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Functional Differences Between ApoC1, ApoC2, and ApoC3

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The human apoCs (ie, apoC1, apoC2, and apoC3) are often portrayed as members of 1 consistent protein family because of their similar distributions among lipoprotein classes, their low molecular weights, and coincident purification. The human apoCs are protein constituents of chylomicrons, VLDL, and HDL. In comparison with the intensely studied apoE, apoB, and apoA1, which play important roles in the development of hyperlipidemia and atherosclerosis, only modest attention has been paid so far to the roles of the apoCs in lipoprotein metabolism. Many of the studies regarding the functional properties of apoCs have been hampered by methodological problems dealing with purification, quantification, and their poorly understood association with hyperlipidemia and other lipoprotein disorders. In the past few years, however, new insights into the metabolic properties of apoCs have been provided, in particular by the technologies of transgenesis and gene targeting in mice.

The present review addresses the influence of apoCs on the major metabolic pathways in lipoprotein metabolism. Therefore, a number of important in vitro and in vivo studies will be discussed that point to a distinct role for each of the individual apoCs in lipoprotein metabolism and human disease.

APOC Genes

The genes coding for human apoC1 and human apoC2 are members of a 48-kb gene cluster on chromosome 19 that also includes the *APOE* and pseudo-*APOC1′* genes.[1–5] It has been reported that the human *APOC1* gene is located either 4.3[2,3] or 5.3[4] kb downstream from the *APOE* gene in the same transcriptional orientation. The *APOC1* gene is ≈4.7 kb and is primarily expressed in the liver, but lower amounts are also found to be expressed in the lung, skin, testes, and spleen (Table 1).[4] One copy of the *APOC1* gene, the so-called pseudo-*APOC1′* gene, is located 7.5 kb downstream from *APOC1*.[1,4] No mRNA products of the pseudo-*APOC1′* gene have been detected in any tissue.[4] *APOC2* spans a region of 3.4 kb and is primarily expressed in the liver and intestine[6–8] (Table 1). An additional gene within the *APOE*/*C1*/*C2* gene cluster, designated the *APOC2*-linked gene, was first discovered in mice.[9] Recently, a similar gene was found in humans.[10] On the basis of its properties and location (555 bp upstream from *APOC2*), this 3.3-kb gene was designated *APOC4*. RNase protection analysis indicated relatively low *APOC4* mRNA levels in the human liver.[10]

The regulation of human *APOC1* gene expression, together with that of the *APOE* gene, is under control of an array of elements found throughout the whole *APOE*/*C1*/*C2*/*C4* gene cluster (for a review, see References 11 and 12). The hepatic control region (HCR), an element located ≈17 kb downstream from the *APOE* gene and ≈9 kb downstream from the *APOC1* gene, was found to regulate the expression of both *APOC1* and *APOE* genes in the liver.[13,14] A second hepatic controlling element within the *APOE*/*C1*/*C2* cluster was identified 27 kb downstream from the *APOE* gene.[15] Recently, it was shown that both HCRs can individually coordinate the hepatic expression of all 4 genes in the *APOE*/*C1*/*C2*/*C4* gene cluster and that the presence of at least 1 of the regions is sufficient for significant liver expression of each of the genes.[16]

The human *APOC3* gene is located in a gene cluster together with the *APOA1* and *APOA4* genes[17] on the long arm of chromosome 11 and is ≈3.1 kb (Table 1).[18–22] The human *APOC3* gene is expressed in the liver and intestine and is controlled by positive and negative regulatory elements that are spread throughout the gene cluster.[23–27] Experiments with transgenic animals have allowed the localization of an element controlling the intestinal expression of *APOC3*, *APOA1*, and *APOA4* in the proximal 5′ human *APOC3* region.[28,29]

Molecular Defects in Human *APOC* Genes and Their Association With Lipoprotein Disorders

Little is known about naturally occurring mutations in the human *APOC1* gene. So far, only 1 study has reported a case of apoC1 deficiency in patients with familial chylomicronemia[30] (Table 2). Because these patients suffered from apoC2 deficiency as well, the chylomicronemia is most likely caused by the apoC2 defect. Remarkably, however, the apoC1/apoC2-deficient patient exhibited markedly decreased levels of cholesterol ester, especially apparent in HDL, which was much more severe than previously reported in cases of apoC2

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TABLE 1. Properties of Human *APOC* Genes and Proteins

Properties	*APOC1*	*APOC2*	*APOC3*	*APOC4*
Chromosomal localization	19q13.2	19q13.2	11q23-qter	19q13.2
Size of gene, kb	4.7	3.4	3.1	3.3
Tissue expression	Liver, lung, skin, testis, spleen	Liver, intestine	Liver, intestine	Low amounts in liver
Length of mature protein, No. of amino acids	57	79	79	102
Molecular mass, kDa	6.6	8.8	8.8	N
Plasma concentration, mg/dL	6	4	12	ND
References	Lauer et al[4] Curry et al[103]	Wei et al[7] Das et al[8]	Protter et al[18] Nestel and Fidge[116]	Allan et al[10]

Chromosomal localization, tissue-specific expression, and other biological properties of the human *APOC* genes and proteins are depicted. N indicates not reported; ND, not detectable.

deficiency.[30] These observations suggest that apoC1 deficiency in HDL may modulate lecithin-cholesterol acyltransferase (LCAT) activity, which is known to catalyze the esterification of free cholesterol in plasma.[31]

