

Lipoprotein apoprotein metabolism

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The study of the plasma lipoproteins in recent years has significantly broadened our understanding of lipid metabolism and lipid transport. Incisive studies into the structure and function of the lipoprotein species have contributed to significant advances in our understanding and management of lipid disorders. All of the lipoproteins are related and interact dynamically within the vascular system. Many of the processes involved in lipoprotein metabolism are regulated by enzyme activity that can influence plasma lipoprotein levels. An understanding of these interactions and interrelationships has been made possible by numerous studies over the past decade.

Until about 10 years ago lipoproteins were classically viewed as isolated families or species as defined either by ultracentrifugation or by electrophoresis. The operational separations by density or electrophoretic mobility allowed for the naming and quantification of various abnormalities, but provided little insight as to the cause of these abnormalities or of the role of the lipoproteins in lipid transport. A more functional view of the lipoproteins awaited the isolation, characterization and metabolic study of the lipoprotein apoproteins. Numerous reviews have appeared over the last several years summarizing our current state of knowledge of the plasma lipoproteins, their composition, structure, and function as well as the structure, distribution, and function of the lipoprotein apoproteins (1–6). It is not our intent herein to provide another comprehensive review of the plasma lipoproteins. Rather we will focus our attention on the functional interrelationships of the lipoprotein apoproteins and the information these relationships reveal about the mechanisms and determinants involved in the lipoprotein metabolism within the vascular bed.

Though there are still some disagreements about nomenclature, in this review we will employ the no-

menclature first introduced by Alaupovic et al. (7). It is now widely agreed that there are at least seven different lipoprotein apoproteins. These apoproteins differ in their primary, secondary, and tertiary structure as well as in their functions and distribution throughout the lipoprotein spectrum (Tables 1, 2). Five of these lipoprotein apoproteins have already been sequenced (8–16) and numerous reports have appeared detailing the unique characteristics that allow these proteins to bind lipid (1–6). Some, like apoprotein B and apoprotein A-II appear to have a primary role in the transport of lipid, while others like apoproteins A-I and C-II serve as activators (coenzymes) for enzyme systems involved in lipoprotein lipolysis and interconversion (1–6). We will review what is known about the metabolism of the individual lipoprotein apoproteins and attempt to relate these apoprotein systems to one another as well as to normal and abnormal lipid transport.

A APOPROTEINS

Physical Properties

Apoprotein A-I (apoA-I) and apoprotein A-II (apoA-II) are the major protein constituents of high density lipoproteins (HDL). These apoproteins are immunologically distinct, can be readily isolated from human HDL, and are easily separated by various

Abbreviations: apo, apoproteins; LCAT, lecithin:cholesterol acyltransferase; VLDL, very low density lipoprotein, $d < 1.006$ g/ml; IDL, intermediate density lipoprotein, $d 1.006$ – 1.019 g/ml; LDL, low density lipoprotein, $d 1.019$ – 1.063 g/ml; HDL, high density lipoprotein, $d 1.063$ – 1.21 g/ml; FH, familial hypercholesterolemia.

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chromatographic techniques (17). ApoA-I is largely helical in structure (18). Glutamine is the carboxy terminal amino acid, aspartic acid is the amino terminal, and isoleucine is absent. ApoA-I has been reported to activate lecithin:cholesterol acyltransferase (LCAT) (19). The amino acid sequence of both A apoproteins has been determined (8–12) (see **Table 1**).

ApoA-I is a single polypeptide chain of 243 amino acid residues, and its amino acid sequence as reported by Brewer et al. (11) differs in several positions from earlier reports (8–10). The reason for these differences remains unclear. ApoA-I can be fractionated into several distinct forms by DEAE ion exchange chromatography (20, 21). Most of this heterogeneity can be eliminated by omitting steps in the purification scheme known to cause aggregation (such as lyophilization or concentration by dialysis). Two polymorphic forms of ApoA-I are still isolated utilizing these latter techniques (6). These forms do not differ in their molecular weight, amino acid composition, or immunologic properties, and the nature of the difference between these two forms remains unknown.

ApoA-II is a single protein of molecular mass of 17,000 daltons. By reduction of a single S–S bond, two identical peptides of molecular mass 8,500 daltons are isolated (12, 22). The peptides consist of 77 amino acid residues, with glutamine as the carboxy terminal amino acid and pyrrolidone carboxylic acid as the amino terminal residue. These peptides lack histidine, arginine, and tryptophan.

The interaction of A apoproteins with lipids has been extensively studied (5). Both apoA-I and apoA-II bind phosphatidylcholine and form protein–phospholipid complexes (18). These complexes contain about 40% protein by weight, are isolated in the HDL density range (1.063–1.21 g/ml), and can incorporate cholesteryl ester. When apoA-II is added to a sonicated mixture of either lecithin or sphingomyelin, 79–90%

of the apoA-II can be isolated in the HDL density range. In contrast, only about 10% of added apoA-I can be recovered under similar conditions (23, 24). These studies suggest that the interaction of apoA-I is less avid with lipid than that of apoA-II.

When apoA-I is complexed with lipid, the amount of secondary structure in apoA-I increases from 53 to 68%. Similar findings have also been reported for apoA-II, suggesting that lipid–protein interactions may increase A apoprotein secondary structure (18). Recently, however, it has been demonstrated that both apoA-I and apoA-II readily self-associate to form oligomers, and that this self-association is accompanied by major changes in secondary and tertiary structure (6, 25–28). These data suggest that changes in secondary protein structure seen with A apoprotein–lipid recombination studies may be due to protein–protein interactions alone.

A apoproteins in lipoproteins

The A apoproteins are predominantly found in the HDL density range in plasma (**Table 2**). About 50% of HDL mass is protein, 30% is phospholipid, and 20% is cholesterol. The phosphatidylcholine to sphingomyelin ratio is 5:1, and the ratio of esterified to free cholesterol is about 3:1. Both A and C apoproteins are found in HDL. ApoA-I and apoA-II constitute about 90% of total HDL protein with an apoA-I to apoA-II ratio of about 3:1 by weight (17). HDL is customarily divided into two density classes: HDL₂ (d 1.063–1.125 g/ml) and HDL₃ (d 1.125–1.21 g/ml). HDL₂ has a mean molecular mass of 360,000 daltons and is composed of 60% lipid and 40% protein. The mean molecular mass of HDL₃ is 175,000 daltons, 55% of which is attributed to the apoproteins. The phosphatidylcholine to sphingomyelin ratio and the ratio of free to esterified cholesterol are higher in HDL₂ than in HDL₃ (29). Published reports of the apoA-I:apoA-II

TABLE 1. Characteristics of human plasma apoproteins^a

Apoprotein	Mol Wt	No. Amino Acids	Carbohydrate	Function	Site of Synthesis
A-I	28,300	243	+	LCAT activation	Intestine, liver
A-II	17,000	154	±	?	Intestine, liver
B	?	?	5%	Triglyceride transport	Liver, intestine
C-I	6,331	57	0	? LCAT activation ? Lipase activation	Liver
C-II	8,837	78	0	Lipase activation	Liver
C-III	8,764	79	+	? Inhibits lipase	Liver
D	22,100	?	?	? Activates LCAT	?
E	33,000	?	?	?	Liver, ? intestine

^a References 1–6.

ratio of HDL₂ compared to HDL₃ are contradictory, indicating variously that it is higher (30–33), lower (34), or identical (35) to HDL₃. Variable dissociation of apoA-I and apoA-II from HDL subfractions during isolation may account for some of these discrepancies. The existence of several different lipoprotein particles within HDL has been reported, with each lipoprotein species containing different apolipoproteins. The presence and composition of lipoprotein families LP-A, LP-B, LP-C, LP-D, and LP-E have been reported within HDL (30, 36, 37). Future research will undoubtedly be directed toward an elucidation of the composition and metabolism of the different lipoprotein species within HDL.

HDL models have been proposed utilizing evidence from chemical, enzymatic, and physical studies, as well as data from nuclear magnetic resonance spectroscopy (38–40). It appears that HDL is a spherical micelle, approximately 90–120 Å in diameter, consisting of lipids with polar groups of the phospholipids at the surface or aqueous interface, and with the globular A apoproteins partially embedded in lipid (40). The major forces in the protein–lipid interaction are apparently hydrophobic. Interactions between apoA-I and apoA-II may be of special importance to the organization of the native HDL (LP-A) molecule.

