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PLASMA LIPOPROTEINS

Edited by R.M.S. SMELLIE

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PREFACE

Understanding of the structures, metabolism and interrelationships of the various classes of plasma lipoproteins has advanced greatly in recent years and order is beginning to appear out of confusion. This is an appropriate moment to take stock of the situation and the Biochemical Society is grateful to the authors for contributing to this Symposium and for helping to place new developments in this field in perspective. Thanks are also due to Professor D. S. Robinson for assistance in planning the programme.

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CHAIRMAN'S INTRODUCTION

By A. T. JAMES

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The striking advances of the last ten years in the isolation, characterization, delipidation and relipidation of the plasma lipoproteins are bringing us closer to an understanding of the nature of types of protein-lipid interaction since the purity of the preparation is such that precise physico-chemical studies can now be made. The bringing together of biochemical, physico-chemical and clinical studies is a particular feature of lipoprotein research and will clearly help in the understanding of lipoidoses and possibly even of degenerative conditions such as Ischaemic Heart Disease.

My own particular interest is in the simplest types of lipoprotein—the fatty acid desaturases which because of their power of introducing a double bond at a specific position in an almost totally inert (relatively speaking) methylene chain show a highly specific protein-lipid interaction. Indeed the closeness of fit of enzyme and substrate is shown by the stereochemistry of the reaction and the failure to interact with methyl substituted fatty acids (with the methyl group on C atoms 5 to 15) must entail a profound protein conformational change on formation of the enzyme-substrate complex. How far this is a model for the common lipoproteins is debatable but does illustrate how the conformation of the fatty chain can play an important part in such interaction. Clearly there are a range of specific and non-specific protein-lipid interactions, sensitive and insensitive to the precise geometry of individual lipid molecules.

THE STRUCTURE AND METABOLISM OF CHYLOMICRONS AND VERY LOW DENSITY LIPOPROTEINS (VLDL)

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There are four major plasma lipoprotein families: chylomicrons, VLDL, LDL and HDL. Their physicochemical properties and composition are summarized in Tables 1 and 2. It is appropriate that a symposium devoted

Table 1. *Physical characteristics of major lipoprotein families*

Lipoprotein	S_f*	Density gm/ml	Mobility†	Molecular Weight	Size Å‡
Chylomicrons	>400	<0·95	Origin	10^3–$10^4 \times 10^6$	750–10,000
VLDL	20–400	0·95–1·006	Pre-β	$5–10 \times 10^6$	300–800
LDL	0–12	1·019–1·063	β	$2·1–2·6 \times 10^6$	205–220
HDL	—	1·063–1·21	α	200,000	75–100

* Lipoprotein flotation rate in Svedberg units (10^{-13} cm/sec/dyne/gm) in a sodium chloride solution of density 1·063 gm/ml (26°C).
† Paper electrophoresis
‡ As determined by electron microscopy

Table 2. *Composition of the lipoprotein families percent of dry weight*

	Chylomicrons	VLDL	LDL	HDL
Lipoprotein Constituents:				
Protein	1–2	10	25	45–55
Triglyceride	80–95	50–70	10	3
Unesterified cholesterol	1–3	10	8	15
Esterified cholesterol	2–4	5	37	22
Phospholipids	3–6	15–20	22	30
Carbohydrate	?	<1	~1	<1

to the plasma lipoproteins begins with a discussion of chylomicrons and VLDL. These two species of lipoproteins are the largest and most heavily lipidated of the lipid transport forms. Their sites of origin have been defined as has some of their lipid transport tasks. New knowledge and insight gained over the last few years leaves little doubt that during their breakdown they give rise to smaller lipoproteins. In fact, at least part of the heavier lipoprotein forms may represent merely the remnants of chylomicron and VLDL metabolism. To appreciate the significance of the above statements, we must begin with some basic definitions and facts. The composition, structure and function of the chylomicrons and VLDL will then be detailed especially in regard to their interrelationship to LDL and HDL and the hyperlipoproteinemic states.

