167].

ApoE in TG-Rich Lipoproteins and LDL Receptor-Related Protein

The LDL receptor-related protein binds apoE but not apoB [44] and is not sensitive to cellular sterol content [168]. There is no common opinion about the influence of Ca^{2+} on lipoprotein–LRP interaction [44, 127, 169, 170]. The interaction of chylomicrons with LRP thought to be influenced by apoE phenotype and E2 phenotype was associated with lower rate of chylomicron clearance [96]. Moreover, apoE2 in β-VLDL interacted with LRP much more efficiently (40% affinity compared to apoE3 or apoE4) compared to binding to LDL receptor (1-2% affinity compared to apoE3) [128]. The existence of LRP in liver has been suggested based on a rapid clearance of chylomicron remnants despite defective LDL receptor in patients with familial homozygous hypercholesterolemia [171] or in Watanabe-heritable rabbits [162]. By analogy with LDL receptor, the reversible binding of apoC to newly secreted chylomicrons and VLDL particles resulted in their diminished clearance by liver, and vice versa, apoC dissociation on LPL-induced remnant formation resulted in increased clearance; apoC were not dissociated and clearance of small remnants by rat liver was not stimulated on apoE addition in the absence of lipolytic degradation [155]. Also, apoE/apoC ratio is thought not to be important in remnant uptake by liver [172]. However, the direct apoE–apoC interaction with the shielding of receptor-binding region of apoE appeared to be involved in uptake of TG-rich lipoproteins and their remnants by perfused rat liver [155]. Also, apoC inhibition of apoE-dependent binding of β-VLDL to LRP was confirmed later [128, 173].

The existence of separate pathway for remnants of TG-rich lipoproteins, however, is still far from definite conclusion; in particular, Bowler et al. [174] suggested the responsibility of LDL receptor for remnant clearance both in hetero- and homozygous Watanabe-heritable rabbits. ApoE-enriched VLDL have interacted with EDTA-resistant (LRP?) and EDTA-sensitive (LDL receptor) binding sites in endosomes of estradiol-treated rats [170], that, according to the authors' opinion, confirmed their earlier hypothesis on apoE involvement in VLDL clearance by LRP and mutant LDL receptor [175]. The specific binding of β-VLDL to rat and human parenchymous cells, independent of LDL receptor and Ca^{2+}, has been observed while LDL receptor is thought to be responsible for β-VLDL binding to HepG2 cells and macrophages [176]. Besides, the suggestion about delayed chylomicron clearance in homozygous Watanabe rabbits [174] is likely to be inappropriate due to a high cholesteryl ester transfer protein activity in rabbits [177]. This protein transfers labeled TG and/or cholesteryl ester molecules used in clearance study to endogenous lipoproteins that could artificially decrease clearance rate [177]. The LRP and LDL receptor probably represent major and minor pathways, respectively, for chylomicron catabolism [128, 159, 176] due to a diminished expression of LDL receptor molecules on Ch-rich diet and LRP-dependent outflow of additional cholesterol.

Significance of VLDL Receptor in Metabolism of TG-Rich Lipoproteins

Takahashi et al. [178] isolated cDNA with the expression of VLDL receptor from cDNA library from rabbit heart, high homology between VLDL receptor cDNA and the LDL receptor gene being identified. The binding of VLDL, β-VLDL, and IDL rather than LDL was induced after cDNA injection into hamster ovary cells defective in LDL receptor. The involvement of this VLDL receptor in uptake of apoE-containing lipoproteins by extrahepatic cells, i.e., in heart, muscle, and adipose tissue with a high expression of receptor mRNA has been suggested [113, 178]. The expression of VLDL receptor in macrophage culture from atherosclerotic plaques in humans and rabbits has been found also [179].

The involvement of VLDL receptor in LPL activity regulation *in vivo* has been suggested based on development of hypertriglyceridemia with concomitant decrease in enzyme activity in mice with knockout VLDL receptor gene [180]. VLDL receptor expression is not down regulated by lipoproteins [113].