The importance of apoC2 as an activator of lipoprotein lipase (LPL) has unequivocally been demonstrated in patients with genetic defects in the structure or production of apoC2, all of whom display high circulating levels of triglycerides (TGs) and are phenotypically indistinguishable from patients with LPL deficiency.[32–36] As summarized in Table 2, sequence analysis of the *APOC2* gene in families with familial hyperchylomicronemia has revealed a variety of molecular defects in this particular gene. In 7 families (Nijmegen, Paris, Barcelona, Japan, Venezuela, Padova, and Bari), a single base change resulted in the introduction of a premature stop that led to the synthesis of truncated forms of apoC2 that were either not secreted or rapidly cleared from the circulation[37–41] (Table 2). A donor splice-site mutation in the first base of the second intron of the *APOC2* gene was found in a Hamburg family and in a neonatal Japanese patient ($APOC2_{Hamburg}$ and $APOC2_{Tokyo}$, respectively). This mutation caused abnormal splicing of *APOC2* mRNA and was associated with low levels of apoC2 in plasma.[42,43] In addition, a variety of single–amino acid substitutions in the *APOC2* gene has been described (Table 2) that either resulted in the inability to initiate apoC2 synthesis[44] or in the production of nonfunctional apoC2.[45–47] For 2 *APOC2* variants ($APOC2_{SanFrancisco}$ and the *APOC2* $Lys_{19}\rightarrow Thr$ mutation), a direct relationship between this mutant form of apoC2 and lipoprotein abnormalities could not be established.[48–51]

Several lines of evidence have implicated apoC3 as possibly contributing to the development of hypertriglyceridemia. A positive correlation has been observed between plasma apoC3 levels and elevated levels of plasma TGs[52–54] and

TABLE 2. Molecular Defects in the Human *APOC* Genes

Gene	Family	Molecular Defect	Lipoprotein Disorder/Abnormality	Protein in Plasma	References
APOC1	N	ApoC1/C2 deficiency	Familial chylomicronemia	0	Dumon and Clerc[30]
APOC2	Nijmegen	Introduction stop codon (Val_{18})	Familial chylomicronemia	0	Fojo et al[37]
	$Paris_2$, Barcelona	Introduction stop codon (Arg_{19})	Familial chylomicronemia	0	Parrot et al[38]
	Japan, Venezuela	Introduction stop codon (Gln_2)	Familial chylomicronemia	0	Xiong et al[39]
	Padova, Bari	Introduction stop codon (Tyr_{37})	Familial chylomicronemia	0	Fojo et al[40] Crecchio et al[41]
	Hamburg, Tokyo	Intron 2 donor splice defect	Familial chylomicronemia	↓	Fojo et al[42] Okubo et al[43]
	$Paris_1$	$Met_{22}\rightarrow Val$	Familial chylomicronemia	0	Fojo et al[44]
	Toronto	$Asp_{69}\rightarrow Thr$	Familial chylomicronemia	→	Connelly et al[45]
	St Michael	$Gln_{70}\rightarrow Pro$	Familial chylomicronemia	→	Connelly et al[46]
	Wakayama	$Trp_{26}\rightarrow Arg$	Familial chylomicronemia	0	Inadera et al[47]
	San Francisco	$Glu_{38}\rightarrow Lys$	Hyperlipidemia	→	Pullinger et al[48]
	N	$Lys_{19}\rightarrow Thr$	Hyperlipidemia	→	Huff et al[49] Hegele et al[50] Zysow et al[51]
APOC3	N	$Lys_{58}\rightarrow Glu$	Hyperalphalipoproteinemia	↓	von Eckardstein et al[60]
	Turkey	$Asp_{45}\rightarrow Asn$	N	→	Lüttmann et al[61]
	Mexico	$Gln_{38}\rightarrow Lys$	Mild hypertriglyceridemia	→	Pullinger et al[62]

Mutations affecting the synthesis, secretion, or structure of the respective apoC proteins. The molecular defect, the related lipoprotein disorder or lipoprotein abnormality, and the presence of the apoC protein in plasma are indicated. 0 indicates absence of apoC protein; ↓, low amounts of apoC protein; and →, presence of apoC protein.

VLDL-TGs.[55] However, structural mutations in the human *APOC3* gene fail to clearly show an association between the mutation and an altered lipid/lipoprotein metabolism. Five genetic variants of apoC3 were identified by the presence of additional bands after isoelectric focusing of VLDL (Table 2). Two of these variants differed from normal apoC3 by their degree of sialylation; ie, 1 was oversialylated[56] while the other was not sialylated at all because of a Thr_{74}→Ala mutation at the glycosylation site.[57–59] Carriers of these mutants were normolipidemic, indicating that the degree of apoC3 sialylation has little or no impact on lipoprotein metabolism. The 3 remaining apoC3 variants represented amino acid substitutions in both the *N*-terminal and *C*-terminal domains of apoC3 (Table 2). The Lys_{58}→Glu mutation was associated with low plasma apoC3 concentrations and atypically large HDL.[60] The number of carriers for this mutation, however, was too small to demonstrate a direct relationship between the mutation and altered lipoprotein levels. The Asp_{45}→Asn variant was found in a Turkish patient who underwent coronary bypass surgery but failed to show a clear association between the mutation and an abnormal lipoprotein metabolism.[61] The *APOC3* Gln_{38}→Lys mutation was observed in a boy of Mexican origin, and family studies in 16 individuals who were heterozygous for this *APOC3* mutation revealed mildly elevated levels of plasma TGs in these subjects.[62] Several studies have also reported a complete apoC3 deficiency in families with an increased prevalence of premature coronary heart disease.[63,64] In addition, 1 family with apoC3 deficiency demonstrated an increased fractional catabolic rate of VLDL.[65] However, in all cases, apoC3 deficiency was associated with an apoA1 deficiency, making it difficult to estimate the exact contribution of the lack of apoC3 to changes in lipoprotein levels.