In normal human plasma, 87% of apoA-I and 90% of apoA-II are found in HDL and trace amounts of each apoprotein are found in the other lipoprotein fractions (33). Twelve percent of apoA-I and 9% of apoA-II are in the 1.21 g/ml infranatant. The amount of A apoproteins found in the 1.21 g/ml infranatant can be enhanced by repeated ultracentrifugation. Apparently as much as 60% of immunoassayable apoA-I can be released from HDL during repeated ultracentrifugation (41).

A apoproteins are also found in lymph chylomicrons. Less than 2% of chylomicron composition is due to protein. ApoA-I and apoA-II account for about 12% of the apoprotein content of chylomicrons isolated from human thoracic duct lymph (42, 43). ApoA-I has been identified as a major apoprotein of rat mesenteric lymph chylomicrons (44) and has been reported

TABLE 2. Apoprotein content of lipoproteins^a

	Chylomicrons ^b	VLDL ^c	LDL ^c	HDL ^c
% of total of lipoprotein protein				
Apoprotein				
A-I	7.4	Trace		67
A-II	4.2	Trace		22
B	22.5	36.9	98	Trace
C-I	15	3.3	Trace	1–3
C-II	15	6.7	Trace	1–3
C-III	36	39.9	Trace	3–5
D				+
E		13.0	Trace	+
Total	100	100	100	100

^a From reference 1–6.

^b Lymph.

^c Plasma.

to comprise 25% of their apoprotein mass (45). After exposure to serum lipoproteins, the content of apoE and apoC in rat mesenteric lymph chylomicrons increases, while apoA-I content decreases (46).

Plasma levels of apoA-I have been reported by several groups that utilized either radioimmunoassay or electroimmunoassay techniques. Reported mean normal values range from 100 to 154 mg% for apoA-I, and 34–83 mg% for apoA-II (32, 33, 41, 47, 48) (Table 3). Differences in values reported may relate to whether or not the assay techniques incorporated a delipidation step and possible variability in antisera. Some groups have reported higher A apoprotein levels in females than in males (48), while others have reported nearly identical levels (32, 33, 41, 47).

ApoA-I and apoA-II plasma levels are less than 30% of normal in patients with LCAT deficiency and less than 25% of normal in untreated hyperchylomicronemic patients (33). Patients with familial HDL deficiency (Tangier disease) have apoA-I plasma levels that are 1% or less of normal, while apoA-II levels are around 7% of normal (33, 49, 50). Most of the apoA-I in these patients is found in the 1.21 g/ml infranatant, while significant amounts of the apoA-II are found in the 1.21 g/ml supernatant (33, 49, 50).

Plasma levels of apoA-I and apoA-II correlate with HDL cholesterol levels (32). Recently low HDL choles-

TABLE 3. Approximate metabolic parameters of plasma apoproteins in humans^a

Apoprotein	Mass Plasma Level	Mass Synthesis	Plasma Half-life	Possible Site of Catabolism
	mg%	mg/day	days	
A-I	120	450–600	5.0	Liver, kidney lysosomes
A-II	40	150–200	5.0	Liver, kidney lysosomes
B	90	850	3.0	Peripheral tissue, liver
C proteins	25	400	0.6	? Liver, peripheral tissue

^a From references 32–34, 36, 37, 48, 59, 73–75, 79–82, 92, 105, 133, 134.

terol levels have been associated with coronary artery disease in population studies (51–54), and it has been suggested that HDL may be important in removing cholesterol from tissue (55). HDL cholesterol levels are elevated in long distance runners (56) and in premenopausal females (57), and can be increased by estrogen (58) or nicotinic acid administration (59). They are decreased in hypertriglyceridemic patients (60) and in subjects on high carbohydrate diets (59, 60).

Metabolism

The site of A apoprotein synthesis in man remains to be determined. In the rat, liver perfusion studies indicate that the liver secretes particles in the HDL density range. These particles differ from plasma HDL in that they are much richer in apoE and poorer in apoA-I content. Incorporation of ^{14}C -labeled amino acids into HDL apoproteins by perfused liver is twelve times higher for apoE than it is for apoA-I when measured as cpm/mg protein (61). More recent rat liver perfusion data indicate that the liver can produce a nascent discoidal HDL particle, which has an apoE:apoA-I ratio of 10:1, while this ratio in spherical plasma HDL is 1:7 (62). Other studies support these observations (63, 64). Since both apoA-I and apoE are easily displaced from lipoprotein particles during ultracentrifugation, it is difficult to know whether the observed density distribution for these proteins is correct (41, 65). These findings, however, do suggest that the liver produces a nascent HDL particle rich in apoE and poor in apoA-I. These nascent particles are discoidal and are rich in free cholesterol, especially when LCAT is inhibited (62, 63).

ApoA-I has been identified as the major apoprotein of rat mesenteric lymph chylomicrons. Immunofluorescent studies indicate that rapid and marked increases in intestinal synthesis of apoA-I occur during lipid absorption (44, 66). This has been confirmed by ^3H leucine incorporation into lymph chylomicron apoA-I during active fat absorption (44). These findings are compatible with previous studies in the rat that suggest that the intestine is a major source of apoA-I (64). Recently it has been reported that human lymph chylomicron apoA-I and apoA-II can serve as precursors for plasma HDL apoA-II (43). After injection of ^{125}I -labeled lymph chylomicrons into plasma, over 90% of apoA-I and apoA-II radioactivity was recovered in HDL within one hour. Studies in the rat suggest that the intestine can produce both chylomicrons and HDL particles that are rich in apoA-I (66). Whether this density distribution exists in lymph fractions separated by means other than ultracentrifugation remains to be determined. The data presented are consistent with the concept that the A apolipoproteins

can enter the plasma on particles of intestinal origin.

Several studies have investigated the metabolic fate of HDL apoproteins in man. Reported plasma HDL half-life values in normal subjects range from 3.3 to 5.8 days (59, 67–69). The half-life of HDL apoproteins is similar if injected as apoHDL or as HDL (68). When injected as pure protein, 90% of the activity is recovered in the HDL fraction, indicating that apoHDL readily reassociates with HDL in plasma (68). ^{125}I -labeled HDL (1.09–1.21 g/ml) kinetics were recently studied in normal subjects and the specific activities of apoA-I and apoA-II in HDL (1.063–1.21 g/ml) were measured serially for at least 14 days. These studies showed that catabolism of apoA-I and of apoA-II within the HDL density range are identical (59). More recently it was reported that radioiodinated apoA-I is catabolized at a faster rate than apoA-II in plasma after intravenous injection of these apolipoproteins associated with HDL (70). However these investigators have subsequently indicated that labeled apoA-I, when reassociated with HDL, is catabolized at a more rapid rate than apoA-I labeled in intact HDL (71). The validity of in vitro labeling of HDL with isolated apoproteins remains to be established.

HDL catabolism is enhanced in nephrotic patients (67) and in hypertriglyceridemic subjects, especially those with hyperchylomicronemia (68). It is also increased in subjects on a high carbohydrate diet (59) and is markedly enhanced in patients with Tangier disease (72). It appears that changes in HDL catabolism may play a major role in regulating HDL levels in the plasma. A multicompartmental model (shown in Fig. 1) for HDL A apoprotein metabolism has been developed utilizing the SAAM computer program (59). This model was generated from plasma and urine radioactivity data collected during ^{125}I -labeled HDL kinetic studies in normal subjects, and it provides the best (least squares) fit for the data (59). The model consists of a plasma and a nonplasma compartment, with exchange between these compartments. In order to simultaneously fit both plasma and urine data, catabolism had to occur from both compartments. The third compartment represents the iodide pool. Almost all catabolism from the plasma and nonplasma compartments passes through the iodide pool. A very small fraction of catabolism decayed directly from the nonplasma compartment. The synthetic entry site could not be resolved from the data and the plasma compartment was arbitrarily chosen as the entry site.

Based on this analysis, mean HDL (1.09–1.21 g/ml) protein synthesis was 8.20 mg/kg per day in eight normal subjects on a balanced isocaloric diet (59). HDL synthesis was not affected in four normal subjects

subsequently studied on a high carbohydrate diet and was only slightly decreased in two subjects given nicotinic acid. Sixty-two percent of the HDL mass was in the plasma compartment and 38% was in the non-plasma compartment. The mean fractional catabolic rate (FCR) from the plasma space (0.113/day) was very similar to the nonplasma FCR (0.122/day). With an 80% carbohydrate diet, the rate of catabolism from the plasma compartment rose by 39.1%; with nicotinic acid treatment, it fell by 42.2%. Changes in the rate of catabolism from the nonplasma compartment were generally opposite to those from the plasma compartment, suggesting that these two catabolic pathways may be reciprocally related. Mean HDL protein (1.09–1.21 g/ml) mass decreased by 20% and mean HDL cholesterol decreased by 32% in the carbohydrate studies because of enhanced plasma catabolism. In the two nicotinic acid studies, HDL protein mass remained unchanged, while HDL cholesterol increased by 12%. In these two studies there was no significant change in catabolic flux (mg/kg per day) but a significantly greater amount was catabolized through the nonplasma pathway (59).

