Anal. Acad. Nac. Cs. Ex. Fís. y Nat., Buenos Aires, Argentina, tomo 50, 1998.

METABOLIC AND CLINICAL SIGNIFICANCE OF PLASMA LIPOPROTEIN FAMILIES DEFINED BY APOLIPOPROTEIN COMPOSITION

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Abstract

The chemical, immunologic and metabolic heterogeneity of operationally defined lipoprotein density classes has necessitated the introduction of an alternative approach to their definition and classification. The discovery and characterization of a number of specific lipid-binding proteins (apolipoproteins) led to the recognition of their role as lipoprotein constituents essential for the structural stability and metabolic and functional properties of plasma lipoproteins. Furthermore, as chemically unique constituents, apolipoproteins (apo) were also found to be the most suitable markers for identifying, differentiating and classifying plasma lipoproteins. The utilization of apolipoproteins as unique chemical markers has established that both low density and high density lipoproteins consist of discrete lipoprotein families of similar density properties but different and specific apolipoprotein composition: the low density lipoproteins (d < 1.063 g/mL) were found to consist of five major apoB-containing lipoprotein families and the high density lipoproteins (d > 1.063 g/mL) of three major apoA-containing lipoproteins. All apoA- and apoB-containing lipoproteins are polydisperse systems of particles heterogeneous with respect to density, size and lipid/protein ratios by homogeneous with respect to qualitative apolipoprotein composition. Lipoprotein families also function as distinct metabolic entities interconnected in their roles as carriers of triglycerides and cholesterol. It appears that apoA-containing lipoprotein families differ in their antiatherogenic, and apoB-containing lipoprotein families in their relative atherogenic potentials. Dyslipoproteinemias are characterized by specific lipoprotein family profiles, the concentrations of which may be determined by newly developed immunologic assays. Measurement of lipoprotein family profiles offers a new means for selecting appropriate therapies targeted, in individual subjects, at decreasing undesirable and/or increasing beneficial lipoprotein families.

Introduction

Soluble plasma lipoproteins recognized as a unique class of conjugated proteins form a polydisperse system of macromolecular complexes consisting of neutral lipids and phospholipids noncovalently bound to specific globular proteins called apolipoproteins. This

Conferencia pronunciada en su incorporación como Académico Correspondiente en Oklahoma City, Oklahoma, Estados Unidos, el 24 de octubre de 1997.

poprotein density classes designated as chylomicrons (d < 0.94 g/mL), very low density lipoproteins (VLDL, d = 0.94 - 1.006 g/mL), low density lipoproteins (LDL, d = 1.006 - 1.063 g/mL), and high density lipoproteins (HDL, d = 1.063 - 1.21 g/mL) [10]. Macromolecular distribution studies showed that each of the major lipoprotein density classes represents a polydisperse system of particles heterogeneous with respect to size, density and lipid-protein composition [11]. The relatively high degree of this heterogeneity, reflected in the spreading of electrophoretic bands and ultracentrifugal boundaries, prompted a further subdivision of VLDL [12,13], LDL [14,15] and HDL [16,17] into several density subclasses. The use of density property as a criterion for separating, characterizing and classifying plasma lipoproteins has had a great impact on the conceptualization of the chemical nature and metabolism of plasma lipoproteins [18,19]. In contrast to the simple electrophoretic classification of plasma lipoproteins recognizing two or three major lipoprotein classes, ultracentrifugal methodology revealed a marked heterogeneity of electrophoretically identified lipoprotein bands. Although it has been assumed that VLDL, LDL and HDL could be related to electrophoretic bands in the pre-β-, β- and α_1- positions, distribution studies have shown that each of the major lipoprotein density classes and corresponding electrophoretic bands consist of a great variety of individual lipoprotein particles that differ from one another in size and density. Determination of lipid and protein composition of several narrow density segments of each of the major lipoprotein density classes showed that differences in densities and sizes are due to changing proportions of neutral lipids and phospholipids and changing lipid/protein ratios of individual lipoprotein particles [11-16,20-26]. In the meantime, results of N-terminal amino acid [27,28] and immunochemical [29,30] analyses indicated the existence of two proteins, one of which was considered to be characteristic of HDL or α_1-lipoproteins and the other of VLDL and LDL or pre-β- and β-lipoproteins. By the end of the 1950's, plasma lipoproteins could be defined as a macromolecular system of polydisperse lipid-protein complexes of noncovalently bound neutral lipids, phospholipids and two specific proteins (α-protein and β-protein) forming discontinuous populations of particles heterogeneous with respect to size, density, electric charge and lipid/protein composition [18]. This definition of lipoproteins was based conceptually both on electrophoretic mobility and density as operational criteria. The popularity of these operational classification systems was enhanced by clinical studies which related derangements of lipid transport to particular lipoprotein density classes or electrophoretic patterns [10,23,31-34]. However, despite compositional heterogeneity, major lipoprotein density classes have been accepted as the fundamental chemical and metabolic entities of lipid transport system. Some of the main reasons for the acceptance of this conceptual view included the emphasis on lipids as important chemical determinants of the polydispersity [11,23] of lipoproteins and as potentially injurious agents in the genesis and development of atherosclerosis [31,33,35,36], the availability of ultracentrifugal methodology for the preparative isolation of lipoproteins [37], and the already developed procedures for quantitative determination of neutral lipids [38,39] and phospholipids [40]. This view of plasma lipoproteins was further strengthened by the disclosure of a metabolic relationship between major lipoprotein density classes [10,23,41].