In addition to the genetic mutations described above, several restriction fragment length polymorphisms (RFLPs) in or around the human *APOC* genes have been identified that are associated with lipoprotein disorders or altered plasma lipid concentrations in humans. One population-based, genetic association study has reported an *Hpa*I RFLP in the *APOC1* promoter,[66] located at a site 317 bp 5′ from the apoC1 transcription initiation site.[67] Recently, it has been shown by cell expression analysis that the promoter carrying the *Hpa*I site in combination with the HCR mediates enhanced gene expression.[68] These results suggest that under certain conditions, the *Hpa*I promoter variant causes overexpression of *APOC1*, which may contribute to the development of hyperlipidemia.

It has been demonstrated that a minor allele (*S2*) of an *Sst*I RFLP in the *APOC3* gene is associated with hypertriglyceridemia in several distinct populations,[69–79] but not in all.[80,81] Furthermore, Shoulders et al[82] reported that healthy carriers of the *S2* allele had higher plasma apoC3 levels than did noncarriers. These results indicate that the *S2* allele may influence plasma TG levels through modulation of *APOC3* gene expression. However, the *Sst*I RFLP is located in the noncoding region of exon 4 of the *APOC3* gene, suggesting that the *S2* allele may modulate plasma TG levels by linkage disequilibrium with other functional sequences in or near the *APOC3* gene. Dammerman et al[79] and Xu et al[83] have identified several polymorphic sites in and around the *APOC3* gene that show strong allelic association with each other and with the *Sst*I site. A detailed overview of these polymorphic sites has recently been published.[84]

Other RFLPs within the *APOA1*/*C3*/*A4* gene cluster such as *Xmn*I and *Pst*I have also been reported to be associated with hypertriglyceridemia[85] or coronary artery disease.[86] In 1 study of selected British families, the *Xmn*I RFLP within the *APOA1*/*C3*/*A4* gene cluster was shown to be linked with familial combined hyperlipidemia (FCH),[87] but this finding has not been confirmed by others.[88,89] FCH is a common inherited disorder of lipid metabolism that is characterized by an overproduction of apoB-100–containing lipoproteins and elevated levels of VLDL and LDL.[90–92] Recently, it was reported that the *Xmn*I polymorphism together with *Msp*I and *Sst*I aggravated hypercholesterolemia and hypertriglyceridemia in FCH probands; ie, a higher frequency of these minor alleles was associated with elevated plasma cholesterol, TGs, LDL cholesterol, apoB, and apoC3 levels.[93] A more detailed analysis of a combination of haplotypes within the *APOA1*/*C3*/*A4* gene cluster showed 2 different susceptibility loci for FCH within this cluster, consisting of an *S2*-bearing haplotype behaving as a dominant trait and an *X2M2* haplotype behaving as a permissive trait.[94] Furthermore, a C_{1100}→T polymorphism in exon 3 of the *APOC3* gene was found to be associated with an increased number of VLDL and IDL particles in the circulation of FCH probands.[95] Altogether, these results suggest that the *APOA1*/*C3*/*A4* gene cluster may contribute to FCH in a rather complex genetic manner, thereby acting as a modifier gene rather than representing the primary cause of FCH.

Further evidence that *APOC3* overexpression may underlie hypertriglyceridemia in humans comes from studies with fibrates, a hypotriglyceridemic class of drugs. Fibrates effectively decrease the apoC3 synthesis rate in humans[54] as well as *APOC3* mRNA levels in isolated human hepatocytes and rat livers via a peroxisome proliferator–activated receptor–dependent pathway.[96–98]

In summary, the characterization of mutations in the *APOC2* gene of patients with hyperchylomicronemia has clearly established an important role for apoC2 as an activator of LPL. In contrast, the mechanisms underlying the hyperlipidemia and hypertriglyceridemia that are suggested as being associated with genetic mutations and polymorphisms of the *APOC1* and *APOC3* genes remain largely unknown.

ApoC Proteins

Nucleotide sequence analysis has indicated that apoC1 is synthesized with a 26-residue signal peptide that is cleaved cotranslationally in the rough endoplasmic reticulum.[99] The remaining single-chain polypeptide of 57 amino acid residues has a molecular mass of 6.6 kDa (Table 1).[100,101] ApoC1 has a high content of lysine (16 mol%) and contains no histidine, tyrosine, cysteine, or carbohydrate.[102] It has been demonstrated that residues 7 to 24 and 35 to 53 of apoC1 are important for the binding to lipoproteins.[102] The plasma concentration of apoC1 in humans is ≈6 mg/dL.[103]

ApoC2 is synthesized with a 22-residue signal peptide that is cleaved cotranslationally in the rough endoplasmic reticulum.[104] The remaining single polypeptide chain of 79 amino acid residues has a calculated molecular mass of 8.8 kDa.[6,104–106] The structure of apoC2 is predicted to contain 3 helical regions between residue 13 to 22, 29 to 40, and 43 to

52, which are thought to be involved in phospholipid binding.[107] Studies using synthetic peptides of apoC2 have shown that LPL interacts with the COOH-terminal amino acids 56 to 79 of apoC2.[108] In line with these data, deletion of the COOH-terminal tetrapeptide residues 76 through 79 impairs the ability of the protein to activate LPL.[109] ApoC2 is present in human plasma at a concentration of ≈4 mg/dL.[8]

ApoC3 is synthesized in the liver and in minor quantities by the intestine as a 99–amino acid peptide. After removal of the 20–amino acid signal peptide in the endoplasmic reticulum, a mature apoC3 protein of 79 amino acids comprises a molecular mass of 8.8 kDa (Table 1).[110] Thrombin cleavage of apoC3 results in an *N*-terminal domain, residues 1 to 40, and a *C*-terminal domain, residues 41 to 79, corresponding to the products of exons 3 and 4, respectively.[111] Structural analysis demonstrated that the binding of apoC3 to surface phospholipids of lipoproteins is mediated by an amphipathic helix at residues 50 to 69 residing in the *C*-terminal domain of apoC3.[112] Isoelectric focusing separates apoC3 into 3 isoforms that differ in their degree of *O*-linked sialylation at the threonine residue in position 74: apoC3–0 (no sialic acid), apoC3-1 (1 mol sialic acid), and apoC3-2 (2 mol sialic acid).[113–115] ApoC3 is the most abundant C apolipoprotein in human plasma, at a concentration of ≈12 mg/dL.[116]