CHYLOMICRONS

Chylomicrons may be defined as lipoproteins of $S_f > 400$ which arise from the gastrointestinal tract and serve to transport glyserides of dietary (exogenous) origin from the intestine into the lymph. Though they are clearly the largest of the plasma lipoprotein species, they are perhaps the least well understood and characterized. Originally, as defined by Gage (Gage, 1920; Gage & Fish, 1924), they were strictly fat particles of intestinal origin. Others since have defined chylomicrons in terms of particle size, electrophoretic mobility, S_f or density (deLalla & Gofman, 1954; Dole & Hamlin, 1962; Oncley, 1963; Scanu, 1965; Hatch & Lees, 1968; Shumaker & Adams, 1969; Fredrickson, Gotto, & Levy, 1971). They range in size from 750–10,000 Å with a molecular weight of 10^3–10^4 million. On paper and agarose gel electrophoresis, they remain at the origin, while on starch and cellulose acetate, they have an α_2-mobility and migrate with VLDL. Their S_f range is 400 to greater than 10^5, and their density is $<0{\cdot}95$ gm/ml. Since endogenous particles (VLDL) can achieve comparable size, mobility S_f and density (Bierman, Porte *et al.*, 1965), the original definition based on their origin is probably best. The chylomicrons are the most difficult lipoprotein species to isolate free of contamination by other proteins and lipoproteins. A single centrifugation in an angle head rotor is not good enough, and repeated washing may produce altered structure and artifacts. Recently, using a stabilizing density gradient and a swinging bucket rotor, chylomicrons have been isolated uncontaminated in a single centrifugation step (Lossow *et al.*, 1969).

COMPOSITION

Chylomicrons consist predominantly of glycerides (80–95%) with smaller amounts of phospholipids (3–6%), cholesterol (3–7%) and protein (0·5–2·5%)

Abbreviations: VLDL = Very low density lipoproteins
LDL = Low density lipoproteins
HDL = High density lipoproteins
apoLP = apolipoproteins

(Table 2) (Lossow *et al.*, 1969; Zilversmit, 1965). The chemical composition of chylomicron particles varies with their size and manner of collection (Yokoyama & Zilversmit, 1965). The larger the chylomicron, the greater its relative content of glycerides and smaller its relative content of cholesterol, phospholipid and protein. Thoracic duct chylomicrons change rapidly on entering the blood stream. The particles rapidly alter their fatty acid composition, phospholipid and protein content, electrophoretic mobility and susceptibility to flocculation in polyvinylpyrolidine (Bierman *et al.*, 1965; O'Hara, Porte, & Williams, 1966).

Table 3. *Composition of the apolipoproteins of the lipoprotein families*

	Chylomicrons	VLDL	LDL	HDL
Apoprotein constituents:				
ApoLP-ala	Unknown	Major	Minor	Minor
ApoLP-ser	Unknown	Major	Minor, if present	Minor
ApoLP-glu	Unknown	Major	Minor, if present	Minor
ApoLDL	Unknown	Manor	Major	Absent
ApoLP-thr	Unknown	Minor, if present	Trace, if present	Major
ApoLP-gln	Unknown	Minor, if present	Trace, if present	Major

"Major" refers to proteins making up 10 percent or more of the total protein.

The nature of the protein component of chylomicrons is uncertain (Table 3). Several plasma proteins, including albumin and gamma globulin, can often be identified immunologically, even after repeated washings, presumably because they are absorbed to the surface of the chylomicrons. The chylomicron apoprotein has not yet been characterized to the extent recently achieved for VLDL and HDL. In the past examination of chylomicrons by the finger-printing technique and amino acid analysis has suggested the presence of HDL-like protein, while immunologic reactions have suggested the presence of LDL protein (Rodbell & Fredrickson, 1959; Middleton, 1956; Levy *et al.*, 1967).

STRUCTURE

Using both the light and electron microscope, chylomicrons appear to be spheres (Lindgren & Nichols, 1960). The increase in relative content of protein, phospholipid and cholesterol as the surface to volume ratio of

chylomicron increases has led to the suggestion that these constituents may occupy the surface of a triglyceride core (Yokoyama & Zilversmit 1965). Indeed, it can be calculated that the surface coverage by protein-phospholipid is constantly about 20% over the entire range of chylomicron sizes (Lossow *et al.*, 1969). Membrane-like structures over a circular lipid droplet with no fine structure has been reported by electron microscopy (Salpeter & Zilversmit, 1968). Similarly, by repeated freezing and thawing or dehydration, surface membrane isolation has been described (Zilversmit 1965; Zilversmit, 1968; Zilversmit, 1969). It remains to be proven that these latter phospholipid-rich structures are not artifacts of preparation. In any event, it is currently best to view chylomicrons as spheroid, consisting of an outer membrane, composed primarily of phospholipid and protein with a core of triglyceride in which some cholesteryl ester is dissolved.