¹²⁵I-labeled HDL kinetic studies were recently completed in two Tangier homozygotes and two obligate Tangier heterozygotes (72). In heterozygotes mean HDL protein synthesis was in the normal range, and mean plasma FCR was about twice normal. ApoA-I and apoA-II HDL specific activity decay curves were parallel in heterozygotes, indicating identical catabolism of the two A apoproteins. In homozygotes HDL catabolism was very rapid; in contrast to normals and heterozygotes, apoA-I was catabolized at a significantly faster rate than apoA-II, and about 50% of the HDL activity was catabolized in the VLDL and LDL density ranges. Mean HDL protein (apoA-I and A-II) synthesis was just below normal in homozygotes, while apoA-I FCR was enhanced over 25-fold and apoA-II FCR was enhanced 8-fold.² These observations indicate that major alterations in HDL catabolism are present in patients with Tangier disease.

The factors that determine HDL catabolism remain to be clearly defined. Studies in animals indicate that liver and kidney lysosomes may play an important role in HDL catabolism (73–75). More recent research is consistent with the concept that extrahepatic tissues may play a predominant role in HDL catabolism (76, 77). What affects and regulates “plasma and non-plasma” catabolism? Why is HDL catabolism enhanced by carbohydrate feeding, and increased in hyperchylomicronemic subjects as well as in patients with

² Schaefer, E. J., R. I. Levy, and H. B. Brewer, Jr. Unpublished observations.

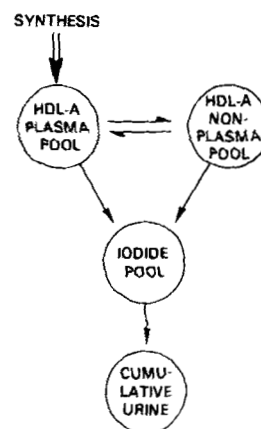


Fig. 1. Model for HDL A apoprotein metabolism.

Tangier disease? The relative contribution of the liver and intestine to human A apoprotein synthesis remains to be firmly established, as does the form in which the bulk of these apoproteins enter the plasma. The data presented are consistent with the concept that chylomicron A apolipoproteins are precursors for HDL A apolipoproteins (43), and that apoA-I and apoA-II are catabolized at similar rates within HDL (59). Finally, more research is required to delineate the composition and metabolism of the various lipoprotein species that appear to exist within the HDL density range (30).

APOPROTEIN B

Physical properties

Apoprotein B (apoB) is the least well characterized of the plasma apolipoproteins (1). Similar to membrane proteins, apoB is not readily soluble in aqueous solutions unless high concentrations of detergents are added (2). It is commonly believed that the detergent-solubilized protein is present in an aggregated form and does not enter either 7% or 10% polyacrylamide gels. The number of apoB molecules in lipoprotein particles is not known, since the exact molecular mass of apoB remains controversial; estimates in the range of 10,000–250,000 daltons have been reported (1). The amino acid composition of apoB is similar to that of other apolipoproteins. Carbohydrates contribute about 5–6% of the total mass of apoB and include mannose, galactose, fucose, glucose, glucosamine, and sialic acid (1). The sequence of one of the glycoside sidechains of apoB has been reported (78).

ApoB in lipoproteins

ApoB has distinct immunological properties and can be quantitated by immunoassay. The concentration of apoB in normal human plasma is between

70 and 100 mg%, mostly in LDL (79–82). ApoB is an integral protein constituent of three lipoprotein species: chylomicrons, very low density lipoproteins (VLDL), and low density lipoproteins (LDL, see Table 2). In abetalipoproteinemia, where apoB is not present in plasma, none of these species can be found (83). ApoB content in lymph chylomicrons and plasma VLDL and LDL averages about 20%, 40%, and 98%, of their total protein masses, respectively. The mass ratio of apoB to soluble proteins (apoC and apoE) increases in VLDL particles of decreasing S_f rates when prepared by centrifugation (84) and of decreasing diameter when prepared by agarose gel filtration (85).

The apoB mass per particle has been investigated in VLDL and LDL subfractions (1, 84, 86). The absolute mass contribution of apoB to VLDL particles of S_f -derived molecular masses ranging from 30.9 to 7.7 million daltons was practically identical. It was similar to an LDL preparation (median particle mass of 2.14 million daltons). The apoB mass contribution to LDL particles with molecular masses varying between 1.8 and 5 million daltons was also identical (87). On the basis of these observations, the apoB content in different VLDL and LDL particles appears to be constant and independent of particle weight and of other lipid and protein constituents. Whether this holds for chylomicrons is unknown since the mass contribution of apoB to chylomicrons of known particle dimension, density, or weight has not yet been reported.

Metabolism

ApoB has been shown to be synthesized in both the liver and intestine in the rat (61–64) and enters the circulation with either chylomicrons or VLDL. These observations appear to be true for man as well. Recently apoB has been identified in human intestine mucosal cells by immunological techniques (88). ApoB appears to be essential for the transport of triglyceride out of the liver and intestine. When no apoB is present in plasma, as in patients with abetalipoproteinemia, no triglyceride enters the bloodstream despite excessive amounts of intracellular triglyceride (83).

The concept that a protein moiety of VLDL may be a precursor of the protein moiety of LDL was suggested by studies carried out in the late 1950's by Volwiler et al. (89) and Gitlin et al. (67). A specific demonstration of the metabolic decay of apoB from VLDL to LDL was not achieved until the late 1960's, using VLDL labeled with ^{125}I in its protein moiety (1, 86, 90, 91). When human plasma VLDL is incubated either with whole plasma or with isolated lipoproteins, the apoB in VLDL does not exchange or

transfer with apoB in other lipoproteins. Following injection of ^{125}I -labeled VLDL into humans, all apoB activity is initially found in VLDL. At later time intervals, apoB activity appears in intermediate density lipoprotein (IDL, 1.006–1.091 g/ml) and finally in LDL, indicating a precursor–product relationship between VLDL-B and LDL-B. Since apoB does not appear in VLDL when labeled LDL is injected into humans (92), these findings unequivocally establish a unidirectional pathway whereby VLDL is delipidated to form LDL.

This lipoprotein interconversion process is dependent upon lipoprotein lipases. Activation of the lipoprotein lipase system by the injection of heparin greatly accelerates the transfer of apoB from large VLDL particles to smaller particles, and eventually to IDL (1, 86). Analysis of small VLDL particles, of S_f 20–60 isolated after injection of ^{125}I -labeled VLDL and heparin into humans, reveals that these immediate products of enhanced lipolysis are almost indistinguishable from IDL particles (S_f interval 12–20) (86). Therefore, one can define various apoB-containing lipoproteins in physiologic rather than operational (density) terms. Accordingly, the intermediate lipoproteins, defined as the immediate products of VLDL lipolysis, are formed following the interaction of VLDL with lipoprotein lipase. The density range of the intermediate lipoprotein thus defined encompasses the lipoprotein particles present in the S_f interval 20–60 as well as those in the S_f interval 12–20 (IDL). LDL is the end product of further delipidation of intermediate lipoprotein particles. The possible contribution of chylomicrons to the apoB subsystem and the possibility that, within each lipoprotein density range, apoB is present in more than one type of particle, or family of particles, remain to be definitively examined.