The operational classification of lipoproteins based on density properties contributed significantly to our knowledge of the lipid composition of lipoproteins in health and disease, but ignored the role of protein moieties (apolipoproteins) as the most probable determinants of the structural stability and functional specificity of plasma lipoproteins. To some extent this situation had been created by the notion from electrophoretic characterization of lipoproteins that plasma lipids were only associated with two proteins, i.e., α_1- and β-protein. Although it had generally been accepted that HDL and LDL each contain a distinct apolipoprotein, the N-terminal amino acid analysis of chylomicrons and VLDL indicated a possible presence of as many as four different apolipoproteins [28,42,43]. Several investigators provided immunochemical evidence for the protein heterogeneity of all ma-

jor lipoprotein density classes [30,44-46] suggesting, in concert with studies on the N-terminal amino acids, that lipoproteins may contain more than two specific proteins. On the other hand, biochemical characterization of genetic disorders of lipid transport demonstrated impressively the essential role of apolipoproteins in the formation of lipoprotein density classes [19,34]. Disorders such as Tangier disease and abetalipoproteinemia were characterized primarily by the virtual absence of an apolipoprotein and only secondarily by the complete absence or low concentration of a corresponding density class. These findings stimulated a considerable interest in the chemistry and metabolism of protein moieties and led in the 1960's and 1970's to discovery of several previously unrecognized apolipoproteins.

The concept of lipoprotein families defined by apolipoprotein composition

The sequence of events leading to the discovery of apolipoproteins has been previously described in detail [19,47] and will only briefly be presented in this article. Chemical and immunochemical studies clearly established that α_1-protein was the characteristic protein moiety of HDL and β-protein that of LDL. It was also demonstrated, on the basis of N-terminal amino acid analysis, that β-protein was also one of the protein constituents of VLDL. In 1964, Gustafson et al. [48] established that partial delipidization of VLDL resulted in the separation of three phospholipid-protein residues which were characterized by aspartic acid, glutamic acid and serine and threonine, respectively, as N-terminal amino acids. The protein moiety of the first phospholipid-protein residue was identified as α_1-protein, the second as β-protein and the third as a new protein referred to as apoC [49]. Brown et al. [50,51] confirmed the occurrence of apoC and demonstrated that it consists of three nonidentical peptides, two of which were characterized by threonine and the third one by serine as N-terminals. Furthermore, the N-serine polypeptide was shown to exist in three isomorphic forms differing with respect to the number of terminal neuraminic acid residues. At approximately the same time, Shore and Shore [52] established that α_1-protein consists of two nonidentical polypeptides, one of which was characterized by aspartic acid and the other by a blocked N-terminus.

The discovery of apolipoproteins necessitated the introduction of a nomenclature that would adequately and unambiguously label the already known apolipoproteins and those which might be discovered in the future. For this reason, we introduced the so-called ABC nomenclature [18,47] in which apolipoproteins are designated by capital letters, their constitutive polypeptides by Roman numerals, and the polymorphic forms by Arabic numbers. Thus, α_1-protein was renamed apoA, β-protein was called apoB and the third apolipoprotein discovered in VLDL was designated apoC. The nonidentical polypeptides of apoA were designated apoA-I and apoA-II and those of apoC were named apoC-I, apoC-II and apoC-III. The isoforms of apoC-III were named apoC-III-0, apoC-III-1 and apoC-III-2.