Little has been reported on how and in which form apoCs are secreted into plasma. Studies by Roghani and Zannis[59] have shown that cell clones expressing the *APOC3* gene exclusively secrete the desialylated form of apoC3 (apoC3-2), suggesting that apoC3-2 must be desialylated after secretion in plasma to produce the monosialo (apoC3-1) and asialo (apoC3-0) forms. Furthermore, it was shown that the intracellular glycosylation of apoC3 is not an absolute prerequisite for its secretion and ability to associate with plasma lipoproteins.[59] Although it has been reported that nascent apoCs are largely secreted in the lipid-poor form by different cell lines in vitro,[59,116] it is likely due to their high affinity toward lipid surfaces that apoCs rapidly associate with VLDL and HDL in plasma.[117–119] A detailed study by Gibson et al[120] showed that apoC3 was found in the broad distribution of particles the size of VLDL, on particles slightly larger than LDL, and on particles slightly larger than HDL. It has been reported that in the fasting state, apoCs are mainly associated with HDL, whereas in the fed state, they preferentially redistribute to the surface of chylomicron and VLDL particles.[121] Similarly, release of LPL and hepatic lipase in subjects intravenously injected with heparin induced a shift in the distribution of apoC2 and apoC3 from VLDL to particles slightly larger than HDL.[122] At least for apoC3, there is also a nonexchangeable pool present on both VLDL and HDL that accounts for 30% to 60% of the total apoC3 mass in each lipoprotein fraction.[123,124]

The relatively low human *APOC4* gene expression in the liver and the total lack of the apoC4 protein in human plasma (Table 1) suggest that apoC4 plays no major role in lipoprotein metabolism. The apoC4 protein sequence was predicted to comprise 127 amino acid residues, which contain a putative 25-residue signal peptide and 2 potential amphipathic α-helical domains.[10] In other species such as the rabbit, it has been demonstrated that apoC4 is secreted at a more substantial level.[125] The rabbit apoC4 protein is synthesized as a 124–amino acid protein that includes a typical signal peptide of 27 residues and has a molecular weight of ≈14 kDa. The mature rabbit apoC4 protein of 97 amino acids is primarily associated with VLDL and HDL.[125]

Interaction of ApoCs With Receptors and Enzymes Involved in Lipoprotein Metabolism

Studies in the early 1980s have demonstrated that enrichment of chylomicrons and VLDL with a mixture of apoCs significantly inhibits their uptake by the isolated, perfused rat liver.[126–133] In line with these studies, it was shown that the apoE-mediated uptake of TG-rich emulsions by HepG2 cells and rat hepatocytes in culture was effectively inhibited by apoC3 and apoC1.[131,133] Ligand blotting assays showed that apoC1 and apoC2 inhibit the apoE-mediated binding of β-VLDL to the low density lipoprotein receptor (LDLR)–related protein (LRP), apoC1's being a more effective inhibitor than apoC2.[134,135] As shown in Table 3, apoC3 had no effect on the binding affinity of β-VLDL to LRP.[135] It is suggested that the inhibitory action of apoC1 on lipoprotein binding to LRP was due to displacement of apoE from the lipoprotein particle. In line with these results, it was shown that synthetic peptides corresponding to the lipid-binding domain of apoC1 were also able to displace significant amounts of apoE from β-VLDL and inhibit the binding of β-VLDL to LRP.[136] Sehayek and Eisenberg[137] reported that apoC1 and apoC2 impaired the apoE-mediated binding of VLDL to the LDLR in cultured fibroblasts (Table 3). In line with the LRP ligand blotting assays, the strongest inhibition of lipoprotein binding to the LDLR was observed with apoC1. In this study, it was concluded that the inhibition of lipoprotein binding to the LDLR occurred through masking or altering the conformation of apoE by apoC1 rather than through displacement of apoE, as suggested by Weisgraber et al.[135]

Previous studies have shown that apoC3 completely abolishes the apoB-mediated binding of lipoproteins to the LDLR (see Table 3). It is suggested that this inhibitory action of apoC3 on lipoprotein binding was due to a masking of the receptor domain of apoB by apoC3.[138,139] An inhibitory effect was also observed for apoC2, whereas apoC1 did not inhibit apoB-mediated binding of lipoproteins to the LDLR.[139] Recent studies have shown that apoCs can also interfere with the binding of lipoproteins to other lipoprotein receptors, including the VLDL receptor[140] and lipolysis-stimulated receptor.[141] The binding of lipoproteins to the VLDL receptor was completely inhibited by apoC1,[140] whereas apoC3 specifically inhibited the binding of chylomicrons and VLDL to the lipolysis-stimulated receptor.[141]

Numerous in vitro studies have investigated the influence of apoCs on the LPL-mediated lipolysis of TG-rich lipoproteins. As shown in Table 3, apoC2 is an essential activator of LPL. However, at high protein concentrations, apoC2 was demonstrated to inhibit LPL activity rather than stimulate it.[142] The mechanism by which apoC2 activates LPL is not fully understood.[143,144] It has been suggested that apoC2 activates LPL after binding of LPL to phospholipids on the surface of TG-rich lipoproteins. On the other hand, apoC2 may also bind directly to LPL. Recent studies by Olivecrona and Beisiegel[145] showed that the lipid binding domain of apoC2 is essential for activation of LPL.