Metabolism of TG-Rich Lipoproteins through Scavenger Receptors

The induction of increased negative charge in LDL with Lys modification is thought to occur on oxidation of lipoprotein phospholipid [181] and to induce toxicity of oxidized LDL *in vitro* [182] and foam cell formation *in vitro* [183] due to the absence of feedback regulation of receptor expression by intracellular sterol content [184]. Kunjathoor et al. [146] suggested that only SR-A and CD36 are the critical contributors to modified lipoprotein uptake in macrophages *in vitro* while recently identified scavenger receptors, including CD68, LOX-1, SR-PSOX, and SREC, play a minor role, if any, in modified LDL uptake by macrophages. Herijgers et al. [185] described LDL receptor as a major pathway for β-VLDL uptake by mouse macrophages with normal and knockout LDL receptor gene: if the LDL receptor would be defective, β-VLDL could be taken up by SR-B rather than LRP. So, LDL receptor probably participates in atherogenesis. These authors have suggested a new function for SR-B, i.e., cholesteryl ester uptake from remnant particles together with cholesterol outflow from the cell; the relative efficiency of these two processes can depend on local content of HDL and remnants.

Scavenger receptor is likely to compete with structurally similar collagen molecules for oxidized LDL [186]. However, as suggested by Brown and Goldstein [186], scavenger receptors could promote additional growth of atherosclerotic plaque due to the induction of secretion of cytokines and growth factors after uptake of oxidized LDL by macrophages. The role of the heparin-binding region of apoE with the excess positive charge may be quite intriguing as competition between apoE, collagen, and scavenger receptor for oxidized LDL may occur on the macrophage surface, i.e., direct apoE–apoB interaction. Our data [1] on extracellular co-localization of apoB and apoE in central zone of atheromatous plaque in thoracic aorta indirectly support this suggestion. This could constitute a direct effect of apoE on scavenger receptor occupation; a more subtle effect may be realized through the expression of scavenger expression at macrophage differentiation and/or activation [187] as apoE seems to possess immunomodulatory properties such as mitogenic lymphocyte stimulation [11]. Stimulated lymphocytes did release lymphokines and γ-interferon, and these agents were able to activate macrophages [188].

$ApoB_{48}$ Receptor in Metabolism of TG-Rich Lipoproteins

The necessity that macrophage $apoB_{48}$-R had to be introduced followed from the fact that although hypertriglyceridemic VLDL with S_f 100-400 bound to LDL receptor [189, 190], neither chylomicrons [191] nor thrombin-treated hypertriglyceridemic $VLDL_1$ [189, 190] interacted with LDL receptor although all three particle types were efficiently taken up by macrophages. Proteolysis of VLDL by trypsin resulted in the increase in their binding by mice $P388D_1$ macrophages [192] and $apoB_{100}$ fragment rather than apoE interacting with receptor molecule [16]. $ApoB_{48}$ receptor determined the efficient uptake of $apoB_{48}$-containing chylomicrons and β-VLDL by human and murine macrophages and $apoB_{48}$-R-transfected Chinese hamster ovary cells [17, 149]. It can be suggested that the ratio of LRP activity in liver to activity of $apoB_{48}$-R in macrophage on Ch-rich diet with suppressed LDL receptor may determine the rate of lipid accumulation in macrophages.

Lipolysis-Stimulated Receptor and Metabolism of TG-Rich Lipoproteins

Lipolysis-stimulated receptor has been described by Yen et al. [193]: the receptor stimulated by oleate and expressed in liver and LDL receptor-deficient fibroblasts interacted with VLDL, chylomicrons, and LDL. The involvement of lipolysis-stimulated receptor as a rate-determining step both in clearance of TG-rich lipoproteins and in distribution of the latter between liver and periphery tissues has been suggested [106].

ROLE OF ApoE IN THE DEVELOPMENT OF HYPERTRIGLYCERIDEMIA

Lipolysis and ApoE

The existence of a VLDL population resistant to lipolysis by LPL in type III dyslipidemia [48] and low lipolysis efficiency of VLDL from E2 homozygotes by LPL or hepatic lipase [194] have been suggested; however, lipolysis could still be activated by addition of LPL to plasma of homozygous patients with type III dyslipidemia with classic Arg158→Lys substitution but has been diminished together with low cholesteryl ester transfer activity in E2 heterozygote with Lys146→Gln substitution [195]. Gibson and Brown [196] have evidenced the decrease in apoE content in TG-rich LPs, small changes within IDL, and incoincident changes within HDL on stimulation or addition of LPL. However, stimulation of hepatic lipase activity in the absence of LPL resulted in the decrease in apoE content in IDL and in increase in HDL, i.e., hepatic lipase specifically attacked apoE-containing IDL.