Particles similar to intermediate lipoproteins can be formed *in vitro* after incubation of VLDL with lipoprotein lipase-rich (postheparin) plasma (93, 94) or purified lipoprotein lipase (95, 96). More recently, an LDL-like particle was similarly produced (96). These *in vitro*-produced particles are poor in apoC (*vide infra*) and contain all the apoB present in the precursor VLDL particles. During lipolysis, precursor particles lose triglyceride, as well as some phospholipids (predominantly phosphatidylcholine) and cholesterol (predominantly unesterified cholesterol). When the contents of lipid and protein constituents of VLDL and postlipolysis VLDL are combined in terms of surface (phospholipids, unesterified cholesterol, and apoproteins) and core (triglyceride and cholesterol esters) concentration and volume, respectively, both particles are surprisingly similar in their

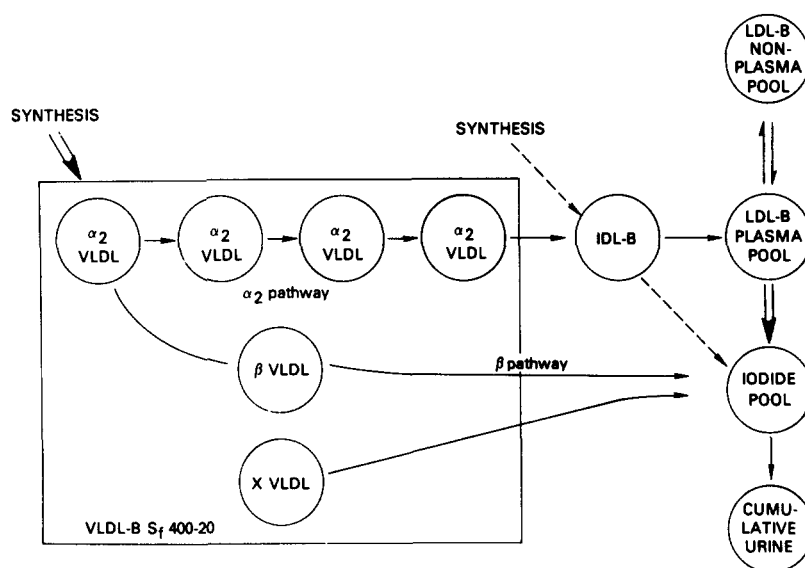


Fig. 2. Model for VLDL-LDL B apoprotein metabolism.

core lipid model structure (1). Thus, the concentration of polar or amphipathic constituents at the surface of the two lipoproteins is similar, and the calculated volume of apolar lipids agrees with the calculated core volume. It seems, therefore, that the interconversion process represents a physico-chemical sequence of substrate-enzyme interaction. Whether a similar sequence of metabolism of VLDL takes place *in vivo* is yet to be determined. However, studies employing *in situ* systems (the supradiaphragmatic rat) (97) and the isolated perfused rat heart (98, 99) have revealed essentially similar changes in VLDL composition and structure during lipolysis initiated by membrane-supported lipase. Recent data are consistent with the concept that "intermediate" lipoproteins are present in both normal and hyperlipidemic subjects without injection of heparin (100). A compositional comparison of these particles with those produced with heparin injection would be interesting.

The biological behavior of the apoB subsystem in rats has also been evaluated using either ^{125}I -labeled or ^{14}C -labeled VLDL (101-103). Similar to the observations in humans, the apoB moiety of rat plasma VLDL is a precursor of the small amount of rat plasma LDL. However, it was found that only a small fraction (10-20%) of the apoB present in VLDL is converted to LDL, whereas the bulk of apoB in VLDL is removed directly from the circulation. In these studies, the liver was the major site of VLDL-B catabolism. Using electron microscopic radioautography, the lipoproteins were found to concentrate at the hepatocyte surface membrane prior to internalization and degradation, indicating possible binding of the particles to a specific

receptor site (104). The studies described above in the rat have shed light on an unsuspected mechanism of regulation of plasma LDL levels, i.e., clearance of VLDL intermediates from the plasma prior to their conversion to LDL. This mechanism doubtless explains the low levels of LDL in rats. The importance of this pathway in other species, including man, remains to be determined. Interference with such a clearance pathway might explain the paradoxical rise in LDL seen in human subjects when hypertriglyceridemia is treated with clofibrate (57).

^{125}I -labeled VLDL metabolism in man has been studied by several investigators. Precise kinetic analysis of the VLDL-B \rightarrow IDL-B \rightarrow LDL-B interconversion is possible only with the use of a multi-compartmental approach. A computer-derived multicompartamental model of the kinetics of apoB in man was derived from the analysis of ^{125}I -labeled VLDL metabolic data studies in 14 subjects (86, 90, 91, 105) (See Fig. 2). The study population consisted of four normal individuals, two type I patients, one type I patient on 60 g/day of medium chain triglycerides (MCT), one patient with type IIb hyperlipoproteinemia, five patients with type III hyperlipoproteinemia, and one type IV patient. Utilizing the VLDL-LDL-B model, kinetic parameters, including transport rates, masses, and rate of synthesis and degradation of apoB were obtained (105).

The kinetics of VLDL-B could not be described by a simple one- or two-compartmental model. At least four compartments were necessary along the normal path of delipidation of VLDL (α_2 path) in order to satisfy the disappearance curves of apoB from

VLDL in normal individuals. An additional VLDL delipidation path (β path) had to be incorporated into the model in order to satisfy the kinetics of VLDL-B in subjects with type III hyperlipoproteinemia. The β path as shown originates from newly secreted VLDL particles. It may, however, be generated at a later step along the normal delipidation chain (α_2 path). The unique features of VLDL-B particles metabolized through the β path were their slow rate of disappearance from the VLDL and IDL density ranges (about one-eighth of that of VLDL-B catabolized via the α_2 path) and their decay directly to catabolic sites, bypassing the LDL density range. In addition, an X-VLDL compartment containing labeled VLDL had to be incorporated into the model to account for rapid decay of labeled apoB from VLDL directly into the iodide pool. Whether X-VLDL represents VLDL particles damaged during preparation or native VLDL-B particles that are directly catabolized from the VLDL density range could not be resolved by the analysis (105).

The apoB moiety of IDL originates from α_2 -VLDL particles and is a direct product of the α_2 path. Direct synthesis of apoB into IDL exists as a possible pathway in the model. ApoB in IDL may be further catabolized by two routes: conversion to LDL or direct removal from the circulation. The kinetics of LDL-B derived from IDL-B can be analyzed using a two-compartmental LDL-B model. ApoB kinetic parameters differ considerably when normal and hyperlipoproteinemic subjects are compared. The α_2 -VLDL-B to IDL-B transport rate in normal subjects varied from 2.8 to 6.2/day (fraction of pool/day), but was slow (0.7–1.4/day) in all hypertriglyceridemic patients. The IDL-B to LDL-B transport rate was similar to the α_2 -VLDL-B to IDL-B transport rate, and was also decreased in hypertriglyceridemic subjects. In all patients, but not in normals, a direct catabolic route of IDL-B from the plasma, bypassing LDL-B, was present. A significant amount (up to 30%) of the VLDL-B was transferred through the β -delipidation path, yielding β -VLDL particles in type III patients. The fractional catabolic rate of β -VLDL-B was similar to LDL-B and varied between 0.4 and 0.8 of the pool/day (105).

The values of apoB synthesis in normals varied between 7.13 and 11.36 mg/kg per day and in patients (excluding type III and type I on MCT) between 4.63 and 11.40 mg/kg per day. All apoB synthesis in normals was via the α_2 -VLDL pathway, as it was for two type I patients and the type IIb and type IV patients. However, in all type III patients (treated and untreated) and in the type I patient on 60 g/day of MCT, a second major route of apoB synthesis through IDL was observed. In some type III patients, the direct IDL-B

synthesis exceeded that of α_2 -VLDL-B synthesis, and the average IDL-B synthesis was 7.08 mg/kg per day as compared to 11.35 mg/kg per day for α_2 -VLDL. Total apoB synthesis was increased (mean, 18.46 mg/kg per day) in all type III patients. Direct apoB catabolism occurred from three lipoprotein classes: 1) LDL (all subjects), 2) IDL (patients only), and 3) β -VLDL (type III patients only). LDL-B degradation did not show any consistent pattern among patients or between patients and normals except that it was decreased in the one type II patient. IDL-B degradation was higher in the type III patients and the type I patient on MCT (range, 321–973 mg/day), than in the other patients (range, 38–160 mg/day).

The computer-based analysis delineated several features unique to type III patients: high apoB synthesis in α_2 -VLDL, direct synthesis of apoB in the form of IDL, and formation of β -VLDL through the β -delipidation path. These abnormalities resulted in elevated apoB synthesis (twice normal) and in a significant contribution by slowly catabolized β -VLDL to the total lipoprotein mass present in the VLDL and IDL density ranges. Type III patients also exhibited a slow rate of α_2 -VLDL delipidation, slow transport of apoB from IDL to LDL, and direct degradation of apoB from IDL, bypassing LDL. These latter features, however, were shared by all other hypertriglyceridemic patients. In a cross-over experiment between type III and type IV patients, the kinetic parameters of the injected VLDL reflected the metabolic state of the recipient individual rather than those of the VLDL donor. It was concluded, therefore, that type III disease is due to a defect in lipoprotein metabolism rather than to a structural difference in the lipoproteins themselves. How to reconcile this conclusion with the recently reported quantitative and qualitative changes of the apoE system in VLDL in type III subjects (106) is as yet unknown. This question can only be resolved when apoE kinetics in normal and hyperlipoproteinemic subjects are examined.