TABLE 3. Effect of ApoCs on Receptors and Enzymes Involved in Lipoprotein Metabolism

Receptors and Enzymes	ApoC1	ApoC2	ApoC3	References
LRP	↓ ↓	↓	0	Kowal et al[134]
				Weisgraber et al[135]
LDLR				
ApoE mediated	↓ ↓	↓	↓	Sehayek and Eisenberg[137]
ApoB mediated	0	↓	↓ ↓	Clavey et al[139]
VLDLR	↓ ↓	N	N	Jong et al[140]
Lipolysis-stimulated receptor	N	0	↓ ↓	Mann et al[141]
LPL	↓	↑ ↑ or ↓	↓ ↓	Havel et al[142]
				LaRosa et al[143]
				Ekman and Nilsson-Ehle[146]
				Brown and Baginsky[147]
				Wang et al[150]
HL	N	↓	↓ ↓	Landis et al[153]
LCAT	↑ ↑	↓	↓	Soutar et al[154]
				Steyrer and Kostner[156]
				Nishida et al[157]
				Liu and Subbaiah[159]
CETP	↓	N	↑	Kushwaha et al[160]
				Sparks and Pritchard[161]

Individual effects of apoC on lipoprotein receptors such as the LDLR, LRP, VLDLR, and Lipolysis-stimulated receptor and enzymes such as LPL, HL, LCAT, and CETP. ↓ ↓ indicates strong inhibition; ↓, moderate inhibition; 0, no effect; ↑, moderate activation; and ↑ ↑, strong activation.

Studies in the early 1970s have indicated that both apoC1 and apoC3 inhibit LPL activity[142,146–149] (Table 3). In a study with hypertriglyceridemic patients, it was shown that apoC3 was 1 of the most specific inhibitors of LPL.[150] Further in vitro kinetic analysis with bovine LPL and purified apoC3 demonstrated that apoC3 displays noncompetitive inhibitory properties against both apoC2 and triolein, indicating that apoC3 exerts its inhibitory effect directly on LPL.[150] In line with these results, McConathy et al[151] used synthetic polypeptide fragments of apoC3 and observed that the *N*-terminal domain of apoC3 is primarily responsible for inhibition of LPL activity. Studies by Ginsberg et al[65] showed that sera from subjects deficient for both apoC3 and apoA1 were able to normally activate human milk LPL at increasing volumes of sera, whereas normal sera effectively inhibits LPL activity at increasing concentrations. Furthermore, addition of purified apoC3 to the apoC3/A1-deficient sera progressively reduced maximal levels of LPL activity, suggesting that apoC3 inhibits the LPL-mediated lipolysis of TG-rich lipoproteins.

In addition to LPL, it has been demonstrated that apoCs can act on several other enzymes involved in lipoprotein processing (see Table 3). In vitro, high concentrations of apoC3 have been shown to inhibit hepatic lipase (HL).[152] In line with this study, apoC3 inhibited the lipolysis of TG emulsions by heparin-immobilized HL in the presence of apoE.[153] An inhibitory effect on the HL-mediated lipolysis of TG emulsions was also observed for apoC2, although to a lesser extent than with apoC3.[153] In the latter study, however, the inhibitory action of apoC3 and apoC2 may have been due to interference of the apoCs with the apoE-mediated binding of the substrate to the lipase-loaded heparin-Sepharose column rather than a direct inhibitory action of the apoCs on HL itself.

ApoCs also appeared to affect LCAT activity (Table 3). Whereas apoA1 is known to be the most powerful LCAT activator, apoC1 was shown to activate LCAT to ≈78% of that of apoA1.[154–156] Both apoC2 and apoC3 were reported to inhibit LCAT activity, probably by displacing the activating apolipoproteins from the lipoprotein surface.[157] Furthermore, LCAT is also able to esterify lysophosphatidylcholine to phosphatidylcholine.[158] This lysolecithin acyltransferase activity was found to be activated by apoC1 as well. In this respect, apoC1 was 70% as effective as apoA1.[159]

It has been reported that in a family of baboons with high plasma HDL cholesterol levels, the transfer of cholesteryl ester from HDL to lower-density lipoproteins is inhibited by a 4-kDa protein.[160] This 4-kDa protein appeared to correspond to the *N*-terminal domain of apoC1. Further in vitro studies demonstrated that a synthetic peptide comprising the 38–amino acid *N*-terminal domain of apoC1 was indeed able to inhibit cholesteryl ester transfer protein (CETP) activity.[160] In addition, the 4-kDa protein was associated with apoA1 on HDL and, to a lesser extent, with apoE on VLDL, thereby resulting in modification of these apolipoproteins. From these data, it was hypothesized that an association of the apoC1 fragment with apoA1 on the surface of HDL and with apoE on VLDL may hamper the accessibility of CETP to these substrate lipoproteins.

Little has been published about the effects of apoC2 and apoC3 on CETP activity. Preliminary studies as discussed by Sparks and Pritchard[161] demonstrate that by using recombinant HDL particles, apoC3 stimulates CETP activity (Table 3).

