Biochemistry and Biology of Plasma Lipoproteins

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Preface

More than 50 years ago a French biochemist, Macheboeuf, working at the Pasteur Institute in Paris, established experimentally that plasma lipids are associated with proteins to form water-soluble complexes that have distinct physical and chemical properties. He was working with horse serum and we now know that the lipoprotein species that he precipitated in half-saturated ammonium sulfate under acidic conditions was a high-density lipoprotein, particularly abundant in this animal species. This was a historical event in the field of lipoprotein research since it came at a time when there was much skepticism about the existence of specific lipoprotein complexes in the circulation. Although the earliest application of the ultracentrifuge to the study of plasma lipoprotein was carried out in 1947 by Pedersen at the Karolinska Institute in Uppsala, it was the classic work carried out at the Donner Laboratory in Berkeley by Gofman and his colleagues in the late 1940s that established the power and the versatility of the ultracentrifugal method for study of the serum lipoproteins. That work generated the nomenclature of the main lipoprotein classes based on their flotational properties. It also described the main features of the lipoprotein distribution in the serum of normal and dyslipoproteinemic subjects. For the first time an attempt was made to establish a relationship between plasma lipoprotein and cardiovascular disease, and an atherogenic value was assigned to certain low-density lipoprotein classes occurring in serum at high concentrations. During this period, the metabolic importance of the plasma free fatty acid and the role of albumin in this process was discovered. Therefore, sufficient information was beginning to emerge to provide a rudimentary understanding of plasma lipid transport and how abnormalities in this system might predispose to atherosclerotic cardiovascular disease.

In the early 1950s, methods for the separation of serum lipoproteins by preparative ultracentrifugation were described, and with some modifications they still remain the basis for much of the work in the lipoprotein field. Electrophoretic methods in their various modes (free boundary, zonal, starch block, paper, agarose, and other supporting media) were also applied to the study of serum lipoproteins, as well as chemical procedures such as polyanion precipitation, Cohn's fractionation, and so on. In an unfortunate turn of events the atherogenic index proposed by Gofman failed to win the general approval of the experts at that time, who felt that levels of total serum cholesterol were as good predictors of cardiovascular disease as lipoproteins. This led to a temporary decline in interest in lipoprotein research essentially until the clinical studies of Fredrickson and his colleagues at the National Institutes of Health. They combined electrophoretic and chemical techniques to develop a classification of lipoprotein disorders based on given sets of phenotypes, which improved considerably our understanding of lipid disorders and also facilitated the dialogue among investigators and clinicians.

In chemical terms, the early work was directed at defining the lipid components of the various lipoprotein classes, whereas the apolipoproteins received comparatively less attention. It was only in the late 1950s that delipidation techniques were developed, and they marked the birth of the apolipoprotein field, which has progressively emerged as one of the most fascinating areas of lipoprotein research. Without their lipid complement, the apolipoproteins became amenable to fractionation. Pure polypeptides were obtained and work on their primary structure led to the important realization that amphiphilic structures are predominant in apolipoproteins and that these structures are involved in lipid binding. These studies also led to an understanding of the chemical basis of the apolipoprotein polymorphism and to the definition of the functional significance of polymorphic forms in terms of lipid binding, enzyme activation, or interaction with cell membrane receptors. Specific polyclonal antibodies have been raised against each of the known apolipoproteins, and they continue to be used widely to quantify these proteins in various body fluids through a series of immunoassay techniques. More recently, monoclonal antibodies have also entered the field of lipoprotein research. Although their true impact is yet difficult to measure, they have already seen important applications that have taken advantage of their high specificity and relative ease of preparation.

Like many other research areas, progress in the field of plasma lipoproteins has been dependent on the development of new techniques. The latest to enter this field have been those of cell and molecular biology. Through the exciting work by Goldstein and Brown we have learned about the role played by low density lipoproteins in regulating cellular cholesterol metabolism and its dependence on what is now

known as the apo B,E receptor. This has generated a large volume of research activities. A second receptor, that for apo E, has also been isolated although its properties are not yet as well established; other receptors are being actively investigated. Equally exciting have been the advances in the studies directed at the clarification of the co- and posttranslational proteolytic events attending the biosynthesis of the plasma apolipoproteins. The cDNA clones of most of the apolipoproteins are now available and the chromosomal localization of most of the apolipoprotein genes has been determined. Clones for the apo B,E receptor and HMGCoA reductase also have recently been identified. The tools for analyzing the genetic basis of lipoprotein disorders are now in hand and soon we may be able to unravel the role that genetic factors play in the pathogenesis of atherosclerotic cardiovascular disease. About 60 years after Macheboeuf's pioneering work, the lipoprotein field is enjoying a period of extensive and exciting productivity that is unlikely to abate in future years. We now know that proteins, whether apolipoproteins, enzymes, or receptors, play an important role in lipid metabolism and they are expected to continue receiving attention at various levels of endeavor.