The continuing search for additional apolipoproteins resulted in the discovery of a minor apolipoprotein referred to initially as "thin line" polypeptide [53]. This apolipoprotein characterized by a relatively high carbohydrate content and occurring mainly, but not exclusively, in HDL was called apoD [54,55]. Three groups of investigators reported independently [56-58] the discovery of an apolipoprotein rich in arginine and present mainly in VLDL and HDL. This minor apolipoprotein was named apoE. Two minor apolipoproteins were identified in HDL. One of these apolipoproteins [59], characterized by a relatively low isoelectric point (pI = 3.7) was designated apoF, and the other, glucosamine-containing polypeptide, was called apoG [60]. An apolipoprotein, first discovered in rat HDL [61], was named A-IV presumably because it was assumed that all apolipoproteins identified in HDL ought to be considered as nonidentical polypeptides of apoA. Although this designation was incorrect according to the rules of ABC nomenclature, it was retained because of its universal acceptance. A homologous protein was also identified in humans, both in triglyceride-rich lipoproteins and in HDL [62]. Schultze et al. [63] identified a plasma glycoprotein, named β2- glycoprotein I,

that was later taught to play an unspecified role in VLDL metabolism [64]. Since this glycoprotein was associated with lipoproteins in all major density classes, had a high affinity for lipids and played a possible role in lipoprotein metabolism [65], it was considered to satisfy the criteria for an apolipoprotein and was named apoH. The acute-phase protein serum amyloid A also referred to as SAA polypeptides was found to occur in low concentrations even in plasma lipoproteins of asymptomatic subjects [66,67]. Because of strong binding of SAA polypeptides to lipids, formation of lipoprotein particles in the presence or absence of apoA-I, and capacity to displace apoA-I and apoA-II from HDL, the SAA polypeptides are now referred to as apoI [47,68]. It remains to be seen whether designation apoI will eventually replace SAA, which is a more traditional one than apoI. The most recently identified lipid-binding protein was also found to occur in its lipoprotein form in HDL and VHDL [69,70]. This apolipoprotein, named apoJ, is a glycoprotein consisting of two disulfide-linked subunits designated apoJa and apoJb or, according to ABC nomenclature, apoJ-I and apoJ-II. The apolipoprotein nature of apoJ was established by the isolation from HDL of its corresponding lipoprotein by immunoaffinity chromatography on an immunosorber with antibodies to apoJ; the isolated lipoprotein contained apoJ and apoA-I as its protein constituents. ApoJ turned out to be identical to previously discovered clusterin, human plasma complement inhibitor (SP 40/40), and testosterone-repressed prostate message-2 protein (TRPM-2) [71].

Since mid-1960's, studies on the protein moieties of lipoproteins revealed the existence of at least fifteen apolipoproteins with a variety of polymorphic forms and genetic variants, and several truncated forms of apoB. The amino acid sequences of all apolipoproteins, with the exception of apoG, have been determined and the corresponding genes have been isolated, sequenced and localized on chromosomes. The detail accounts of these accomplishments have been described in several reviews and publications [71-75]. Structural studies [76] have identified lipid-binding domains of most of the apolipoproteins and indirectly provided evidence for their capacity to maintain lipids in water-soluble forms. In addition to their structural role, apolipoproteins, especially apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D and E, have also been shown to play crucial roles in the metabolism of lipoproteins such as secretion, activation of lipolytic enzymes, retardation of premature removal and recognition of binding or removal sites on hepatic and extrahepatic cellular surfaces [77-80]. Due to the growing awareness of the importance of apolipoproteins for structural and metabolic properties of lipoproteins, it was necessary to develop procedures for their quantitative determination. Because of the complexity of plasma lipoproteins, the reliable analytic methodology had to be based on specific and sensitive immunologic procedures. Various immunoassays have been described and successfully applied to measurement of most apolipoproteins, with the possible exception of apoG [81]. The quantitative determination of apolipoproteins has already been shown to be more useful than measurements of lipids for diagnostic purposes and chemical characterization of lipoprotein particles [82,83].