METABOLISM

Chylomicron synthesis

Chylomicrons serve to transport dietary triglycerides from the sites of lipid absorption in the small intestine via the thoracic duct into the bloodstream and ultimately to the sites of utilization in the tissues. They are produced in the intestinal mucosa in response to ingestion of dietary fatty acids with chain length of C_{12} or greater (Fredrickson, Levy, & Lees, 1967). These fatty acids are reassembled into glycerides in the mucosal cell and coalesce into particles called chylomicrons with the addition of surface stabilizing cholesterol, phospholipid and protein (Zilversmit 1968). The precise morphological and physiological details of chylomicron formation and movement from the base of the jejunal epithelial cell into the lymphatics are still not clear. The chylomicrons apparently move out of the intestinal mucosa cell golgi into the lymph channels, filter through lymph nodes and eventually reach the plasma by way of the thoracic duct (Fig. 1) (Dole & Hamlin 1962). The small protein component of chylomicrons appears to be essential for its formation and function. In the rare familial disorder, abetalipoproteinemia, the peptide moiety of LDL (which is also a part of the protein complement of chylomicrons) is apparently not synthesized; chylomicrons are not formed, and triglycerides accumulate in the intestinal mucosa (Fredrickson *et al.*, 1971; Salt *et al.*, 1960). De novo synthesis of LDL protein has been demonstrated from the intestine (Windmueller & Levy, 1968; Kessler *et al.*, 1970), and it is likely that the defect in abetalipoproteinemia rests with faulty de novo intestinal LDL protein synthesis, since plasma LDL apparently cannot enter the cell and be re-utilized for new chylomicron formation (Lees & Ahrens, 1969).

The appearance of turbidity in normal plasma in the nonfasting state is due to chylomicrons. Their presence in the plasma 12–16 hr after the last meal is distinctly abnormal. In the absence of certain gastrointestinal

disorders which lead to delayed absorption of dietary fat, fasting chylomicronemia is usually the result of impaired chylomicron metabolism (Levy & Langer, 1969).