The topics mentioned in the preceding overview were covered in a series of lectures given to graduate students in Biochemistry at the University of Chicago during the spring of 1983. With suitable updating, they are now presented in this book. Its preparation was prompted by the need for ready access to background material as an aid to students in the classroom, in the library, and at the laboratory bench. We have attempted to provide a selective coverage of important subjects in the lipoprotein field but not to produce an exhaustive treatise. In consequence, the reader will find inevitable omissions that should be viewed not as a lack of appreciation of significant work by highly qualified investigators but as a need to distill and condense much valuable information into a volume of a manageable size. The authors have responded well to our request for conciseness and clarity and we wish to thank them for their fine and timely contributions.

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Plasma Lipoproteins: An Overview

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INTRODUCTION

The plasma lipoproteins are water-soluble complexes containing specific proteins, apolipoproteins, and lipids, free cholesterol and esters, phospholipids, and triglycerides (1). These lipoproteins all recognize a common structural organization that is characterized by a hydrophobic core composed of cholesteryl esters and triglycerides surrounded by a relatively more hydrophilic shell comprised of proteins, phospholipids, and unesterified cholesterol projecting their hydrophilic domains into the aqueous environment. A model for high-density lipoproteins proposed in 1977 by Shen et al. (2) is shown in Fig. 1. The most commonly adopted classification of plasma lipoproteins is based on their behavior in the ultracentrifuge in high-salt media (Fig. 2). According to their flotational characteristics, the lipoproteins have been classified as very low density (VLDL), low density (LDL), and high density (HDL), each exhibiting density heterogeneity and differences in size and protein lipid distribution (2). This property is exploited in their separation by isopycnic density gradient ultracentrifugation, where each lipoprotein bands in an equilibrium region where its buoyant density spent coincides with that of the salt medium. Chylomicrons, which accumulate in the plasma after a fatty meal, are usually not classified according to their hydrated density; they have a very high flotational rate and thus move rapidly to the top of the collection tube even in the absence of a gravitational field.

Plasma lipoproteins can also be separated by electrophoretic methods in various supporting media. According to their electrophoretic migration, they can be classified as pre-beta- (VLDL), beta- (LDL), and alpha-lipoproteins (HDL) (Fig. 3). Chylomicrons stay at the origin.

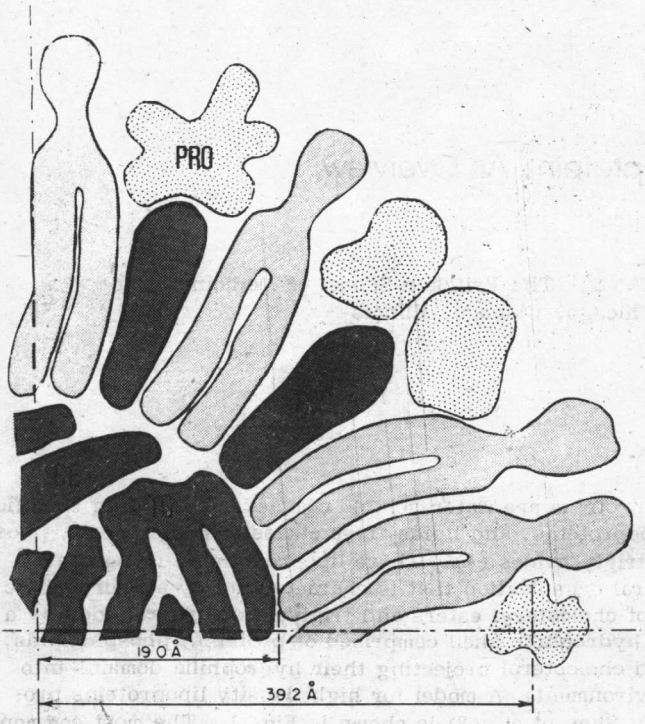


Fig. 1 Structural model of HDL_3 according to Shen et al. (2). PL, phospholipids; FC, free cholesterol; PRO, protein, CE, cholesteryl esters; TG, triglycerides.