The influences of LPL and hepatic lipase on lipoprotein metabolism are mediated by both enzyme (with subsequent apoE activation) and ligand activities in receptor binding. The interaction of lipoprotein with cell surface seems not to be receptor-dependent; however, subsequent endocytosis and degradation is. The direct interaction of LPL with all the LDL receptor family has been suggested [197]; hepatic lipase proved to interact with LRP [198] and to promote VLDL–LDL receptor interaction independently from enzyme activity. The presence of apoE in sufficient quantities is thought to play a key role in the interaction of TG-rich lipoproteins with LDL receptor or LRP besides promotion of the interaction by LPL and hepatic lipase [199]; however, apoE can inhibit the interaction of VLDL with heparan sulfate proteoglycans that in turn is important in subsequent lipolysis by LPL [200], i.e., apoE can influence VLDL binding to heparan sulfate proteoglycan and receptor in a reciprocal manner. The following reaction with a cooperative effect can be suggested:

(LPL (hepatic lipase) + TG-rich lipoprotein = = complex) + receptor = internalization,

and the action of apoE may be negative and positive for the first and second reactions, respectively.

The influence of apoE on lipolysis remains to be established, both activation [201] and inhibition [202] having been described. The inhibiting effect was observed in E2 transgenic mice [203] and in E3 transgenic rabbits [204]. However, Ebara et al. [205] suggested the involvement of apoC-III in the inhibition of lipolysis with subsequent development of hypertriglyceridemia, independent from apoE content, in apoC-III transgenic/apoE-knockout mice. The situation with recessive or dominant expression of type III dyslipidemia and the influence of isoform on lipolysis became more complex with the observation of de Man et al. [200]; first, about the same efficiency of lipolysis of VLDL both from homozygotes with classic E2 isoform and from heterozygotes with apoE3-Leiden by enzyme in solution that contradicted [206] and, second, about the diminished efficiency of lipolysis by LPL–heparan sulfate proteoglycan complex due to a decrease in VLDL binding compared to particles with E3 isoform. The binding and lipolysis decrease were practically identical in both cases, which cannot explain the different penetration of two isoforms in type III dyslipidemia development. De Beer et al. [207] observed the inhibition of VLDL lipolysis by E2 isoform with Lys146→Gln substitution with conserved binding to LDL receptor, i.e., the major defect was thought to be connected with the impairment of lipolysis.

ApoE and ApoC

Each of the C apolipoproteins inhibited both the binding of β-VLDL to LRP [128, 173] and the binding of VLDL and IDL to LDL receptor [167]; however, the relative importance of changes remained to be established, the B_{100}/B_{48} ratio, besides E/C ratio, having been suggested to control additionally IDL clearance [208, 209]. ApoC-III is a glycoprotein with 8.8 kD molecular mass and is synthesized in liver and intestine; it remains associated with HDL in normolipidemia and with TG-rich lipoproteins in hypertriglyceridemia [210]. Apolipoprotein content in plasma and TG-rich particles is correlated with plasma TG content [211], and variations in regions flanking the apoC3 gene are associated with hypertriglyceridemia [212]. The decrease in apoC-III content was associated with low plasma TG [213] and vice versa with high plasma TG on apolipoprotein hyperexpression in apoC-III transgenic mice [214]. ApoC-III inhibited both LPL activity *in vitro* [156, 215], the interaction of TG-rich lipoproteins with LPL [216], and their uptake by liver [155] due to the decrease in clearance of TG-rich particles through LDL receptor [217], lipolysis-stimulated receptor [218], heparan sulfate proteoglycan [205], and LRP [128]. ApoC-III inhibited hepatic lipase activity also [219]. The increased apoC-III synthesis has been suggested to be responsible for apolipoprotein accumulation in VLDL, lipolysis inhibition, and the decrease in clearance of $apoB_{100}$ and of whole particle in hypertriglyceridemia and type III dyslipidemia [220]; however, this opinion contradicted the data of Huang et al. [203] about the contribution of apoE in the development of hypertriglyceridemia through displacement of enzyme activator apoC-II [156]. Marcoux et al. [221] suggest the increased apoC-III content in plasma in hypertriglyceridemia is due to apolipoprotein accumulation in TG-rich-lipoproteins originating from apoC-III transfer from HDL and diminished lipolysis. The remnant content in both types of hypertriglyceridemia, i.e., IV and IIb, is increased also; lipolysis index apoC-III(HDL)/apoC-III(apoB-containing LPs) has been inversely correlated with the remnant content, which may be important due to the high atherogeneity of IDL [222, 223] and, vice versa, the diminished efficiency of lipolysis may result in atherogenic phenotype B [224]; so, apoC-III may contribute to remnant accumulation. The apoC-III content in HDL has not been related with plasma TG or remnant level, probably due to the large apoC-III pool that does not participate in the exchange between HDL and TG-rich lipoproteins; the latter suggestion contradicts, however, other data of these authors [220].