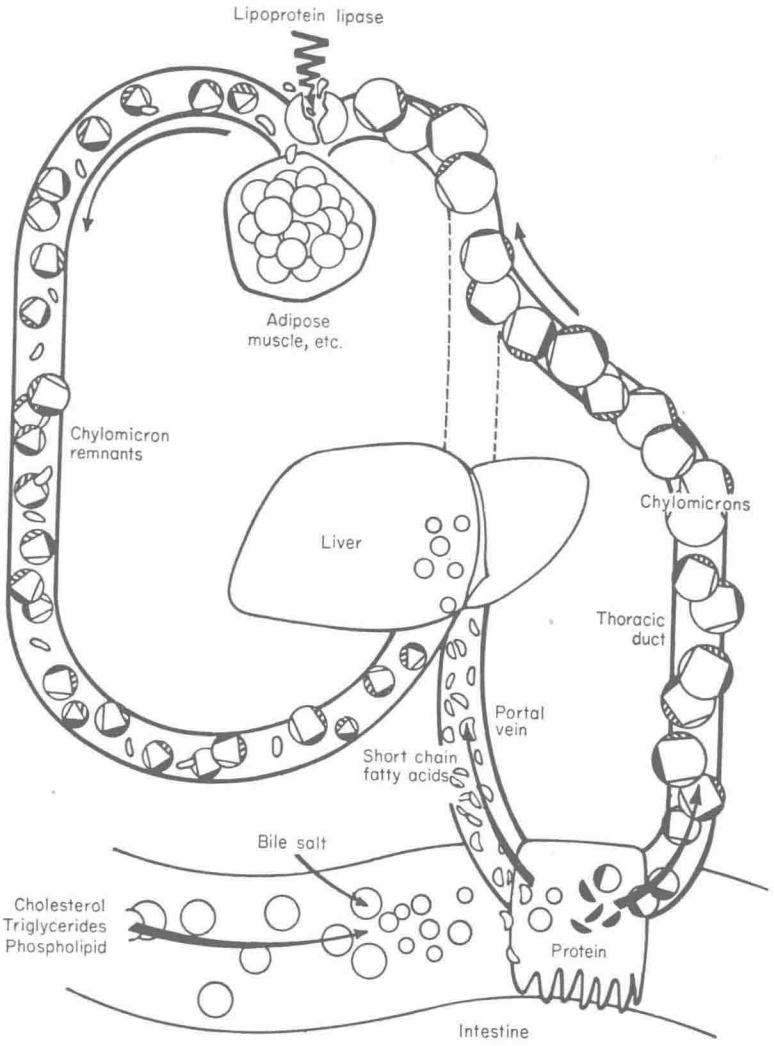

FIG. 1. Schematic representation of chylomicrons' metabolism.

Chylomicron catabolism

Chylomicrons are rapidly removed from the plasma. The uncertain nature of chylomicron protein and the preponderance of lipid in this macromolecule has precluded rigorous investigation of the metabolism of chylomicron protein. When chylomicrons containing labeled triglycerides are infused into humans, they disappear with a half-life of 5–15 min (Table 4) (Nestel,

1964; Dole & Hamlin 1962; Havel, 1965; Scow, 1970). The mechanism of removal of triglycerides from the plasma has been the subject of many reviews (Dole *et al.*, 1962; Havel, 1965; Scow, 1970; Nikkila, 1969); it is only partially understood. The fatty acids of chylomicron glycerides find their ways into glycerides and phospholipids in cells located all over the body with the exception possibly of the brain; some are rapidly oxidized.

Table 4. *Turnover of plasma lipoproteins*

Lipoprotein	Concentration mg/100 ml (Fasting Plasma)	T 1/2	Fractional Catabolic Rate % I.V. pool/ day	Apoprotein Synthetic Rate mg/kg/day	% I.V.
Chylomicrons	None	5–15 min*	—	—	—
VLDL	20–400	6–12 hr	100–250†	—	—
LDL	200–400	2–4 days	35–50	12–18	75
HDL	125–425 (males) 250–650 (females)	3–5 days	25–35†	10–20†	60†

* T 1/2 of glyceride turnover.
† Estimated from the literature.

Most of the glyceride fatty acid goes to skeletal muscle and adipose tissue, lesser amounts can be found in liver, spleen, heart and other tissues (Fredrickson, McCollester, & Ono, 1958; Bragdon & Gordon, 1958; Borgström & Jordan, 1959; Olivecrona, 1962; Robinson, 1964; Olivecrona & Belfrage, 1965; Fredrickson & Levy, 1971). Precise localization of chylomicron clearance is difficult because of the rapid transport of free fatty acid released from glyceride of one organ to another.

With the possible exception of the liver, it appears that most of the chylomicron glyceride is *hydrolyzed* during the removal process. This hydrolytic process is mediated by a group of lipolytic enzymes. Recent experiments have been interpreted by Redgrave to suggest that chylomicrons are degraded by hydrolysis initially in the environment of adipose tissue and skeletal muscle in such a way that the fatty acid components of the triglyceride enters the adjacent muscle and adipose tissue while *chylomicron remnants* relatively enriched in cholesterol, phospholipid and protein are then cleared by the liver (Fig. 1) (Redgrave, 1970). This hypothesis is compatible with the observation that chylomicron cholesterol and cholesteryl ester is essentially all removed by the liver (Quarfordt & Goodman, 1967).

The initial hydrolysis of chylomicrons probably involves the rich supply of the enzyme lipoprotein lipase that is present in the capillary bed of adipose tissue and muscle (Robinson, 1964). This enzyme is essentially absent from fasting human plasma. The intravenous administration of small amounts of

heparin results in the appearance of lipolytic activity in the plasma often denoted as PHLA. The uptake of chylomicron remnants by the liver might not involve hydrolysis. In the liver capillaries, as opposed to that in other tissues, there is no basement membrane, and there are gaps in the endothelium large enough to admit chylomicrons to the subendothelial space of Disse (Majno, 1965). Phagocytosis by Kupfer cells and other cells of the reticuloendothelial system may provide an alternate pathway for chylomicron clearance.

It is likely that glycerides in VLDL and other lipoproteins are removed by the same hydrolytic step postulated for chylomicrons. Though the glycerides in VLDL are substrates for lipoprotein lipase, their rate of clearance is slower, however, than that of chylomicrons. This may be attributable to size differences, for larger chylomicrons are reported to be removed more rapidly than smaller ones.