Several subspecies comprise each liproprotein class. For some of them there are questions whether they are real or artifactual, but in general they appear to have distinct functions. Plasma lipoproteins represent dynamic structures subject to exchange and transfer processes as well as remodeling; however, as ultracentrifugal isolates they can be considered discrete entities. Each of them is associated with apolipoproteins in varying concentrations and distribution (Fig. 4).

The main characteristics of each main lipoprotein class are outlined below.

CLASS	SIZE Å	DENSITY gm/ml	S_f (1.063)	PLASMA CONC. mg/100ml	APPROXIMATE COMPOSITION (% WEIGHT) 10 30 50 70 90
CHYLOMICRONS				0 to 50	
VLDL	700	0.96	400		
LDL_1	400	1.019	12	225	
LDL_2	200	1.063	0	350	
HDL_2	120	1.125		100	
HDL_3	75	1.21		200	
$VHDL_1$		1.25		10	
$VHDL_2$	?	>1.25		?	

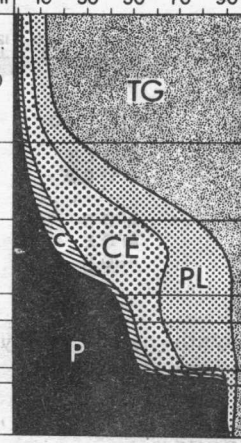


Fig. 2 Schematic distribution of the major classes of plasma lipoproteins according to their flotation in the analytical ultracentrifuge. For each lipoprotein class, the apolipoprotein components are listed.

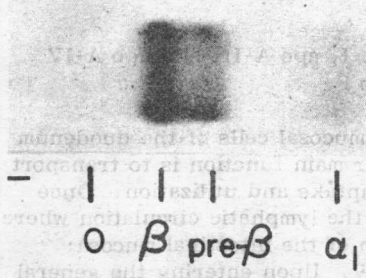


Fig. 3 Agarose gel electrophoretic protein of a human serum. Staining with Sudan black. The chylomicrons, if present, would remain at the origin.

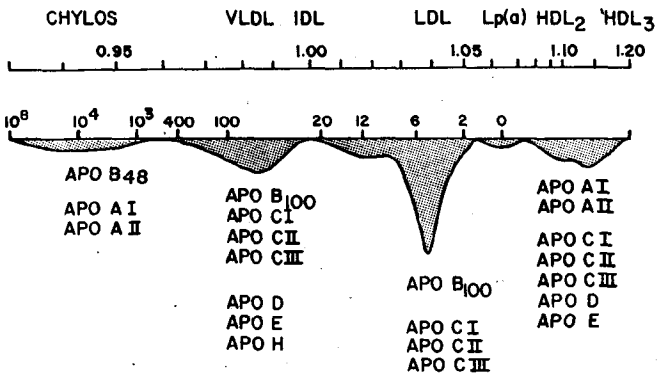


Fig. 4 A synthesis of the physical and chemical properties of the major plasma lipoproteins.

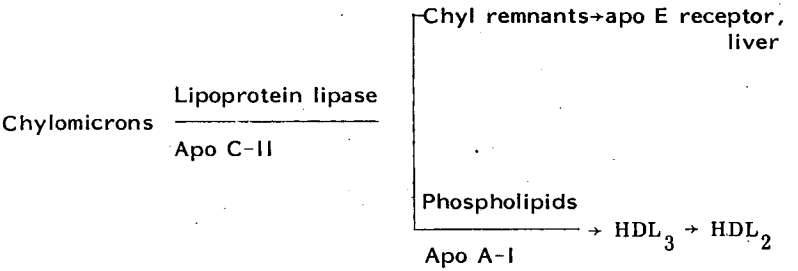
CHYLOMICRONS

Source: intestine
Density: <0.95 mg/ml
Size: 800–1000 A
Total lipid, % total particle mass: 98
Lipid classes, % total lipids:
Triglycerides ∿90
Phospholipids ∿8
Cholesterol ∿5
Protein, % total particle mass: 2
Major components: apo B48, apo A-I, apo A-II, and apo A-IV
Minor components: apo Cs and apo E