¹²⁵I-labeled VLDL apoB kinetics have recently been compared with ¹³¹I-labeled LDL kinetics in a variety of subjects by two groups of investigators (107–110). Kinetic parameters from published data are summarized in **Table 4**. Since these parameters were derived assuming one VLDL-B pool, it is difficult to compare these results with the data just cited. However, similar to the data obtained from multicompartmental analysis, all VLDL-B was converted to LDL in normals and this pathway accounted for all LDL synthesis. Degradation of VLDL-B without conversion to LDL-B was a feature in many of the hyperlipoproteinemic subjects in these studies as well (107, 108). Conversely, in two FH homozygotes, a significant

TABLE 4. Apoprotein B kinetic parameters

	VLDL-B Synthesis	LDL-B Synthesis	LDL-B Fractional Catabolic Rate	Study
	mg/kg/day	mg/kg/day	day ⁻¹	
Normals (10)		14.4	0.46	Langer et al. (92)
Type IIA Heterozygotes (10)		15.0	0.24	
Normals (5)		11.6	0.31	Packard et al. (112)
Type IIA Heterozygotes (3) ^a		14.6	0.17	
Type IIB Heterozygotes (3) ^a		16.6	0.15	
Normals (4)		19.6	0.52	Simons et al. (111)
Type IIA Homozygotes (4)		33.9	0.20	
Type II Homozygotes (2)	10.7	18.6		Thompson et al. (114)
Type II Heterozygote (1)	13.0	14.8		
Normals (7)	15.3	12.2	0.41	Sigurdsson et al. (107–109)
Type II Heterozygotes (2)	13.0	12.7	0.18	
Type V	33.1	13.6	0.65	

^a Members of same family.

amount of VLDL-independent apoB synthesis was necessary to account for all LDL-B synthesis (110).

Numerous LDL kinetic studies have been carried out (67, 89, 92, 107–116). The kinetic behavior of labeled LDL-B is best described by a simple two-compartmental model consisting of a plasma and a non-plasma compartment (see Fig. 2). This model predicts that synthesis and degradation of LDL-B occurs from the plasma compartment and that LDL-B is reversibly distributed and freely exchangeable between the compartments. The kinetic parameters of LDL-B, as derived from a two-compartmental analysis of turnover studies of ¹²⁵I-labeled LDL in normal humans and patients with familial hypercholesterolemia (FH), are presented in Table 4. These data indicate that patients with FH have a defect in LDL-B catabolism. This catabolic defect in FH patients appears to be independent of changes in LDL pool size (113, 114). LDL-B synthesis appears to be increased in FH homozygotes and to be normal or only slightly increased in FH heterozygotes (92, 108–112). LDL metabolic studies in one FH homozygote before and after portacaval shunt are consistent with the concept that this procedure decreases LDL synthesis (115). Nicotinic acid also appears to decrease LDL synthesis (113), while cholestyramine enhances LDL catabolism in FH patients (116).

ApoB appears to be intimately involved in the VLDL–LDL delipidation cascade. Throughout this pathway, smaller and heavier particles are formed from larger and lighter lipoprotein particles. An important facet of the interconversion process is that each product particle contains all the apoB molecules present in the precursor particle. Therefore, as the particles become smaller, they become relatively enriched with

apoB. It can be calculated that the mean concentration of apoB in an average LDL particle (mol/unit surface area) is several times greater than in an average VLDL particle. The possible physiologic implication of this increased concentration is not clear; it may play an important role in the eventual catabolism, including the interaction of the lipoprotein with enzymes or tissue cell surface receptors. The specific site(s) of LDL removal in man is not clear. Hepatectomy in pigs does not retard LDL-B catabolism (117), suggesting that peripheral tissues rather than the liver are important in LDL catabolism. Data from rat studies are also consistent with this concept (118). Several cell types in tissue culture have been shown to have specific surface receptor sites to bind and eventually catabolize LDL (119, 120). Coincident with binding of LDL to its receptor site there is suppression of 3-hydroxy-3-methylglutaryl CoA enzyme activity (the rate-limiting enzyme in cholesterol synthesis) within the fibroblast. It has been reported that FH patients are deficient in or lack the “high affinity” LDL receptor site (119, 120). Recently an FH patient has been identified who is unable to internalize LDL into the fibroblast following binding to the receptor (121). LDL receptor studies have recently been reviewed (122). The suggestion has been made that FH homozygotes metabolize LDL solely via a scavenger pathway, the nature of which remains to be elucidated.

The data discussed above delineate the major pathways of VLDL–LDL apoB metabolism in man. ApoB enters the circulation with VLDL and is converted to LDL through a chain of delipidation steps, LDL being the final breakdown product of VLDL metabolism. The clearance of apoB from the circulation occurs in the form of LDL particles. In dyslipoproteinemic

states it appears that variable amounts of apoB may enter the circulation with either IDL or LDL, and that variable amounts of apoB may be cleared directly from the plasma while in the VLDL or IDL density range.

C APOPROTEINS

Physical properties

The C apoproteins are a group of low molecular weight proteins present in plasma lipoproteins. In the human, three distinct proteins comprise this group: apoC-I, apoC-II, and apoC-III. The amino acid sequences of all three proteins have been reported (13–16). The latter protein appears in three different forms containing two moles of sialic acid per mole of protein, one mole, or none, respectively. All three apoproteins are soluble in aqueous solutions. The C apoproteins avidly bind phospholipids above their transition temperature and form protein–lipid complexes with either phosphatidylcholine, sphingomyelin, or lysolecithin (5, 123–126).

The C apoproteins play an exceedingly important role in the metabolism of triglyceride-rich lipoproteins. ApoC-II is a specific protein cofactor necessary for triglyceride hydrolysis by lipoprotein lipases of extrahepatic origin (127–129). Phospholipids also appear to be necessary for optimal lipolysis. The presence of a lipoprotein lipase activated specifically by apoC-I has also been reported (130, 131). ApoC-I has also been reported to activate LCAT (2). ApoC-III may inhibit lipoprotein lipase (132), although all three of the C apoproteins may partially inhibit triglyceride hydrolysis when present in surplus amounts. Because of their impressive role in the metabolism of lipoproteins, the C apoproteins are sometimes referred to as the “functional apoproteins” of the plasma lipoproteins.

ApoC in lipoproteins

ApoC-II and apoC-III levels in normal plasma are approximately 5 mg/dl and 14 mg/dl, respectively (133, 134). The levels of both these apoproteins are increased in hypertriglyceridemic subjects. However, the amount of apoC-II per mg VLDL protein and its lipoprotein lipase activation per mg VLDL protein are reduced in hypertriglyceridemic patients, which may account for the impaired VLDL catabolism noted in these subjects (133). Recently a patient with hyperlipidemia secondary to marked apoC-II deficiency has been reported (135). Plasma apoC-I levels have not been reported, but lipoprotein composition studies suggest that apoC-I levels may be similar to those of

apoC-II. Variable amounts of apoC can be found with all plasma lipoproteins. ApoC comprises more than 60% of the total protein mass of chylomicrons, 40–80% of the VLDL apoproteins, 2–10% of the HDL apoproteins, and is also present in trace amounts in the LDL density range (1). The relative contribution of apoC to the protein mass of several different VLDL density subfractions shows that smaller, denser particles contain less apoC relative to apoB than do larger, lighter particles. Very small amounts of apoC relative to apoB are found in LDL (1). Since LDL is formed from VLDL, almost all of the apoC molecules present in a VLDL particle are removed from the lipoprotein during the formation of LDL.

In fasting-human plasma, the C apoproteins are mainly found in VLDL and HDL, predominantly HDL₂ (1.063–1.125 g/ml) (29). ApoC constitutes about 50% of the total VLDL protein and only 5% of HDL protein; however, its mass is evenly divided between the two lipoprotein classes in the fasting state. This even distribution is due to the HDL protein mass (about 150 mg%) as compared to the VLDL protein mass (about 10 mg%) in fasting plasma. In hypertriglyceridemic subjects, the C apoproteins are redistributed from HDL to VLDL (133).