VLDL

VLDL comprises a family of macromolecules rich in endogenous triglycerides with a broad S_f range of 20–400, isolated between density 0·95 and 1·006 gm/ml (Table 1). They are roughly spherical particles with a diameter from 280 to 800 Å and a molecular weight of 5 to 10 $\times$ 10^6. VLDL migrate with pre-β or α_2 mobility on most media but with decreasing density and increasing size they mimic chylomicrons in physicochemical behaviour. VLDL then, like chylomicrons, are heterogeneous in size, density and S_f; at the lower end of their density ranges they overlap with chylomicrons and at their highest end they merge with LDL.

COMPOSITION

The predominant lipid of VLDL is glyceride which comprises approximately 50–70% of the molecule by weight with smaller amounts of phospholipids (15–25%), cholesterol (15–20%, 1:1 free/ester) and protein (7–12%) (Levy, Lees, & Fredrickson, 1966). The ratio of phospholipid to protein is the highest found in any of the plasma lipoprotein families (Table 2). Subfractions of VLDL have been measured by density gradient centrifugation using a fixed angle (Gustafson, Alaupovic, & Furman, 1965) or a swinging bucket rotor (Hatch *et al.*, 1967). The results are relatively comparable. The less dense particles have more glyceride and less protein; the particles of higher density have more protein and less triglyceride.

The VLDL protein has recently been fractionated into four major constituents (Tables 3, 5) (Brown, Levy, & Fredrickson, 1969; Brown, Levy, & Fredrickson, 1970*a*, *b*). Several other components, most probably minor constituents or contaminants, are often also observed (Shore & Shore, 1969). After complete delipidation of VLDL with ethanol:ether (3:1) and resolubilization of its proteins with decyl sulfate, several distinct apoproteins can

be demonstrated. With a combination of gel filtration using Sephadex G-150 and DEAE cellulose ion exchange chromatography, these apoproteins may be isolated and characterized (Brown *et al.*, 1969; Brown *et al.*, 1970*a*, *b*). Approximately 40% of the VLDL protein appears to be identical with the major protein component of LDL (apoLDL). Approximately 50% of the human VLDL protein is made up of three small proteins. For simplicity, they are best defined currently in terms of their carboxyterminal amino acids (Tables 3–5). ApoLP-ser is rich in lysine and does not contain tyrosine, histidine, cysteine and cystine; it has a molecular weight of 7,000 daltons.

Table 5. *Some characteristics of the small VLDL proteins*

	ApoLP-val	ApoLP-glu	ApoLP-ala$_1$	ApoLP-ala$_2$
Mobility	γ	β_1–α_2	α_2	α_2–α_1
Approx. Molecular Weight	7,000	10,000	10,000	10,000
C-Terminal*	Serine	Glutamic acid	Alanine	Alanine
N-Terminal*	Threonine	Threonine	Serine	Serine
Missing AA.s*	Cys, Tyr, His	Cys, His	Cys, Ileu	Cys, Ileu
ORD and CD	α helix	Random	Random	Random
Sialic Acid	0	—	1 mole/mole	2 moles/mole
Approx. % of apoVLDL	10	10	30	
Approx. % of apoHDL	1–3	1–3	4–10	

C Terminal = carboxylterminal amino acid.
N Terminal = aminoterminal amino acid.
Missing AA's = absent amino acid.
ORD = optical rotary dispersion.
CD = circular dichroism.

Circular dichroism and optical rotatory dispersion are consistent with a high content of α helix. ApoLP-glu does not contain cystine, cysteine or histidine and has a molecular weight of about 10,000 daltons. Its configuration is primarily that of a random coil. ApoLP-ala, the third and most plentiful of the VLDL small proteins, exists in at least two forms, apoLP-ala$_1$ and apoLP-ala$_2$, containing one and two moles of sialic acid per mole of protein, respectively. ApoLP-ala lacks isoleucine, cysteine and cystine, has a molecular weight of about 10,000 daltons and has predominantly a random coil configuration.

In addition to these major apoproteins, VLDL, immunochemically, appears to contain minor quantities of the major HDL apoproteins, apoLP-gln and apoLP-thr. The amounts of the different apoproteins in VLDL appears to vary depending on the source of the VLDL, its density and the