The chylomicrons are synthesized in the mucosal cells of the duodenum and jejunum during fat absorption. Their main function is to transport the triglycerides to the cellular sites of uptake and utilization. Once synthesized by the intestine, they enter the lymphatic circulation where they retain the apolipoprotein composition of the intestinal mucosa: apo B48, apo A-I, apo A-II, and apo A-IV. Upon entering the general circulation via the thoracic duct, they acquire additional apolipoproteins, namely apo Cs and apo E, from their interaction with other lipoproteins. By acquiring apo C-II, they become suitable for attack by the enzyme lipoprotein lipase at the levels of the endothelial cell surface; within minutes they are transformed into remnants which are structures impoverished in triglycerides through lipolysis and enriched in apo Cs and cholesteryl esters through the action of the cholesteryl

ester exchange/transfer protein. These remnants are taken up by the liver via the apo E receptor whereas the surface components, mostly apo A-I and phospholipids, are transferred to HDL_3, contributing to the transformation of this lipoprotein subspecies into HDL_2. Thus, chylomicrons enter in the process of remodeling plasma HDL.

Scheme of Metabolism of Plasma Chylomicrons



From the above it is apparent that once they reach the general circulation, chylomicrons undergo restructuring by partial lipolysis and then reenter the cell for degradation. This explains why in familial disorders where either lipoprotein lipase or its cofactor apo C-II is absent, very high levels of chylomicrons accumulate in the plasma.

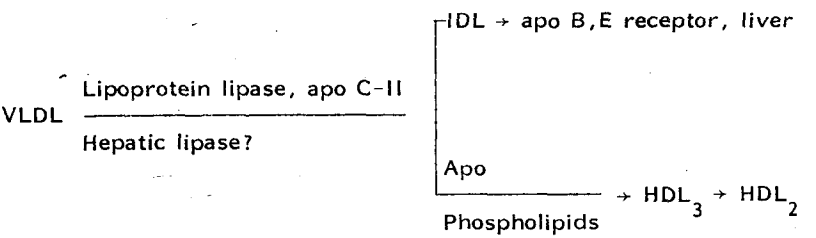
VERY-LOW-DENSITY LIPOPROTEINS

Source: liver
Density: 0.95–1.006 g/ml
Size: 280–800 A
Lipid content, % particle mass: 90
  Lipid class, % total lipids:
    Triglycerides ∿60
    Cholesterol ∿17
    Phospholipid ∿20
Protein content, % particle mass: 10–12
  Major apolipoproteins: apo B100, apo Cs, and apo E
  Minor apolipoproteins: apo A-I, apo A-II, and apo B48

The liver synthesizes and secretes essentially all of the VLDL particles present in the fasting plasma. VLDL are heterogeneous in size and density: each subspecies differs in chemical composition and likely metabolic fate. All of the VLDL particles undergo hydrolysis by lipoprotein lipase and are transformed first into intermediate-density lipoproteins (IDL) and then into LDL. The role of hepatic lipase in this

lipolytic cascade has not been clearly established. There is no loss of apo B during these conversions, whereas the minor apolipoproteins together with some phospholipids and unesterified cholesterol are transferred to the HDL class. Thus, following lipolysis there are two avenues of degradation for plasma VLDL: uptake as remnants via the apo B,E receptor in the liver cells, and transfer of surface components to heavier lipoproteins.

Scheme of VLDL Metabolism in the Plasma



The VLDL particles are known to migrate electrophoretically in the pre-B position. However, in man following a diet rich in cholesterol and also in experimental animals, a VLDL class with β-migration appears in the circulation. Patients with familial type III hyperlipoproteinemia or dysbetalipoproteinemia also exhibit a β-migrating VLDL. These VLDL species contain about 40% triglycerides and 35% cholesterol, mostly cholesteryl esters, apo B (both apo B100 and apo B48), and apo E; they are taken up by the cells by a dual mechanism—the apo E and the apo B,E receptors.

LOW-DENSITY LIPOPROTEINS

Source: plasma
Density: 1.006–1.063 g/ml
Size: 200–250 A
Lipid content, % particle mass: 75
  Lipid classes, % total lipids:
    Cholesterol ∿60
    Phospholipids ∿30
    Triglycerides ∿10
Protein content, % particle mass: 25
  Major protein: apo B100
  Minor proteins: apo Cs and apo E

Two main subclasses of LDL are recognized: LDL-1, d 1.006–1.019 g/ml, and LDL-2, d 1.019–1.063 g/ml. Additional LDL subclasses can