Abvances in LIPID RESEARCH

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ADVANCES IN LIPID RESEARCH

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PREFACE

This volume of *Advances in Lipid Research* completes the second decade of publication of our review series. We have attempted to blend reviews of established work in the lipid field with articles dealing with emerging areas of lipid research. In this volume we have collected seven essays relating to several aspects of cholesterol metabolism as well as other areas of research to which lipid metabolism is pertinent.

The first article deals with the significance of human plasma apolipoproteins and lipoproteins in health and disease and contains a review of methods of apolipoprotein quantification. The next article relates cholesterol metabolism to plasma lipoprotein metabolism. The third contribution is an extremely thorough review of lecithin:cholesterol acyltransferase (LCAT) and its role in the regulation of endogenous cholesterol transport. The next two articles are concerned with pharmaceutical agents used in treatment of hyerlipidemia and atherosclerosis. The first of these agents is nicotinic acid, which, with its derivatives, has been used for over 25 years. The other substance is heparin, which also has a long history of use in treatment of lipid abnormalities and research. The sixth article deals with the lipids of actinomycetes, an interesting and important class of microorganisms. The final essay describes the role of sterols in sperm capacitation. It describes a regulatory role for sterols that will probably be new to most lipidologists.

RODOLFO PAOLETTI
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polipoproteins and Lipoproteins of Human Plasma: Significance in Health and in Disease

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I. Introduction

According to the definition by Alaupovic (1971), an apolipoprotein is defined as a lipid-binding protein, consisting of a single or of multiple

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polypeptides, with the capacity to form soluble, polydisperse lipoprotein particles; the latter are considered lipoprotein families. Among the criteria which define a certain apolipoprotein, the following seem to be of importance: (1) distinct chemical, physical, and immunochemical properties; (2) capability to bind or interact with serum lipids; (3) capacity to form discrete lipoprotein particles; and (4) integral importance for the transport or metabolism of lipids. Thus certain enzymes which have been shown to bind quite selectively to lipoproteins, as for example alkaline phosphatase to LpX, are not apolipoproteins since the interaction is not specific and the enzyme is not of importance for the general lipid metabolism. β_2-Glycoprotein I, on the other hand, is included in this article since it is believed to be of relevance for the lipolytic system, although the major part of it is present in the plasma in lipid-free form.

In 1969, when I joined the group of P. Alaupovic, we were concerned with convincing other researchers in this field of the existence of apolipoprotein C (apoC), the third immunochemically distinct entity in the lipid transport system. Today, the situation has changed dramatically. Not only has the number of known distinct units grown exponentionally, but we also have learned a great deal about the existence of isoforms, allotypes, and apolipoproteins present either in small concentrations or occurring only to an appreciable extent in diseased conditions. The nomenclature of these polypeptides, as well as that of lipoproteins in general, has often been handled in a rather arbitrary way and added to the confusion of individuals not directly acquainted with the field. One of the goals of this article, therefore, will be to strictly denominate lipoproteins, apolipoproteins, and the different classes and subclasses in terms of what they in fact represent: density classes, electrophoretic fractions, families or subfamilies, or just single polypeptides or isoforms thereof. In exceptional cases, trivial names such as "proline-rich polypeptide" might not be avoidable.

II. Classification of Lipoproteins and Nomenclature

The lipoproteins and apolipoproteins of various density classes have been treated extensively in review articles which may be consulted for specific questions (Kostner, 1975, 1981; Osborne and Brewer, 1977; Alaupovic, 1982; Assmann, 1982). Lipoproteins consist of different proportions of various lipids in addition to a number of specific polypeptides or apolipoproteins. As a consequence, physicochemical properties of intact lipoproteins vary to a considerable extent. Fractions which may be homogeneous or uniform according to one method may be mixtures of several subunits which can be demonstrated by special procedures only. This

microheterogeneity is subject to change under certain metabolic, hyperlipoproteinemic, or dyslipoproteinemic conditions. It thus seems unavoidable that lipoproteins are investigated by a combination of physicochemical and immunochemical procedures.

A. Lipoprotein Density Classes

According to the concentration maxima and minima which occur along a density gradient, lipoproteins are divided into chylomicrons (CYM) with a density of <1 g/ml, very low density lipoproteins (VLDL) with $d < 1.006$ g/ml, intermediate-density lipoproteins (IDL) with $d = 1.006–1.019$ g/ml, low-density lipoproteins (LDL) with $d = 1.019–1.063$ g/ml, high-density lipoproteins (HDL) with $d = 1.063–1.21$ g/ml, and very high density lipoproteins (VHDL) with $d = 1.21–1.25$. Almost all of these density classes have been subfractionated by stepwise ultracentrifugation at increasing densities or in a density gradient giving rise to density subclasses (DeLalla and Gofman, 1954; Laggner *et al.*, 1977b; Patsch *et al.*, 1978). As will be shown later, there exists no single density class which might be considered as immunochemically homogeneous, and distinct entities from any given density fraction can be separated. The most homogeneous fraction in this respect is LDL, which under normal fasting conditions may contain more than 90% of lipoprotein B (LpB).