Tomiyasu et al. [225] separated both VLDL and IDL particles into apoE-containing and apoE-free and further into "light" and "dense" particles. ApoE-containing particles contained a lot of apoC-III and catabolized slowly; on decrease in apoC-III content the rate of catabolism increased and lipolysis rate of "dense" VLDL without apoE was higher compared to apoE-containing ones, i.e., apoE inhibited the lipolysis. These authors in their next paper [226] suggested the accumulation of apoC-III-

containing VLDL, both with and without apoE, in hypertriglyceridemia and suggested the inhibition of lipolysis either by apoE or apoC-III. Waterworth et al. [227] observed for a Czech population the association of TG content in remnants of TG-rich particles with apoE phenotype, with two polymorphisms of the C3 gene, the first being associated with high TG level while the second one with diminished response to insulin and increased apolipoprotein expression, and with polymorphism of C1 gene responsible for increased apolipoprotein expression; cholesterol content in these particles was correlated with apoE phenotype and C1 genotype that corresponded the influence of apoC-I on the remnant binding to receptors (gene and apolipoprotein are designated by Arabic and Roman numerals, respectively).

Involvement of ApoE in Clearance of TG-Rich Lipoproteins

Weisgraber et al. [228] noted two striking examples of accelerated atherosclerosis development, namely, type III dyslipidemia and familial hypercholesterolemia due to the impairment of remnant clearance and LDL receptor, respectively. Remnant content in obese patients was shown to perhaps not correlate with atherosclerosis progression [229], i.e., remnants may be an TG-independent risk factor; however, according to Marcoux et al. [230], Ch and TG content in remnant-like particles have been strongly positively correlated with fasting plasma TG level in patients with all types of hypertriglyceridemia, i.e., types III, IIb, and IV. The half-time of chylomicron clearance in patients with type III dyslipidemia was shown to be considerably higher compared to normolipidemics and patients with type IV hypertriglyceridemia [231] and the impairment of chylomicron catabolism was not correlated with the size of the TG pool for both types of hypertriglyceridemia. Zhao et al. [232] studied in detail the lipoprotein profile in type III dyslipidemia and observed an increase in Ch content in VLDL and IDL particles, their enrichment by cholesteryl esters, and the decrease in LDL content. Cholesterol content in IDL and LDL was not correlated with plasma Ch level, but strong positive correlation between plasma and $VLDL_1$ or $VLDL_2$ cholesterol levels was observed. The increase in VLDL particles in E2 heterozygotes should be pointed out [233]. Cholesterol and TG content in VLDL fraction and plasma apoE level were both increased in patients with E2 (Lys146→Gln) allele and the existence of this allele largely explained the variation in apoE content and in (VLDL + IDL) – Ch/TG ratio [234]. However, VLDL and IDL particles from the patients with this allele were more TG-enriched compared to E3-Leiden-bearing patients. It is possible that different metabolic defects contribute to dominant expression of two alleles in these carriers of type III dyslipidemia.