Many experiments have demonstrated that after incubation of VLDL and HDL *in vitro*, labeled apoC molecules introduced with one of the lipoproteins distribute to the other, unlabeled lipoprotein (86, 90, 91). Upon induction of alimentary lipidemia, apoC (measured as the lipoprotein lipase activator) moves from HDL to chylomicrons (136). Chylomicron clearance is associated with transfer of the activator protein back to HDL. Abrupt initiation of lipolysis following the injection of heparin into humans previously injected with ¹²⁵I-labeled VLDL causes a prompt decrease of plasma VLDL triglyceride levels and a transfer of labeled apoC-II and apoC-III to HDL (apoC-I was not labeled in these experiments and could not be followed) (86). ApoC-II and apoC-III were transferred back to VLDL when newly secreted VLDL particles were again found in the circulation 6 hr after the injection. These studies suggest that apoC molecules are transferred from chylomicrons and VLDL to HDL and vice versa during secretion and degradation of triglyceride-rich lipoproteins.

Metabolism

We will discuss apoC as one entity, because we currently lack sufficient data to delineate the metabolism of the individual C apoproteins. Relatively little is known about synthesis and degradation of apoC in man. Experiments carried out in rats have shown that very little, if any, apoC is secreted with newly synthe-

sized lipoproteins of intestinal origin (64). ApoC is definitely synthesized and secreted by the liver (64). When the liver is perfused with radioactive amino acids, both VLDL and HDL isolated from liver perfusate contain labeled apoC. Whether apoC is secreted from the liver with VLDL or HDL or both is not clear. ApoC is found in the plasma of patients with either abetalipoproteinemia or familial HDL deficiency (Tangier disease) (50, 83). These observations as well as the disparate parameters of apoC (Table 3) suggest that apoC metabolism is independent of that of apoB and apoA. In rats, apoC can be identified in VLDL particles isolated from Golgi cisternae (137). It seems, therefore, that apoC molecules are channeled from their intracellular site of synthesis to the Golgi, where they become associated with nascent lipoprotein particles present in this system. Since the distribution of labeled apoC between VLDL and HDL parallels the relative mass of apoC in these lipoproteins, it has been postulated that this represents an exchange phenomenon.

When ^{125}I -labeled VLDL is incubated with either plasma or HDL, VLDL apoC specific activity decreases and, simultaneously, an increase in HDL apoC specific activity occurs (93, 138). The manner in which this exchange takes place has not been elucidated; it appears to be independent of phospholipid exchange between VLDL and HDL (138), but the possibility that apoC exchanges as apoprotein cannot be excluded. In vitro, the amount of apoC transferred from VLDL to HDL during incubation of ^{125}I -labeled VLDL with lipoprotein lipase-rich (postheparin) plasma was found to be proportional to the extent of triglyceride hydrolysis (93). Since the specific activity of ^{125}I -labeled apoC in the VLDL during the incubation was similar to that of particles incubated with normal plasma, the transfer of apoC from VLDL to HDL seen with lipolysis must have been due to a net transfer. A chemical analysis of postlipolysis VLDL after approximately 80% of VLDL triglyceride was hydrolyzed disclosed that the number of apoC molecules in the product was only 7% of that in the initial particles. Assuming that all of the apoC molecules are present at the surface of either intact or postlipolysis particles, then the calculated concentration of apoC (moles/unit surface area) decreased to about one-fifth of the original concentration during lipolysis. None of the apoB in VLDL is removed from the particles during lipolysis, and the calculated concentration of apoB at the surface of these particles increases 3-fold. Therefore, the molar ratio of apoB to apoC at the surface of a VLDL particle may increase 15-fold or more during lipolysis (94). ApoB and apoC in lipoproteins may play important roles in different metabolic pathways (i.e., inter-

action with tissue cells and interaction with lipoprotein lipase sites, respectively). The changing pattern of apoB to apoC at the lipoprotein surface as observed during lipolysis may determine subsequent lipoprotein catabolism through a variety of metabolic pathways.

The physicochemical basis of the association, dissociation, and exchange of apoC among lipoproteins is as yet poorly understood. Although apoC forms stable complexes with phospholipids, it has not yet been demonstrated that similar complexes are present in lipoproteins or are produced during their metabolism. An attempt to isolate apoC subunits, and to investigate some of the determinants of the associations of apoC with triglyceride-rich lipoproteins, was undertaken recently. VLDL- ^{125}I -labeled apoC was incubated with purified lipoprotein lipase in the absence of plasma and lipoproteins. ApoC units were removed from the VLDL density range and were found in the aqueous phase of an albumin-containing buffer (95). Thus, although partially degraded particles were the only lipoproteins present in the mixture, apoC was found in a lipoprotein-free form. Since the presence of plasma in the incubation system did not affect the magnitude of removal of apoC from VLDL when related to the degree of lipolysis, it was concluded that the dissociation of apoC was solely dependent on the features of the partially hydrolyzed particles. Part of the apoC found in the albumin-buffer phase was probably associated with lipids, since it was isolated with the fraction of d 1.04–1.21 g/ml. The remainder was poorly lipidated and was found in the fraction of $d > 1.21$ g/ml (95).

In subsequent studies, VLDL labeled biosynthetically with ^{32}P -labeled phospholipids, ^3H cholesterol, ^{14}C -labeled fatty acid triglycerides, and ^{125}I -labeled apoC was used. Upon initiation of lipolysis, it was found that the fate of lipids and apoproteins was complex.³ Some of the phospholipids (predominantly lecithin) were hydrolyzed to lysocompounds and were found associated with the albumin-buffer fraction of $d > 1.21$ g/ml. Another part of the VLDL phospholipids containing both lecithin and sphingomyelin was removed from the VLDL density range and was recovered in the density range of 1.04–1.21 g/ml. Only small amounts of cholesterol were removed from VLDL when less than 50% of the triglycerides were hydrolyzed. With more pronounced lipolysis, however, cholesterol, predominantly in the unesterified form, was also removed from VLDL to the buffer fraction of d 1.04–1.21 g/ml. These studies suggest that apoC removal from triglyceride-rich lipoproteins occurs, at

³ Eisenberg, S. Unpublished observations.

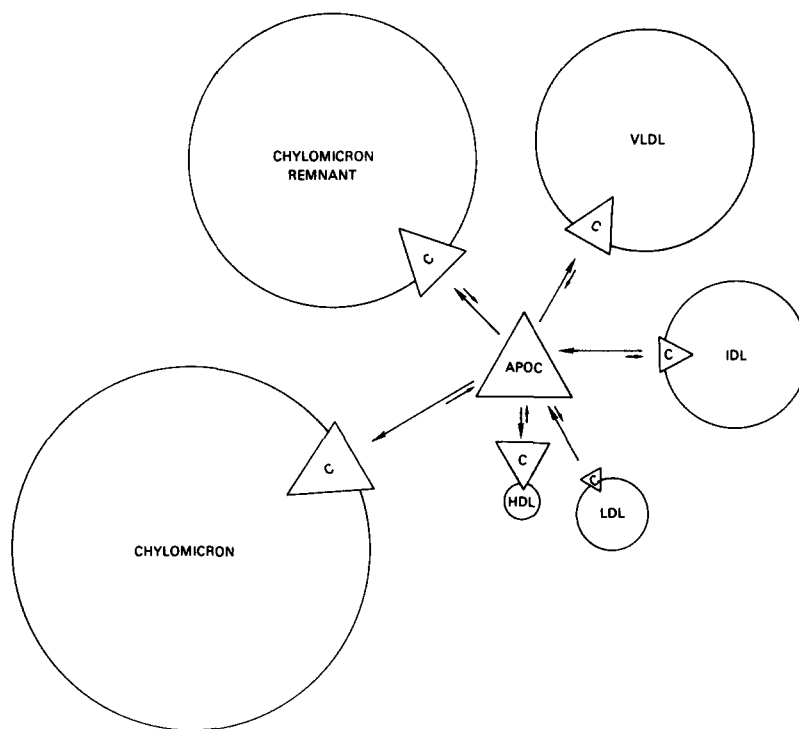


Fig. 3. C apoprotein interactions with lipoproteins.

least in part, in the form of a small lipid-protein complex of d 1.04–1.21 g/ml. In the presence of plasma, the apoC units removed from VLDL are found predominantly with HDL, presumably due to protein-lipoprotein interaction. More recently, very similar sequences of removal of lipid and apoprotein constituents were observed with a membrane-supported lipoprotein lipase system, using labeled VLDL and the isolated perfused rat heart (97).

On the basis of all these observations, we suggest that the C apoproteins are in a dynamic association-dissociation equilibrium with lipoprotein particles. Since apoC can definitely be displaced from lipoproteins to an aqueous environment, we suggest that its equilibrium among lipoproteins takes place through a water-soluble form. According to this view, a specific configuration of lipids at the surface of lipoproteins determines the association constant. Triglyceride-rich lipoproteins have a high affinity for apoC molecules; upon lipolysis, the association constant becomes progressively smaller and is the smallest with LDL. A schematic representation of possible apoC lipoprotein interactions is shown in Fig. 3.