The spectacular advances in the chemistry and metabolism of apolipoproteins influenced markedly the conceptualization and classification of plasma lipoproteins. One of the obvious questions was how to reconcile the growing number of apolipoproteins with only two major electrophoretic bands or four major lipoprotein density classes. Specifically, how are apolipoproteins distributed along the entire density spectrum and how are they localized on individual lipoprotein particles within each of the major lipoprotein density classes. Answers to these questions were made possible through the introduction and use of qualitative and quantitative immunologic techniques [47]. Results of these studies have demonstrated that apoA-I and apoB are the major apolipoproteins [82-84] distributed monomodaly along the lipoprotein density spectrum with the former encompassing the HDL density range and the latter the VLDL and LDL density ranges; however, these two apolipoproteins were found to overlap slightly within a density segment of approximately 1.050 - 1.125 g/mL [84]. All other apolipopro-

teins were shown to occur in significantly lower concentrations than apolipoproteins A-I and B [82-84]. The apoC polypeptides and apoE display a bimodal distribution along the density spectrum with peak concentrations in VLDL and HDL [84]. Characteristically, all other apolipoproteins are distributed mainly within the HDL and VHDL ranges, although small amounts of these apolipoproteins may also be detected in VLDL and LDL [64,67,85]. These findings indicated a marked apolipoprotein heterogeneity of major lipoprotein density classes contributed mainly by minor apolipoproteins. Further immunologic analyses showed that subfractions of each of the major lipoprotein density classes had a different apolipoprotein composition excluding the possibility of an equal distribution of apolipoproteins on individual lipoprotein particles of any given density class. This uneven distribution of apolipoproteins on individual lipoprotein particle suggested the possible presence within VLDL, LDL and HDL of several types of lipoproteins of similar density properties but different apolipoprotein composition [15,18,47, 86-88]. Indeed, in a number of studies discrete lipoprotein particles characterized by specific composition of apolipoproteins were isolated from whole plasma or lipoprotein density classes by sequential immunoprecipitation [88-90] or immunoaffinity chromatography [47,69,87,91-99]. Additional evidence for the lipoprotein heterogeneity of major lipoprotein density classes was provided by kinetic studies which showed differences in kinetic parameters between subfractions of the same density class [100-104]. Taken together, these findings showed clearly that lipoprotein density classes are heterogeneous with respect to physical-chemical properties, lipid-protein composition, kinetic parameters and the content and composition of apolipoprotein-defined lipoprotein particles. Although the identification of discrete lipoprotein particles added another dimension to the complexity of plasma lipoproteins, it also disclosed apolipoproteins as chemically unique markers for differentiating and characterizing distinct families of lipoprotein particles.

To account for the chemical and metabolic heterogeneity of major lipoprotein density classes, we have introduced an alternative system for the classification of plasma lipoproteins by taking advantage of the chemical uniqueness of apolipoproteins as a differentiating criterion [18,47]. According to this classification system, plasma lipoproteins are viewed as a mixture of discrete lipoprotein families, each of which is characterized by a unique qualitative apolipoprotein composition. Lipoprotein families which contain a single apolipoprotein are called *simple lipoprotein families* and those which contain two or more apolipoproteins are referred to as *complex lipoprotein families*. All simple and complex lipoprotein families are polydisperse systems of particles which differ from one another in density, size and lipid-protein composition but are characterized by the same qualitative apolipoprotein composition. Polydispersity of lipoproteins is due to the capacity of apolipoproteins to bind varying amounts of neutral lipids and phospholipids which, in turn, are the main determinants of density and size of corresponding lipoproteins. The magnitude of polydispersity and the distribution of each lipoprotein family along the density gradient depend on the concentrations of lipids to be transported and processes for their degradation and removal [47].

There are two major classes of lipoprotein families, one of which is characterized by apoA (apoA-I + apoA-II) and the other by apoB as characteristic apolipoproteins. The apoA-containing lipoproteins occur mainly in HDL and apoB-containing lipoproteins in VLDL and LDL. The third lipoprotein class consist of minor lipoprotein families characterized by apolipoproteins A-IV, C, D, E, F, G, H, I and J; minor lipoprotein families such as LpE, Lp-A-IV, LpF, LpI and LpJ occur in HDL and VHDL [47].

The fractionation of apoA-containing lipoproteins by sequential immunoaffinity chromatography [105] has revealed the occurrence of three lipoprotein families including lipoprotein A-I (Lp-A-I), lipoprotein A-I:A-II (Lp-A-I:A-II) and lipoprotein A-II (Lp-A-II). The fractionation of apoB-containing lipoprotein families [105] showed that this class of lipoproteins consists of five distinct families including lipoprotein B (Lp-B), lipoprotein B:E (Lp-B:E), lipoprotein B:C (Lp-B:C), lipoprotein B:C:E (Lp-B:C:E) and lipoprotein A-II:B:C:D:E