B. Electrophoretic Fractions

There is a great variety of methods in current use which separate lipoproteins according to charge. Electrophoretic methods on the one hand may be subdivided, whether or not the supporting media cause sieving effects of lipoproteins. On the other hand, isoelectric focusing (IEF) of intact lipoproteins proved to be the most powerful procedure for exhibiting microheterogeneities (Kostner *et al.*, 1969). The most common electrophoretic procedures using paper, cellulose acetate, or agarose gels separate the lipoproteins into α-lipoproteins, β-lipoproteins, and pre-β-lipoproteins. By the use of refined methodology it has been impossible to split all these electrophoretic fractions into three to five bands (Papadopoulos, 1978). Depending on their position in the gel or on the paper strip, names such as pre-β_1-lipoprotein, α_2-lipoprotein, fast- or slow-β-lipoprotein, inter-β-lipoprotein, and many more have been created by numerous authors. Since the electrophoretic migration of subfamilies depends so much on individual systems, I consider electrophoresis reliable only for defining or quantitating major fractions, unless separations are performed by specialists in one and the same laboratory.

Lipoprotein density, as well as electrophoretic, classes are composed of a number of subfractions which have been demonstrated by various procedures. Since apolipoproteins are not covalently bound to lipids and secondary complex formation within the bloodstream may be a common feature of all hydrophobic material, any separation procedure applied may cause artifact formation and may fail to mirror the true *in vivo* situation. However, there are methods which are considered "safe," which possibly disrupt only weak linkages. Among these, ultracentrifugation, precipitation and electrophoresis, and column chromatography in the absence of detergents and dissociating agents have been successfully applied for the purification of intact lipoprotein entities, so-called lipoprotein families.

C. Lipoprotein Families

Lipoprotein families are characterized by their protein moiety. They behave as homogeneous fractions, metabolically as well as physicochemically. At a density of approximately 1.020 g/ml, some of the lipoprotein families are complexed by neutral lipids, predominantly triglycerides, and fall apart during the action of lipoprotein lipase. Today we know almost 10 distinct lipoprotein families, most of them being found in the HDL class. Table I lists most of these lipoprotein families and relates them to density fractions, on the one hand, and to electrophoretic classes on the other.

The most abundant family in human serum is LpB, the major fraction of LDL. Since more than 90% of LDL consists of LpB, which migrates as β-lipoprotein, the terms LDL, β-lipoprotein, and LpB have frequently been used interchangeably. This might be quite safe under normal conditions but leads to tremendous errors in diseased states. Lipoprotein A is the major lipoprotein of HDL. Lipoprotein C exists in HDL in a free form (Kostner and Alaupovic, 1972) but is complexed with other lipoproteins in VLDL and chylomicrons. Lipoproteins D, E, F, G, and H are considered

Table I
LIPOPROTEIN FAMILIES OF HUMAN PLASMA

Lipoprotein family	Major density class	Electrophoretic mobility
LpA	HDL	α
LpB	LDL (VLDL)	β (pre-β)
LpC	HDL (VLDL)	α (pre-β)
LpD	HDL	α
LpE	HDL (VLDL)	α_2 (pre-β)
LpF, LpG	HDL	α
Lp(a)	HDL_1	pre-β_1

o be minor lipoprotein families as far as concentration is concerned, but ney may be of great importance for lipoprotein metabolism.

III. Apolipoproteins of Plasma and Lymph

A. Major Apolipoproteins

According to the concept of Alaupovic (1982), lipoprotein families consist in their protein moiety of one or several related polypeptides which, as it turned out, probably after they had been detected and denominated, behave very similarly metabolically. At the beginning, apolipoproteins were named simply according to their occurrence in lipoprotein families (Table II). In the case of LpA it was possible to separate nonidentical subunits, which were called apoA-I, apoA-II, and possibly apoA-III, after delipidation (Kostner and Alaupovic, 1971; Kostner, 1974b). The protein moiety of LpB consists of two, probably identical subunits (apoB). There is still some dispute as to whether the protein moiety of LpB is homogeneous. Apolipoprotein B has a molecular weight of 550,000 and is manufactured primarily in the liver. The apoB of intestinal origin has approximately half that molecular weight and has been called B-48, in contrast to the liver apoB which was designated B-100. Information is lacking on how B-100 and B-48 interrelate.