The kinetics of apoC metabolism in human plasma lipoproteins have been studied using radioiodinated VLDL as the tracer lipoprotein (1, 86, 90, 91, 105). Polyacrylamide gel electrophoresis was used to follow radioactivity decay of the two labeled apoC moieties,

apoC-II and apoC-III. A multicompartamental model (Fig. 4) for apoC metabolism was derived from the analysis of ^{125}I -labeled VLDL metabolic studies in normal and hyperlipoproteinemic subjects. The analysis of the data was carried out in conjunction with labeled apoB and a complete agreement between the apoB and apoC systems was therefore required (105). Within the resolution of the data, the model consists of four compartments in VLDL, identical to the normal VLDL α_2 delipidation path; and IDL compartment; and two HDL compartments, plasma and non-plasma. ApoC exchange occurs only between HDL and the first compartment of VLDL in this model. In all subsequent delipidation compartments along the α_2 path, apoC is assumed to be transferred to HDL. ApoC synthesis may take place through either VLDL or HDL or both; apoC clearance takes place from HDL. An interesting feature of the analysis is an initial disappearance of labeled apoC from the plasma compartment, as part of the injected X-VLDL material (vide supra), and its reappearance in the circulation at later time intervals, associated with newly formed chylomicron or VLDL particles.

Based on the model, the fractional catabolic rate of plasma apoC varied between 1.5 and 2.5/day, with an apoC plasma half-life ranging from 10 to 18 hr (90, 91, 105). Synthesis varied between 100 and 400 mg/day if apoC entered as VLDL, or between 150

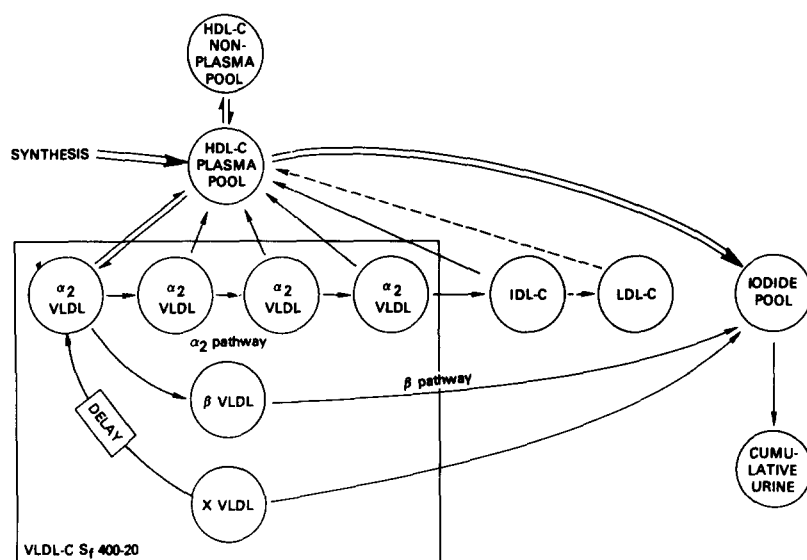


Fig. 4. Model for C apoprotein metabolism.

and 660 mg/day if the apoC synthetic entry site was HDL. No consistent differences in apoC synthesis or catabolism were noted between normal and hyperlipoproteinemic subjects (105).

Little is known about the degradation of apoC. Since intermediate lipoprotein particles and LDL contain very little apoC, these two lipoproteins seem to play a small role in the clearance of apoC units from the circulation. HDL may serve as a major vehicle for the clearance of apoC. However, the half-life in the normal human circulation of apoC is less than 24 hr, whereas that of apoA-I and apoA-II is about 5 days (See Table 3). A possible explanation for the discrepancy between the circulating half-lives of apoC and apoA is that apoC is preferentially associated with HDL particles of very short lifetime. There are no data as yet to support this hypothesis. Alternatively, it is possible that apoC units are cleared from the circulation independently of either apoB or apoA. According to this hypothesis, the clearance of apoC may take place at the stage of transfer between triglyceride-rich lipoproteins and HDL or as water-soluble forms. Although this hypothesis is obviously compatible with the available data on the clearance of apoC from the circulation, it has not yet been tested experimentally.

In contrast to apoB, the C apoproteins appear to move readily between HDL and newly synthesized triglyceride-rich particles. The liver is the major site of apoC synthesis (64). Whether apoC enters the circulation with VLDL, HDL, or both remains to be definitively determined. The sites of apoC catabolism are also unknown. Finally, it is not unreasonable to assume that the individual C apoproteins are metabolically distinct. Studies on the kinetics of each C apoprotein

will hopefully enable us to more fully understand the role of each of these proteins in lipoprotein metabolism.

APOPROTEIN E

Physical properties

The arginine-rich protein, or apoprotein E (apoE), as isolated from human plasma VLDL, has a molecular mass of approximately 33,000 daltons. It is rich in arginine (about 10% of total amino acids) (139–142) (See Table 1). It is present in several polymorphic forms, as separated by either ion exchange chromatography (142) or isoelectric focusing (106). The composition and structural differences between the different forms of apoE have not yet been defined.

ApoE in lipoproteins

ApoE levels in normal human plasma are approximately 10 mg% as measured by electroimmunoassay, and they are increased two-fold in patients with Type III and Type V hyperlipoproteinemia (37, 143). ApoE is found in both VLDL and HDL, and it constitutes about 13% of VLDL protein mass in man (Table 2) (85). ApoE seems to be primarily associated with VLDL of slow electrophoretic mobility (β -VLDL) and small particle size (85, 100) and with HDL₂ (65). It comprises about 20–25% of rat HDL protein. ApoE levels in rat plasma have been measured by radioimmunoassay (62, 144). A large proportion of apoE in lipoproteins (30–40%) is displaced upon a single ultracentrifugation and is found with the water-soluble proteins of $d > 1.21$ g/ml (65, 144).

ApoE is elevated in VLDL isolated from subjects

with type III hyperlipoproteinemia (141) and is also increased in abnormal lipoproteins isolated from cholesterol-fed animals (β -VLDL, HDL-C) (145). A VLDL fraction of β -mobility (β -VLDL), rich in cholesteryl esters and poor in triglycerides, constitutes the predominant pathological lipoprotein found in the plasma of patients with type III hyperlipoproteinemia (141). ApoE constitutes about 20% of the apoprotein mass of β -VLDL. Further separation of apoE into constituents (E-I, E-II, and E-III) can be achieved by isoelectric focusing (106). Of interest is the report that β -VLDL from type III patients is deficient in apoE-III, a constituent that comprises 39% of the total apoE mass of normal VLDL (106).

Metabolism

In nascent lipoproteins isolated from the perfused rat liver, apoE constitutes more than 50% of the total HDL protein mass and is also found in significant amounts in VLDL (61–63). When LCAT is inhibited, most of the apoE is found in HDL (63). In humans with alcoholic hepatitis and LCAT deficiency, HDL levels are decreased and apoE constitutes 40% of the HDL protein mass (146, 147). No apoE is found in VLDL. When plasma from LCAT-deficient patients is incubated with LCAT, apoE is transferred from HDL to VLDL. Similarly, after recovery from hepatitis, the apoE content in HDL protein of these patients fell to 1% and normal amounts of apoE were found in VLDL (146). These *in vivo* changes were associated with an increase in HDL levels.

When VLDL is exposed to lipoprotein lipase, much of the apoE appears to remain with the partially degraded particles. About 70% of the proteins of an apoE-rich fraction were still found associated with post-lipolysis particles (93, 94). ApoE content is increased in VLDL of slow pre-beta mobility in normal human plasma (100, 141). ApoE, therefore, may contribute significantly to the apoprotein content of intermediate lipoproteins. Yet apoE is found in only minute amounts in LDL. Evidently, apoE is removed from the intermediate lipoprotein density range prior to the formation of LDL. Whether apoE is present in all IDL particles and is specifically removed during LDL formation, or is present in especially high amounts in some IDL particles that are cleared from the circulation and do not subsequently form LDL, is not known. In rats, degradation of radioiodinated VLDL is accompanied by a transfer of apoE activity to HDL, as determined by polyacrylamide gel electrophoresis (101, 102); however, the extent of this transfer is impossible to assess. It is interesting to note that when a radioiodinated human VLDL apoprotein fraction rich in apoE is mixed with human plasma, about 20% of the

radioactivity is found associated with VLDL, 50% with HDL, and 30% with the plasma protein fraction of $d > 1.21$ g/ml (91). In the rat, an intravenous injection of a similar apoprotein fraction resulted in a distribution of radioactivity of about 15%, 30%, and 55% among VLDL, HDL, and the 1.21 g/ml infranantant, respectively (101). Recent studies in man are consistent with the concept that apoE turnover is similar to apoC turnover within VLDL (148).