Huang et al. [235] showed that hypolipidemia in transgenic mice with low expression of apoE2 could be transformed into type III dyslipidemia in two ways, namely, by $apoB_{100}$ coexpression or apoE2 hyperexpression, i.e., the ε2 recessive effect was visualized. Remnant catabolism only through LRP was suggested. However, the involvement of the other pathways in remnant clearance has been suggested by others, i.e., through LDL receptor in apoE3-Leiden transgenic mice [236] or through both LDL receptor and LRP in mice [237, 238]. The latter suggestion probably reflects the saturation of LDL receptor by lower VLDL concentrations [237] and "secretion–capture" mechanism of remnant uptake suggested for β-VLDL and stimulated by apoE was rejected [239].

Inconsistent results have been obtained for the influence of apoE4 on lipoprotein clearance. The equal [240] or lowered [241] efficiency of the clearance of TG-rich lipoproteins through LDL receptor were described for E4/3 heterozygotes in comparison to E3/3 homozygotes, but increased LDL production through VLDL lipolysis for both groups was postulated in these studies. It should be pointed out that observed clearance impairment (probably due to decreased apoE4 accessibility compared to apoE3) contradicts the hypothesis of Davignon et al. [10] on the increased remnant clearance and LDL receptor suppression in subjects bearing ε4 allele; the preferential apoE4 accumulation in VLDL [242] and more rapid apoE4 catabolism compared to apoE3 [243] was thought not to contradict decreased clearance of apoE4-containing VLDL due to apoE exchange between VLDL and HDL. According to Gerritsen et al. [244], apoE4 expression in human E2-transgenic/mouse E-knockout mice resulted in an increase in TG synthesis in VLDL and VLDL accumulation; however, the expression of truncated E4-202 apolipoprotein without C-domain resulted in decrease in plasma TG and Ch content due to apolipoprotein binding to lipoproteins with subsequent promotion of their clearance. The increase in clearance of large TG-rich particles in normolipidemics was associated also with ε4 allele compared to (ε2 + ε3) alleles [245]. The authors also postulated activation of lipolysis by apoE with subsequent decrease in TG content in apoE-containing VLDL. The opinion of these and other authors [246-248] seems not to be compatible with the known correlation of plasma apoE and plasma TG content in hypertriglyceridemia with normally functioning LDL receptor.

Human apoE expression in apoE-knockout mice resulted in a decrease in plasma cholesterol content and apoE-dependent increase (E3 < E4 < E2) of TG content in plasma and VLDL; TG and apoE content correlated with each other, but TG synthesis rate was not influenced by isoform [249]. Similarly decreased content of VLDL with apoE3 compared with apoE4 was observed by Hopkins et al. [250]. The increased plasma TG content in

apoE4- compared to apoE3-bearing patients should be pointed out, which coincides with the existence of VLDL remnants in ε4 heterozygotes [251] and decreased clearance [241, 243]. These data coincide well with the increased post-prandial TG content among E2/2 and E4/4 phenotypes compared with E3/3 in the general population [252]; HDL-Ch level in men correlated reciprocally, i.e., was the lowest among E2/2 and E4/4 subjects. However, the same plasma lipid levels were observed in transgenic E4/4 mice compared to E3/3 ones [253], but cholesterol, apoE, and apoB_{48} content within VLDL fraction in E4/4 animals was twice that in E3/3 ones. It is interesting that LDL receptor binding of VLDL particles from two lines did not differ but clearance was twofold lower in E4/4 mice; the first result contradicts, while the second coincides with other data [241]. The progression of atherosclerosis on Ch-rich diet was more prominent in E4/4 animals, and a direct link between VLDL clearance and atherosclerosis progression was supposed.

The activating action of apoE on TG synthesis seems to be firmly established; however, the influence of apolipoprotein and its isoforms on the activities of lipolytic enzymes, together with the relative role of LDL receptor and LRP in receptor-mediated remnant clearance remains to be unambiguously described. The ratio between pro- and anti-atherogenic actions of apolipoprotein included in various particles is controlled by many competitive factors. The structural organization of the apoE molecule influences lipoprotein metabolism in least by three different ways: first, by amino acid substitution(s) influencing apoE receptor-binding activity; second, by self-association of apolipoprotein molecules on the particle surface; and finally, by protein–protein and/or protein–lipid interactions that influence the enzyme activities involved in lipoprotein metabolism. If the first way seems to be studied thoroughly, then the study of the second and the third ways is still only at its beginning.

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