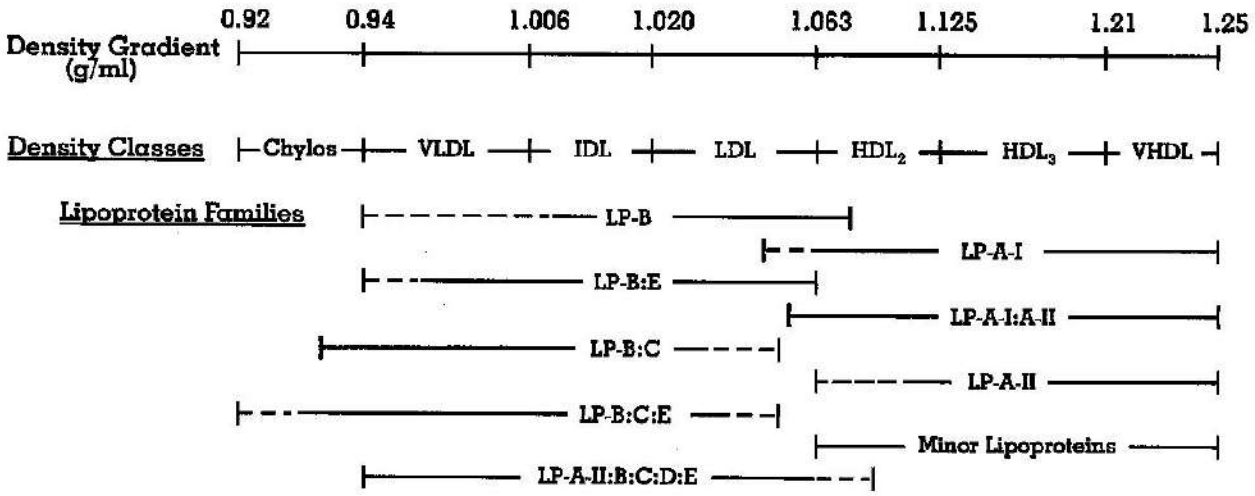

Fig. 1: The relationship of apoA-I- and apoB-containing lipoprotein families to major lipoprotein density classes. The lines under lipoprotein families designate the approximate density boundaries with solid lines depicting the actual and with broken lines the possible localization of each lipoprotein family. Abbreviations: Chylos = chylomicrons, VLDL = very low density lipoproteins, IDL = intermediate density lipoproteins, LDL = low density lipoproteins, HDL_2 = high density lipoprotein subfraction with d = 1.063 - 1.125 g/mL, HDL_3 = high density lipoprotein subfraction with d = 1.125 - 1.21 g/mL, VHDL = very high density lipoproteins, L = lipoprotein.

(Lp-A-II:B:C:D:E) [Figure 1]. As shown in Figure 1, apoA-containing lipoprotein families and minor lipoprotein families overlap within the HDL density range, and apoB-containing lipoprotein families within the VLDL, IDL and LDL density regions. The line under each lipoprotein family indicates their actual and potential distribution along the density gradient; as stated before, the magnitude of the distribution of lipoprotein families within each density segment will depend on their rates of secretion and degradation. Figure 1 also illustrates clearly why the overlap of polydisperse lipoprotein families is the cause of heterogeneity of lipoprotein density classes regardless of whether they encompass wide or narrow density segments.

The lipid composition of all three major apoA-containing lipoproteins is characterized by high percentages of phospholipids (60 - 80%) and varying cholesterol ester/free cholesterol ratios and triglycerides. Approximately 20 - 25% of these lipoprotein families contain minor apolipoproteins as integral protein constituents [106].

The lipid composition of Lp-B particles is characterized by cholesterol esters as the main neutral lipid constituent regardless of their density properties [47]. On the other hand, Lp-B:E, Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E families contain triglycerides as the most characteristic neutral lipid; however, with increasing densities of these complex lipoprotein families, the relative content of triglycerides decreases and that of cholesterol esters increases [47]. The apolipoprotein composition of complex apoB-containing lipoproteins also changes with increasing densities; the relative content of apoB increases and those of apoC polypeptides and apoE decrease [47].