These studies suggest that apoE enters the circulation with nascent HDL particles and is then transferred to VLDL during the cholesterol esterification process. ApoE may then recycle back to HDL, so that its metabolism in plasma may be similar to that of the C apoproteins. What role apoE plays in the metabolism of triglyceride-rich VLDL or IDL particles remains to be determined. Further apoE kinetic studies will hopefully help to define apoE metabolism.

OTHER APOPROTEINS

Apolipoprotein D, also known as “thin-line peptide,” has been isolated from HDL₃ and has a molecular mass of 22,700 daltons (149). Together with lipid components, apoD forms its own lipoprotein particle (36). It is a minor protein constituent within the HDL density range. An additional apoprotein, apoA-III, also designated as thin-line peptide has been isolated from HDL (150). ApoA-III differs from apoD in that cystine is absent in this protein. ApoA-III has been reported to activate LCAT (151). Recently a new apolipoprotein (F) has been reported within HDL, of molecular mass 26,000–30,000 daltons (152). ApoF is reported to carry its own complement of lipid and to form a distinct lipoprotein species within HDL. Further work is required in order to define the composition and metabolism of these apoproteins.

CONCLUSIONS

The many observations cited above clearly reveal that the plasma lipoproteins are undergoing dynamic and constant change as they travel through the vascular system. The apoprotein content of both newly formed VLDL and chylomicrons changes dramatically as these lipoproteins enter the bloodstream and interact with other circulating lipoproteins (43, 46, 86, 90, 91). Both species appear to pick up apoprotein C and E. Then as lipolysis occurs and the triglyceride content of the particles rapidly diminishes, VLDL and chylomicrons release much of the C (and ?E) apoprotein (43, 86, 93, 136). During this active process the B apoprotein appears to remain with its parent particle and thereby comprises an increasingly larger por-

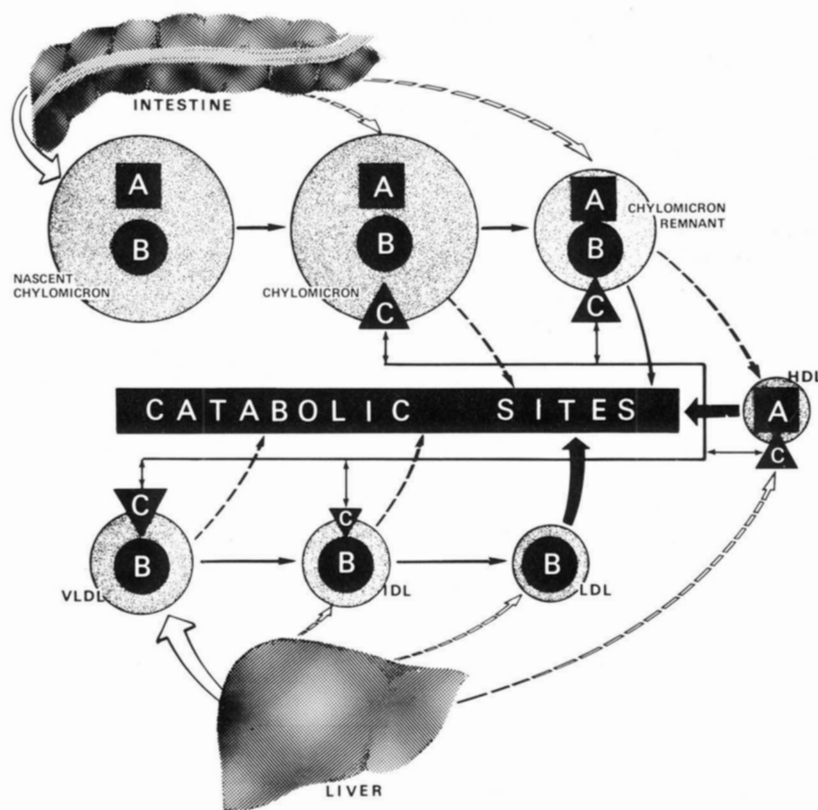


Fig. 5. Conceptual overview of A, B, and C apoprotein metabolism; dotted pathways have not been well defined experimentally.

tion of the total lipoprotein protein mass as VLDL is metabolized to IDL and ultimately to LDL. Recent data on human chylomicron apoprotein metabolism are consistent with the concept that most of chylomicron apoB is removed from plasma prior to the formation of IDL or LDL, while almost all chylomicron apoA-I and apoA-II are metabolized to form HDL apoA-I and apoA-II (43). A schematic overview of apoprotein metabolism is shown in **Fig. 5**.

The functional role of the A apoproteins in man is far from clear. ApoA-I clearly catalyzes the LCAT reaction (19) but what role cholesterol esterification plays in lipoprotein interconversion remains to be defined. Cholesteryl ester deposition occurs in Tangier disease. In this rare inherited disorder, apoprotein A catabolism is abnormal and rapid (72), and apoprotein A levels are extremely low (33, 49, 50). Whether the tissue cholesteryl ester deposition is secondary to the extraordinary A apoprotein catabolism or is due, as some suggest, to lack of receiver apoprotein A in the plasma remains conjectural. The C apoproteins appear to move rapidly from delipidating chylomicrons and VLDL to HDL. Although A apoprotein catabolism appears to be increased when triglyceride flux increases, a direct apoprotein A–C relationship

has not been established. In fact, when LDL (apoB) is present in excessive amounts (as in the homozygous form of familial hypercholesterolemia), more C apoprotein is found in LDL than in HDL.⁴ Though A apoprotein (and HDL) levels appear to correlate inversely with plasma triglyceride levels in normal and hyperlipoproteinemic subjects (and in Tangier disease chylomicron clearance is delayed and carbohydrate-induced hypertriglyceridemia is exaggerated) the precise role of A apoprotein in intravascular triglyceride clearance is unknown.


The sites of apoprotein B synthesis in man seem clear, as does the primarily structural, or core, role of the B apoprotein. The liver and intestine—the only sites of apoprotein B synthesis—are also the only plasma sources of triglyceride, even though essentially all the cells of the body can synthesize triglyceride. In the inherited disorder abetalipoproteinemia, neither chylomicrons nor VLDL are produced by livers or intestines often laden with triglyceride, and the plasma is devoid of apoprotein B, IDL, and LDL (83).

C apoproteins are synthesized only by the liver and rates of production appear to be independent of the

⁴ Shaefer, E. J., R. I. Levy, and H. B. Brewer, Jr. Unpublished observations.

rates of both the A and B apoproteins (64, 105). Whether C apoproteins are released from the liver alone or with HDL, VLDL, or both is not clear. In contrast to the B apoprotein, the C apoproteins transfer and exchange rapidly between lipoprotein species. At least one of the C apoproteins, C-II, is essential in intravascular lipolysis. The central importance of apoprotein C-II has recently been dramatically demonstrated in man by the report of a patient with absent apoC-II who had life-long hyperchylomicronemia (type I hyperlipoproteinemia) despite normal lipoprotein lipase activity (135). The functional roles of apoproteins C-I and C-III require further study, as do the source and role of apoprotein D.

In the rat and other lower animals the liver appears to be the major source of apolipoprotein E (61-65). Its role is unclear and still to be explained are the observations that its concentration in the plasma increases with cholesterol feeding in animals (145, 153) and is extremely high in type III hyperlipoproteinemia (37, 143), a disorder in which a deficiency of one of the polymorphic forms of apoprotein E has been reported (106).

Many more of the molecular events involved in lipid transport are understood and can be described. These, in turn, have shed light on the mechanism behind the abnormal lipoprotein concentrations seen in the different hyperlipoproteinemias. This new knowledge, however, has only emphasized how much more there is to learn. The movement from a description of lipoproteins by density or electrophoretic class to a visualization of interacting lipoprotein apoproteins is just the end of the beginning. Further study of the lipoprotein apoproteins, their structure, function and metabolism, as well as their concentrations in various disease states (now readily measurable by immunoassays), promises to unravel further the mechanisms of normal and abnormal lipid transport. Ultimately, these insights will vastly improve the approach(es) to disorders of lipid transport. 

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