Together with apoA and apoB, the apolipoproteins of the C family are considered as the major apolipoproteins of plasma and lymph of fasting

Table II
Apolipoproteins of Human Serum

Apolipoprotein	Synonym	Molecular weight $\times 10^{-3}$	Major density class
AI		28.5	HDL
AII		17.5	(HDL_2), HDL_3
AIII	D	22	HDL, VHDL
B		550	LDL
CI		7	Chylomicron, VLDL (HDL)
CII		8.5	Chylomicron, VLDL (HDL)
CIII		8.5	Chylomicron, VLDL (HDL)
D		22	HDL_3, VHDL
E		36	VLDL, IDL, HDL_c
F		30	HDL
G		72	VHDL, d = 1.21 g/ml bottom
(a)	Sinking pre-β	65	HDL_1

Table III
APODIPOPROTEINS FORMING NO SEPARATE LIPOPROTEIN FAMILY AND PROTEINS ASSOCIATED WITH LIPIDS AND LIPOPROTEINS

Apolipoprotein	Synonym	Molecular weight $\times 10^{-3}$	Major density class
AIV		46	Chylomicron, d = 1.21 g/ml bottom
H	β_2-Glycoprotein I	54	Chylomicron, d = 1.21 g/ml bottom
PRP	Proline-rich protein	74	Chylomicron, d = 1.21 g/ml bottom
S (SAA)	Serum amyloid antigen	10–15	HDL (VLDL)
D_2		7	HDL
E–AII complex		46	HDL_c

man. Apolipoprotein C is a mixture of three immunochemically distinct units which are of great importance for the action of lipoprotein lipases (LPL). The apolipoproteins which were detected later because of their much lower serum concentration are apoD, -E, -F, -G, and -H. They are listed in Table II together with the major apolipoproteins. Among these proteins I have listed apo-a, the characteristic antigenic determinant of lipoprotein-a [Lp(a)]. Lipoprotein-a was detected by Berg (1963) and was considered at that time as a genetic variant of β-lipoprotein or, better, LpB. Today we know that the a-peptide is inherited as a quantitative genetic trait and can be demonstrated in the plasma of almost every individual. Apolipoprotein-a has the highest molecular weight of all the apolipoproteins detected so far. Table III lists another class of proteins associated with lipids. They may be of equal importance for lipid metabolism or transport, yet separate lipoprotein families of them have not been defined until now.

B. ISOFORMS AND GENETIC VARIANTS

Apolipoproteins purified to homogeneity by electrophoretic criteria have been shown in the past to be heterogeneous by isoelectric focusing. Since in many cases pooled human plasma was used as starting material, there was some confusion as to whether the separated bands represented isoforms comparable to isoenzymes, or whether genetic variants might have been separated. As it turned out, both possibilities do occur. In a general fashion they might be called isoelectric subspecies. With respect

to this it must be mentioned that even minute structural alterations or aggregations which occur during purification cause a tremendous heterogeneity in isoelectric focusing experiments. This can be caused by carbamylation in the presence of urea, interaction of NH_2 groups with aldehydes (which are created by oxidation of unsaturated lipids), deamidation, or proteolysis. Thus whenever such isoforms are to be demonstrated, a most gentle treatment of the fractions is mandatory. The most prominent isoelectric subspecies as well as genetic variants of plasma apolipoproteins are listed in Table IV.

1. Subspecies of ApoA

According to Zannis *et al.* (1980), six different isoelectric subspecies of apoA can be demonstrated by a combination of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and IEF. Some of them are biosynthesized primarily in the intestine, and others are synthesized in the liver. Apolipoprotein AI_4 is the most abundant in fasting plasma, followed by apoA-I_5. The other isoforms occur in rather small amounts only.

In addition to these isoforms, a variety of genetic variants have been described in the literature. One of them might be the apoA-I of Tangier patients exhibiting a polypeptide with altered lipid-binding properties (Assmann, 1979). Another example demonstrating that structural alterations of apolipoproteins may lead to dyslipoproteinemias is the "Milano" variant described by Franceschini *et al.* (1980). This apoA-I, in contrast to

Table IV
ISOELECTRIC SUBSPECIES AND GENETIC VARIANTS OF HUMAN PLASMA APOLIPOPROTEINS

Apolipoprotein	Isoprotein (partially deriving from different organs)	Genetic variants (allotypes)
AI	AI_1–AI_6	Tangier, Milano, Marburg, Giessen
B	B-100, B-74, B-48, B-26	Ag: a_1, c, d, g, h, i, t, x, y, z
CI	CI_1, CI_2	
CII		CII^0, CII^1
CIII	$CIII_0$–$CIII_3$	
E	E_1–E_4 (sialylated forms)	E^2, E^3, E^4
AIV		AIV^1, AIV^2
H	H_1–H_4	