Metabolism of lipoprotein families

Lipoprotein families characterized by specific apolipoprotein composition also have distinct metabolic properties [47,106]. The turnover rate of apoA-I in Lp-A-I has been found to be faster than that of apoA-I in Lp-A-I:A-II [107]. The *in vivo* metabolic studies have shown that LpE, LpE:A-I and LpE:A-I:A-II particles isolated from HDL are catab-

olized more rapidly than Lp-A-I and Lp-A-I:A-II suggesting that the presence of apoE changes markedly the metabolic pathways of lipoprotein families within the HDL density region [108]. It appears that Lp-A-I, but not Lp-A-I:A-II, promotes sterol efflux from cultured human fibroblasts [109] or mouse adipocytes [110]. Lp-A-I particles bind to various cell membranes with greater affinity than Lp-A-I:A-II particles [111] and may play a role as carriers of lecithin:cholesterol acyltransferase (LCAT) and cholesterol ester transfer protein (CETP) in the esterification and transfer of cholesterol in plasma [112,113]. On the other hand, Lp-A-I:A-II particles may be more efficient than Lp-A-I as acceptors or donors of apoC polypeptides and apoE during the lipolytic degradation or formation of triglyceride-rich lipoproteins [84,114].

Differences in metabolic properties have also been found among apoB-containing lipoprotein families. Lp-B:E particles bind to LDL receptors with greater affinity than Lp-B particles [115,116]. However, the binding to LDL receptors of Lp-B:C and Lp-B:C:E particles with E2/E2 phenotype was found to be negligible suggesting that, in contrast to Lp-B and Lp-B:E, these lipoprotein families have little or no effect on the regulation of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity [116,117]. Conversely, Lp-B:C particles have a greater affinity for human THP1 macrophages than Lp-B or Lp-A-II:B:C:D:E particles and their uptake seems to be mediated by an unsaturable mechanism [118]. Lipoprotein lipase was shown to have a greater affinity for Lp-B:C:E than Lp-B:C or Lp-A-II:B:C:D:E as a triglyceride-rich substrate [96].

There is still a scarcity of information on the formation of lipoprotein families. Using HepG2 cells as a hepatic model, we have shown that Lp-A-I and Lp-A-I:A-II particles are secreted into the medium in approximately equal amounts [119]. Cheung et al. [120] have confirmed this finding and showed that both lipoprotein families consist of discoidal and spherical particles. Lp-A-I particles with apolipoproteins A-IV, apoC polypeptides and apoH are probably formed in the intestine [64].

The apoB-containing lipoproteins secreted into the HepG2 cell medium were identified as Lp-B and Lp-B:E particles [94]. The contents of apoC polypeptides were too small to be detectable. The size distribution of both lipoprotein families was similar to that of plasma LDL. However, in contrast to plasma LDL, both lipoprotein families contained high percent contents of triglycerides (56 - 80%) and low percentages of cholesterol esters (15 - 22%). These findings suggest that Lp-B and Lp-B:E may be the precursors for the extracellular formation of complex Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E particles, the three main vehicles for transporting triglycerides in the plasma compartment [47,106]; the acquisition of apoC polypeptides may possibly occur in the space of Disse with Lp-A-I and especially Lp-A-I:A-II particles serving as donors of apoC polypeptides. The lipolytic degradation of complex triglyceride-rich lipoproteins, Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E results in the formation of Lp-B particles as the finite remnants of this conversion process and dissociation of apoC polypeptide, apoE and some other minor apolipoproteins which are transferred and bound to Lp-A-I and Lp-A-I:A-II particles [121]. However, the mechanism and regulating factors responsible for the intracellular assembly and extracellular modification of lipoprotein families remains an important and fruitful area for future exploration.

Clinical significance of lipoprotein families

The function of lipoproteins is to transfer exogenous and endogenous triglycerides and cholesterol from their sites of absorption or formation to their sites of storage and utilization. This function is carried out by triglyceride-rich lipoproteins of intestinal and hepatic origin through a series of enzymic conversion reactions resulting in the release of triglyceride fatty acids and generation of cholesterol-rich remnant lipoproteins. Under normal conditions, the input and output of triglyceride-rich lipoproteins are balanced with little or no change in their plasma levels. However, an increased influx and/or decreased efflux of triglyceride-rich lipoproteins from the plasma compartment have been