TABLE 4. *APOC*-Transgenic Mouse Models

Gene	DNA Construct	Tissue Expression	Phenotype	References
Overexpression				
Human *APOC1*	20.8 kb; *APOC1/APOC1′/HCR*	Liver	TC ↑ TG ↑	Simonet et al[162]
	27 kb; *APOE*3L/APOC1/APOC′/HCR*	Liver	TC ↑ ↑ TG ↑ ↑	Jong et al[163]
	10.4 kb; *APOC1/HCR*	Liver	TC ↑ TG ↑	Shachter et al[164]
	18 kb; *APOC1/APOC′/HCR*	Liver/skin	TC ↑ TG ↑	Jong et al[165,169]
Human *APOC2*	8.4 kb; CYPIA1 promoter/*APOC2*	Brain, liver, intestine	TC ↑ → TG ↑ ↑	Shachter et al[172]
Human *APOC3*	6.7 kb; *APOC3*	Liver, intestine	TG ↑ ↑	Ito et al[173]
	10.5 kb; *APOC3*	Liver, intestine	TC ↑ → TG ↑ ↑	de Silva et al[174]
Mouse *ApoC3*	4.7 kb; *ApoC3*	Liver, intestine	TC ↑ TG ↑ ↑	Aalto-Setälä et al[175]
Human *APOC4*	*APOE* promoter/*APOC4/HCR*	Liver, kidney, spleen, brain, lung	TC→ TG ↑	Alan and Taylor[180]
Knockout				
Mouse *ApoC1*	12 kb; hygro B^r/HSV-tk	...	TC→ TG ↑ →	van Ree et al[170]
				Jong et al[171]
Mouse *ApoC3*	12 kb; Neo^r/HSV-tk	...	TC ↓ TG ↓	Maeda et al[179]

APOC-transgenic and knockout mice were generated by using the constructs as indicated above. TC indicates total cholesterol; TG, triglycerides; ↓, decrease; →, no change; ↑, increase; ↑ ↑, strong increase; *APOC1′*, *APOC1* pseudogene; *APOE*3L*, *APOE*3*$_{Leiden}$ gene; CYPIA1, cytochrome P450 IA 1; hygro B^r, hygromycin B resistance gene; Neo^r, neomycin resistance gene; and HSV-tk, herpes simplex virus thymidine kinase gene.

In summary, in vitro studies have demonstrated that apoCs have an inhibitory or stimulatory effect on a variety of receptors and enzymes involved in lipoprotein metabolism (Table 3). These data suggest a complex role for apoCs in human disease. However, it is important to know which of these in vitro effects extends to the in vivo situation, because several in vitro effects of apoCs on receptors and enzymes may appear nonspecific or secondary, ie, due to the displacement of other activating or inhibiting components of the lipoprotein particle.

Transgenic Mouse Models Overexpressing or Lacking ApoC1

Studies relating to the in vivo metabolism of apoCs have been hampered in humans owing to the highly complex nature of lipoprotein metabolism that can be influenced by multiple genetic and environmental factors. To study the in vivo functions of the individual apoCs in lipoprotein metabolism against a defined genetic background and under strictly controlled environmental conditions, several laboratories have created mouse models lacking or overexpressing the respective *APOC* genes through the technologies of transgenesis and gene targeting. As shown in Table 4, *APOC1*-transgenic mice were generated by using different DNA constructs that all contained the 154-bp HCR that directs expression of the human *APOC1* gene to the liver. Human *APOC1*–transgenic mice exhibited elevated levels of cholesterol and TGs owing to an accumulation of VLDL-size particles in the circulation.[162–165]

To investigate the mechanisms underlying the hyperlipidemia in human *APOC1*–transgenic mice, in vivo turnover studies were performed using labeled VLDL. The clearance of both VLDL TG and VLDL apoB was severely hampered in hyperlipidemic human *APOC1*–transgenic mice,[163–165] suggesting that apoC1 interferes with either the lipolysis or hepatic uptake of VLDL. The findings that (1) VLDL from *APOC1*-transgenic mice bound as efficiently to heparin-Sepharose as did VLDL from wild-type mice,[164] (2) the in vitro lipolysis by LPL of VLDL TG fractions isolated from *APOC1*-transgenic mice was not impaired, and (3) the in vivo extrahepatic lipolysis of VLDL TG in *APOC1*-transgenic mice was not different from that in wild-type mice[165] indicate that apoC1 does not interfere with lipolysis of VLDL TGs in vivo. Furthermore, it was demonstrated that the production rate of VLDL TGs in *APOC1*-transgenic mice is not different from that in control mice.[164,165] In conclusion, the elevated lipid levels in the plasma of *APOC1*-transgenic mice are primarily due to an impaired uptake of VLDL by the liver rather than to an enhanced production or disturbed lipolysis of VLDL.[163–165]

Overexpression of apoC1 in LDLR-knockout mice leads to extremely elevated levels of plasma cholesterol and TGs compared with cholesterol and TG levels in LDLR-knockout mice.[165] These results suggest that apoC1 inhibits the alternative lipoprotein clearance pathway. The fact that overexpression of the receptor-associated protein (RAP) greatly enhances serum cholesterol and TG levels in $LDLR^{-/-}$ mice whereas it does not alter serum lipid levels in *APOC1*/$LDLR^{-/-}$ mice indicates that RAP and *APOC1* overexpression act on the same pathway in inhibiting the clearance of VLDL remnants by the liver. Because RAP overexpression is known to block LRP, it can be concluded that apoC1 inhibits the uptake of lipoproteins via LRP in vivo, thereby sustaining the in vitro findings that apoC1 is the most efficient apoC for inhibiting the binding of VLDL to the LRP.[135,136]

The in vitro observation that apoC1 is a potent activator of LCAT suggests that the increases in VLDL/IDL and LDL cholesterol observed in human *APOC1*–transgenic mice[164,165] may also partly result from an increase in the cholesterol esterification rate. Increased LCAT activity, as found in transgenic mice overexpressing human LCAT, has been reported to elevate HDL cholesterol esters levels.[166–168]

However, the findings that the free to total cholesterol ratios were unchanged in *APOC1*-transgenic mice[164] and that HDL cholesterol esters were not significantly elevated in *APOC1*-transgenic mice compared with wild-type mice[165] argue against an LCAT-mediated elevation in cholesterol levels in *APOC1*-transgenic mice.

