Advances in Lipid Research

Volume 17

Edited by

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PREFACE

One of the most intriguing current problems in the lipoprotein field, the metabolic role of high-density lipoproteins (HDL), is focused upon in the opening chapters of this volume. Almost 30 years ago Barr suggested that α-lipoprotein (HDL) levels played a possible predictive role in coronary heart disease. He hypothesized that a high α/β-lipoprotein ratio (HDL/LDL) predicted relative freedom from coronary disease and the converse predicted susceptibility. The role of HDL in cholesterol transport [as a component of the lecithin–cholesterol acyl transferase (or LCAT) system] was articulated by Glomset in the late 1960s and reviewed by him in this publication (1973). Other investigators have demonstrated that HDL actually transports cholesterol from cells. The role of HDL in cholesterol transport and HDL metabolism are the subjects of the first two chapters. The third chapter discusses cholesterol metabolism in various types of clinical hyperlipidemias, and addresses cholesterol turnover as a function of hypercholesterolemia, hypertriglyceridemia, or a combination of the two. Polyunsaturated fats are known to exert a hypocholesterolemic effect in man. The mechanism(s) underlying this observation is the subject of the fourth chapter.

Many lipids are susceptible to oxidation. Polyunsaturated fatty acids are the most readily oxidized, but even cholesterol is converted to oxidation products such as the 7- or 25-hydroxy derivatives. The topic of lipid peroxidation in mitochondrial membranes and its effects on alterations in cellular processes is discussed at length in the volume's fifth chapter. The final contribution also relates to membrane phenomena, but its thrust is toward the study of membrane structure by means of membrane-cooperative enzymes. The application of allosterism as a tool for membrane research also is explored.

RODOLFO PAOLETTI
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Body Cholesterol Removal: Role of Plasma High-Density Lipoproteins[1]

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I. Introduction

The human body is abundantly equipped with mechanisms for the provision and maintenance of tissue cholesterol levels. Cholesterol absorption increases with dietary intake, with no apparent upper limit (Borgstrom, 1968). In times of reduced intake the liver and small intestine increase their synthesis of cholesterol (Dietschy and Wilson, 1970; Grundy *et al.*, 1969; Dietschy and Gamel, 1971). Absorbed cholesterol is transported to the liver as chylomicron remnants (Redgrave, 1970; Nervi *et al.*, 1975; Andersen *et al.*, 1977) and hepatic cholesterol is either secreted as very low-density lipoproteins which are catabolized in part to low-density lipoproteins (Eisenberg and Levy, 1975), or secreted into bile as free cholesterol or its metabolites, the bile salts (Small *et al.*, 1972; Grundy *et al.*, 1974). LDL delivers cholesterol to the peripheral tissues, where specific cell surface receptors bind, internalize, and degrade the LDL particles, providing the cell with cholesterol (Brown and Goldstein, 1976; Goldstein and Brown, 1977). In the absence of an adequate supply of lipoprotein cholesterol, the cholesterol synthesis pathway is activated in peripheral tissues (Andersen and Dietschy, 1976).

Although a great deal is known about cholesterol synthesis and delivery, there is relatively little information about the role of a removal mechanism in the regulation of tissue cholesterol levels. That regulation is exerted by cholesterol removal involving plasma high-density lipoproteins has at this time the status of a good working hypothesis. The evidence can be summarized as follows:

1. In epidemiological studies plasma levels of HDL are correlated inversely with the incidence of atherosclerotic cardiovascular disease.
2. There is probably an inverse correlation between levels of HDL cholesterol and tissue cholesterol pools.
3. HDL and HDL-like particles can remove cholesterol from cells in tissue culture.
4. HDL is a preferred substrate for plasma lecithin:cholesterol acyltransferase, an enzyme which converts surface cholesterol into core cholesterol ester, thereby creating a gradient for transfer of membrane unesterified cholesterol into HDL.

This review will present our views of HDL structure and metabolism, particularly as it might be related to cholesterol homeostasis. Emphasis is placed on the composition and structure of HDL, the physical structure of the individual components of HDL, and the different HDL recombinants. These structural considerations are intimately related to the formation and

function of plasma HDL. Much of our discussion is based on *in vitro* or physiclogical experiments conducted with both human and nonhuman subject matter. Consequently, many of our conclusions are speculative and are intended as a starting point for further experimental testing. Comprehensive reviews of lipoprotein metabolism (Eisenberg and Levy, 1975) and structure (Scanu and Wisdom, 1972; Jackson *et al.*, 1976; Morrisett *et al.*, 1975; Smith *et al.*, 1978) have been published.