identified as main pathophysiologic mechanisms leading to hypertriglyceridemia [122]. The increased formation and/or decreased removal of cholesterol-rich remnant lipoproteins result in hypercholesterolemia which may or may not accompany typically hypertriglyceridemic states [122]. Deranged lipid transport processes are of great clinical significance, because they are considered as one of the main factors responsible for the genesis and development of atherosclerosis [10,33,35,123,124]. Dyslipoproteinemias seem to be characterized by specific concentration profiles of apoA- and apoB-containing lipoprotein families. The apoA-containing lipoprotein families can be quantified by several immunologic procedures including immunoprecipitation [125], immunoaffinity chromatography [126,127], enzyme-linked differential-antibody immunosorbent assay [128], and differential electroimmunoassay [129]. In normolipidemic subjects, Lp-A-I, but not Lp-A-I:A-II, levels are significantly higher in women than men [47]. It has been estimated that 35 - 40% of plasma apoA-I is present in Lp-A-I and 55 - 60% in Lp-A-I:A-II [47]. The Lp-A-II particles account for 5 - 15% and Lp-A-I:A-II particles for 85 - 95% of total apoA-II not associated with apoB [97]. Although there are relatively few data available on the levels of apoA-containing lipoprotein families in dyslipoproteinemias, it appears that the levels of Lp-A-I and Lp-A-I:A-II particles in hypercholesterolemia are similar to those of normolipidemic subjects [130,131], but are reduced to varying degrees in both primary and secondary hypertriglyceridemia and combined hyperlipidemia [132-134].

An accurate measurement of apoB-containing lipoprotein families may only be achieved by sequential immunoprecipitation or immunoaffinity chromatography [47,82, 135]. There are, however, simpler procedures developed for the measurement of different groups rather than individual apoB-containing lipoprotein families. These procedures are based on the differential-antibody immunoassay developed originally by Koren et al., [128]. One of these assays is designed to measure apoB-containing lipoprotein families which also have apoC-III as a protein constituent [130,135]; these assays provide data on the levels of Lp-B + Lp-B:E and on the levels of Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E (these three lipoprotein families are referred to as $Lp\text{-}B_{complex}$ or $Lp\text{-}B_c$ particles). The other assay [130] is designed to measure lipoprotein families which contain apoE and apoB as protein components (Lp-B:E + Lp-B:C:E and Lp-A-II:B:C:D:E). The determination of apoB-containing lipoprotein families by various aforementioned assays has demonstrated that five major lipoprotein families occur in normolipidemic, hypercholesterolemic and hypertriglyceridemic subjects, albeit in different concentrations [47,82,130,131,136,137]. The Lp-B is the main apoB-containing lipoprotein family in normolipidemic subjects and 90 - 95% of total Lp-B particles are present in the LDL density range; however, Lp-B particles may also occur in VLDL and IDL [82]. Men have higher levels of Lp-B than women [82]. Hypercholesterolemic patients have high levels of Lp-B particles in LDL in comparison with normolipidemic subjects. However, they also have relatively high levels of lipoprotein families which contain apoB and apoE as apolipoprotein constituents [82]. In contrast, hypertriglyceridemic stats are characterized by relatively high levels of Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E families and normal or slightly elevated concentrations of Lp-B [82,136]. These results suggest that the differences between normal and dyslipoproteinemic states result mainly from quantitative rather than qualitative composition and distribution of apoA- and apoB-containing lipoprotein families.

Apolipoprotein B is generally considered to be a marker of atherogenic and apoA-I a marker of nonatherogenic lipoproteins. The recognition that individual apoA- and apoB-containing lipoprotein families have distinct apolipoprotein composition and metabolic properties has raised the question as to whether or not lipoprotein families may also possess different nonatherogenic or atherogenic potentials. Although the entire HDL class has been thought to be nonatherogenic, results of recent studies have suggested that there may be differences in the antiatherogenic properties between two major apoA-containing lipoprotein families. The finding that the concentration of Lp-A-I, but not Lp-A-I:A-

II, particles was significantly lower in normolipidemic subjects with angiographically documented coronary artery disease than in subjects without coronary artery disease has suggested that the "protective" capacity of Lp-A-I may be greater than that of Lp-A-I:A-II [138]. Although there were reports suggesting no significant difference in the antiatherogenic potentials of Lp-A-I and Lp-A-I:A-II [139-141], a case-control study of lipoprotein particles in two populations (Northern Ireland and France) at contrasting risk for coronary artery disease has shown that a high risk profile characterized by low levels of Lp-A-I was more frequent in the population of Northern Ireland than France [142]. The results of a recent study on a subset of the Monitored Atherosclerosis Regression Study [143] provided additional evidence for the potentially protective role of Lp-A-I. Further studies are needed to show unequivocally the clinical significance of Lp-A-I particles.