In addition to hyperlipidemia, it has recently been reported that *APOC1*-transgenic animals exhibit several abnormalities, consisting of elevated plasma free fatty acid levels, epidermal hyperplasia and hyperkeratosis, atrophic sebaceous glands, lack of sebum, and (subcutaneous) adipose tissue.[169] These results suggest an additional role for apoC1 in epidermal lipid synthesis as well as adipose tissue formation.

Because transgenic mice overexpressing *APOC1* develop hyperlipidemia, a hypolipidemic phenotype was expected in *ApoC1*-knockout mice. It was, however, surprising to observe that *ApoC1*-knockout mice had normal serum lipid levels on a chow diet (Table 4).[170] Only when fed a high-fat and high-cholesterol diet did apoC1-deficient mice develop hypercholesterolemia. In vitro binding experiments revealed that apoC1-deficient VLDL was a poor competitor for LDL binding to the LDLR, suggesting that total apoC1 deficiency leads to an impaired receptor-mediated clearance of remnant lipoproteins.[170] Later, these results were confirmed in a more detailed characterization of these *ApoC1*-knockout mice, demonstrating that an impaired in vivo hepatic uptake of VLDL is the primary metabolic defect in apoC1-deficient mice.[171]

In summary, whereas overexpression of human *APOC1* in transgenic mice predominantly inhibits the uptake of VLDL particles by the liver, the absence of endogenous mouse *ApoC1* in mice appears to have the same effect, though to a lesser extent. It has been suggested that apoC1 may impair VLDL clearance either directly, by a specific interaction between apoC1 and the hepatic receptor, or indirectly, as caused by an apoC1-induced displacement of apoE from the lipoprotein particle.[164,165] On the other hand, it is suggested that the impaired interaction of apoC1-deficient VLDL with hepatic receptors is due to an enrichment of the VLDL particle with apoA1 and apoA4.[170,171]

Transgenic Mice Overexpressing Human ApoC2

Transgenic mice overexpressing human *APOC2* were generated by using a vector containing the human *APOC2* gene joined to a cytochrome P450 CYPIA1 promoter[172] (Table 4). This promoter is normally silent in intrauterine life but can lead to transgene expression after administration of β-naphthoflavone. Strikingly, transgenic mice overexpressing human apoC2 were hypertriglyceridemic, due to an accumulation of TG-rich VLDL particles in the circulation. This hypertriglyceridemia was shown to be caused by impaired clearance of VLDL TGs.[172] This finding suggests that high levels of apoC2 interfere with either the peripheral lipolysis of VLDL or the uptake of the VLDL particle by the liver. The observation that *APOC2*-transgenic mice accumulate large, TG-rich VLDLs and have only minimally elevated levels of plasma cholesterol is most consistent with a defective LPL-mediated hydrolysis of VLDL TGs in these mice rather than an impaired hepatic VLDL uptake. The observation that VLDL isolated from APOC2-transgenic mice showed decreased binding affinity to heparin-Sepharose suggests that these lipoprotein fractions may be less accessible to cell surface–bound LPL[172] and therefore sustains the hypothesis that excess apoC2 on the VLDL particle inhibits LPL activity in vivo. These results are in striking contrast to the human studies discussed earlier, in which it was shown that apoC2 is the physiological activator of LPL. Altogether, these data suggest that apoC2 may play a complex role in plasma TG metabolism; ie, apoC2 activates LPL, most likely at low protein concentrations, whereas at high protein levels, apoC2 directly inhibits VLDL lipolysis.

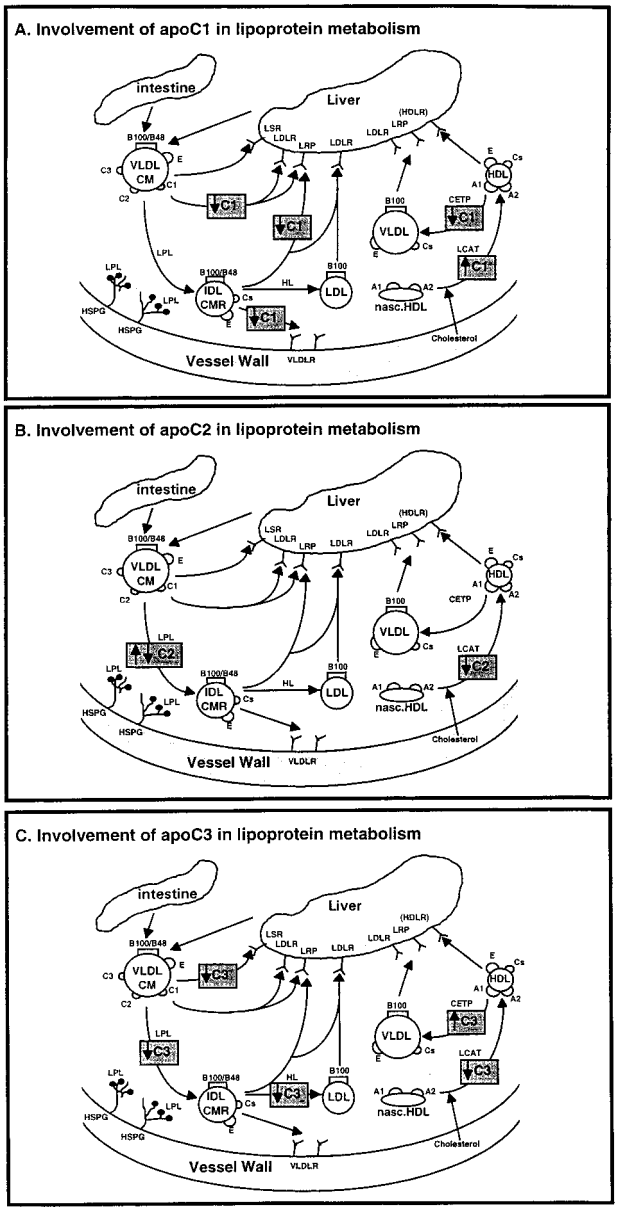
Transgenic Mouse Models Overexpressing or Lacking ApoC3