There are two separate issues regarding the atherogenic character of apoB-containing lipoprotein families. The first one pertains to the question as to whether all or only some of the apoB-containing lipoprotein families are atherogenic. If, indeed, all apoB-containing particles are atherogenic, then the second issue relates to the possible differences in relative atherogenic potentials of individual apoB-containing lipoproteins. It has generally been accepted that cholesterol-rich apoB-containing lipoproteins of decreasing size and increasing densities (corresponding to LDL or Lp-B particles) may have the greatest atherogenic potential. However, several metabolic [144-146] and clinical [147-152] studies have provided evidence that partially delipidized triglyceride-rich lipoproteins (small VLDL and IDL) may be as atherogenic as the cholesterol-rich LDL or Lp-B particles. The results of the MARS trial [143] have demonstrated that, in the presence of significantly reduced Lp-B particles, increased concentrations of intact or partially delipidized triglyceride-rich particles such as Lp-B:C, Lp-B:C:E and Lp-A-II:B:C:D:E ($Lp\text{-}B_{complex}$) contribute significantly to the progression of atherosclerotic lesions. It appears that, among the complex apoB-containing lipoprotein families, partially delipidized Lp-B:C and Lp-A-II:B:C:D:E particles may be more atherogenic than Lp-B:C:E and Lp-B:E particles. Due to a low affinity for the LDL receptors [116,117] or a relatively low substrate affinity for lipoprotein lipase [96], the residence times in the circulation of the former lipoprotein families may be longer than those of the latter ones. Furthermore, our preliminary results suggest that rates of neutral lipid and apoB accumulation in human THP1 macrophages are higher for Lp-B:C than Lp-A-II:B:C:D:E or Lp-B [118]. These results suggest that, with the possible exception of intact chylomicrons and large VLDL [153], all apoB-containing lipoprotein families are potentially atherogenic, and that Lp-B:C particles may have the highest relative atherogenic capacity. This latter suggestion has been further supported by a recently completed ancillary Cholesterol and Recurrent Events (CARE) study showing that statistically most powerful predictors of the recurrent cardiovascular events in patients with previous myocardial infarction are VLDL particles that contain apoB and apoC-III as protein constituents [154].

Atmeh et al. [155] first showed that lipid-lowering drugs may affect the levels of plasma lipoprotein families in a specific manner. These authors have established that probucol decreases and nicotinic acid increases the concentration of Lp-A-I particles with little or no effects on the levels of Lp-A-I:A-II particles. Cholestyramine [130] and simvastatin [131] were shown to exert a similar effect on Lp-A-I particles in patients with hypercholesterolemia. In contrast, fenofibrate administration lowered significantly the levels of Lp-A-I and raised the levels of Lp-A-I:A-II [131]. Statins and fibrates are two main groups of hypolipidemic drugs affecting the levels of apoB-containing lipoprotein families. Whereas statins are drugs of choice for lowering cholesterol-rich Lp-B particles [135, 143], fibrates are more effective than statins in reducing complex triglyceride-rich apoB-containing lipoprotein families such as Lp-B:C and Lp-B:C:E particles [131,156,157].

What are the advantages of lipoprotein family concept in comparison with the concept of operationally defined lipoproteins? By emphasizing apolipoproteins as the main determinants of structural and functional

properties of lipoproteins, the lipoprotein family concept provides an open-ended framework capable of incorporating the existing or any new information about the apolipoprotein-defined lipoproteins and their metabolic interactions into an integrated view of lipid transport processes. Dissecting of operationally defined lipoprotein density classes or electrophoretic bands by a combination of chemical and immunologic techniques showed that apolipoprotein-defined lipoproteins are the fundamental structural, metabolic and functional entities of plasma lipoprotein system. According to this view, normal and defective lipid transport processes ought to be regarded, measured and evaluated in terms of lipoprotein families rather than individual lipid or apolipoprotein constituents. Differences in the chemical, metabolic and pathophysiologic properties between distinct apoA- or apoB-containing lipoprotein families reside in intact lipid-protein assemblies and not in an isolated constituent of corresponding lipid or protein moieties. When taken in conjunction with recent findings that hypolipidemic drugs and dietary measures may have selective effects on the levels of individual lipoprotein families, the measurement of apoA- and apoB-containing lipoprotein families may become a useful means not only for identifying patients with increased risk for coronary artery disease and its clinical consequences but also for selecting specific therapies targeted at decreasing the levels of potentially harmful and/or increasing the levels of beneficial lipoprotein families.

Acknowledgments

The author thanks Ms. Kathy Hampton for her excellent assistance in manuscript preparation and Ms. Peggy L. Anderson for her skillful preparation of the figure.

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Manuscrito recibido en febrero de 1999.