Two laboratories have reported the generation of human *APOC3*–transgenic mice by using DNA fragments of different sizes, both of which resulted in high levels of human *APOC3* mRNA in the liver and intestine[173,174] (Table 4). Human *APOC3*–transgenic mice exhibited very elevated levels of VLDL TGs. Recently, it was reported that mouse *ApoC3*–transgenic mice are also hypertriglyceridemic.[175] Human and mouse *APOC3*–transgenic mice had impaired clearance of VLDL TGs, concomitant with a decreased VLDL apoE to apoC ratio.[174–176] Because crossbreeding of human *APOC3*–transgenic mice with human *APOE*–overexpressing transgenic mice normalizes plasma TG levels,[174,175] it was concluded that the delayed clearance of VLDL TGs in *APOC3*-transgenic mice was due to the low amount of apoE relative to apoC3 on the VLDL particle. More recent studies, however, have shown that the hypertriglyceridemia in *APOC3*-transgenic mice is most probably caused by an excess of apoC3 rather than by the apoC3-induced displacement of apoE. *ApoE*-knockout mice normally accumulate large amounts of VLDL that is enriched in cholesterol ester but relatively poor in TG.[177] Crossbreeding of *ApoE*-knockout mice with transgenic mice overexpressing human apoC3 resulted in a massive accumulation of TG-rich VLDL-size particles,[178] indicating that it is the amount of apoC3 that causes hypertriglyceridemia.

From in vitro binding studies, it was suggested that excess apoC3 inhibits the binding of VLDL to the LDLR.[174,175] However, the prolonged residence time of the predominantly enlarged, TG-rich VLDL particles in *APOC3*-transgenic mice implies that apoC3 impairs the hydrolysis of VLDL TGs. In line with this observation, VLDL isolated from *APOC3*-transgenic mice displayed decreased binding affinity to heparin-Sepharose.[164,175] In addition, the observations that apoC3-deficient mice are protected from postprandial hypertriglyceridemia and exhibit reduced serum lipid levels compared with control mice also points to an inhibitory action of apoC3 on VLDL lipolysis.[179]

Transgenic Mice Overexpressing Human ApoC4

The recently identified human *APOC4* gene was overexpressed in transgenic mice[180] (Table 4). Under normal conditions, the *APOC4* gene is poorly expressed in human liver, most likely as a consequence of a TATA-less promoter.[10] Therefore, to enhance liver expression of the human *APOC4* gene in mice, a vector was constructed containing human



Schematic representation of the effects of apoC1 (A), apoC2 (B), and apoC3 (C) on the major metabolic pathways in lipoprotein metabolism. The stimulatory (↑) and inhibitory action (↓) of the individual apoCs on lipoprotein lipolysis, clearance, and hepatic uptake is depicted.

APOC4 cDNA and the HCR element under control of the human *APOE* gene promoter. Human *APOC4*–transgenic mice were hypertriglyceridemic compared with their non-transgenic littermates, owing to an accumulation of TG-rich VLDL particles. Because there was little change in serum cholesterol levels in these transgenic mice, apoC4 may interfere with the clearance of VLDL TGs via an inhibitory effect on lipolysis in a way similar to that discussed for apoC2 and apoC3.[180] The fact that apoC4 is totally absent in human plasma indicates no major modulating role for apoC4 in VLDL TG metabolism in humans.

Conclusions

Clinical evidence, as well as in vitro data and in vivo work on transgenic mouse models, have demonstrated that each of the individual human apoCs effectively modulates lipoprotein metabolism. As schematically depicted in panel A of the Figure, apoC1 inhibits the uptake of TG-rich lipoproteins via hepatic receptors, particularly the LRP. As a consequence, the presence of apoC1 on the lipoprotein particle may prolong their residence time in the circulation and subsequently facilitate their conversion to LDL.

ApoC2 is an important activator of LPL and is required for efficient lipolysis of TG-rich lipoproteins in the circulation. The total absence of apoC2 or defects in its structure severely hamper LPL-mediated lipolysis of TG-rich lipoproteins, resulting in strongly elevated levels of plasma TGs. In contrast, excess apoC2 on the lipoprotein particle has been demonstrated to inhibit LPL-mediated hydrolysis of TGs (panel B of the Figure).

At least from in vivo studies with *APOC3*-transgenic mice, it appears that apoC3 inhibits the lipolysis of TG-rich lipoproteins by hampering the interaction of these lipoproteins with the heparan sulfate proteoglycan–LPL complex (panel C of the Figure). Subsequently, the poorly lipolyzed apoC3-containing lipoprotein particles may accumulate in plasma because of their lower binding affinity to hepatic receptors as a consequence of their lipid composition, large size, or the presence of apoC3 on the particle. These results suggest that the amount of apoC3 on the lipoprotein particle is a strong modulator of plasma TG metabolism and may contribute to hypertriglyceridemia in the human population.

Several in vitro studies have shown that apoCs can also modulate enzymes that are involved in the transport of cholesterol from extrahepatic tissues to the liver (the Figure). Although these specific functions remain to be established in vivo, it has been demonstrated that apoC1 can effectively activate LCAT. In contrast, both apoC2 and apoC3 have been reported to inhibit LCAT activity, most likely as a consequence of displacing the activating components of the HDL particle. CETP, which mediates the transfer of cholesterol ester from HDL to apoB-containing lipoprotein particles, was shown to be inhibited by apoC1, whereas apoC3 was reported to activate this process.

In conclusion, human apoCs have been demonstrated to have distinct effects on the major metabolic pathways in lipoprotein metabolism, implying that changes in human *APOC* gene expression may play an important role in the etiology of human hyperlipidemias.

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