II. Composition and Structure of High-Density Lipoproteins

A. Composition

Human HDL is operationally defined as the class of lipoproteins isolated between densities 1.063–1.125 g/ml (HDL_2) and 1.125–1.21 g/ml (HDL_3) in the preparative ultracentrifuge. A minor component, HDL_1, has been isolated between density 1.050 and 1.063 g/ml. HDL_2 consists of ~ 40% apoprotein and 60% lipid and HDL_3 of 55% protein and 45% lipid. The lipids comprise 44% phospholipid, 6% cholesterol, 28% cholesteryl ester, and 16% triglyceride (Scanu and Wisdom, 1972). By sedimentation equilibrium the molecular weights of HDL_2 and HDL_3 are 320,000 and 175,000, respectively (Scanu and Granda, 1966). Values of 360,000 and 184,000 have been obtained by small angle X-ray scattering studies.

The principal apoproteins include apoA-I (MW ~ 28,000), apoA-II (MW ~ 17,000) and the smaller C-apolipoproteins (MW 5000–8000). ApoA-I and apoA-II together comprise about 90% of the apoprotein. Other minor components that have been identified in human HDL include apoD (also called "thin-line" polypeptide; MW 22,100) (McConathy and Alaupovic, 1976), apoE (MW ~ 34,000; also known as the arginine-rich peptide), apoF (MW 26,000–32,000; Olafsson *et al.*, 1978), and two threonine-poor apoproteins (MW 40,000 and 10,000; Shore *et al.*, 1978). A subfraction of human HDL_2 contains an apoprotein of MW 48,000 termed "pro-arginine rich" apoprotein, because on disulfide reduction it dissociates into two subunits one of which appears to be the arginine-rich apoprotein and the other apoA-II (Weisgraber and Mahley, 1978). Rat HDL and probably the HDL of LCAT-deficient subjects contain a minor constituent apoA-IV (MW ~ 46,000; Swaney *et al.*, 1977; Utermann *et al.*, 1974).

Although there are reports that the ratio of apoA-I to apoA-II is identical in HDL_2 and HDL_3 (Friedberg and Reynolds, 1976) or higher in HDL_3 (Albers and Aladjem, 1971), the majority of workers have found a higher ratio in HDL_2 (Kostner *et al.*, 1974; Kostner and Alaupovic, 1972; Bornt

and Aladjem, 1971). Cheung and Albers (1977) have reported an apoA-I/apoA-II weight ratio of 5.1 (men) or 6.1 (women) in HDL_2 and 3.7 (men) or 3.8 (women) in HDL_3. An increase in the ratio of HDL_2/HDL_3, such as occurs in women compared to men (Cheung and Albers, 1977) or in long-distance runners (Krauss *et al.*, 1977), results in an increase in the apoA-I/apoA-II ratio in plasma.

The Schlieren pattern of HDL in the analytic ultracentrifuge shows two major peaks. The $F^{\circ}_{1.2}$ 3.5–9 subclass approximates HDL_2 and the $F^{\circ}_{1.2}$ 0–3.5 subclass HDL_3. Anderson *et al.* (1977) have recently identified three subclasses of HDL by equilibrium density ultracentrifugation, of densities 1.063–1.10 g/ml (108–120 Å), 1.100–1.25 (97–107 Å), and 1.125–1.063 (85–96 Å). Having developed a method for resolving HDL Schlieren patterns into contributions from these three different components, they have estimated their relative contributions to plasma HDL in a normal population sample (Anderson *et al.*, 1978). These studies show that HDL_3 levels in plasma are relatively constant [158 ± 30 mg/dl (SD)]. The density 1.100–1.125 (HDL_{2a}) and 1.063–1.100 (HDL_{2b}) are highly correlated with plasma HDL levels. Individuals with HDL levels less than 100 mg/dl have mainly HDL_3; increases in total HDL up to 200 mg/dl are due to increases in HDL_{2a}; increases up to 475 mg/dl are due to HDL_{2a} and HDL_{2b}; and with HDL levels in excess of 475 mg/dl there are additional faster floating components in the Schlieren pattern. Thus, increases in plasma HDL levels are due to the incremental build-up of subclasses of increasing Sf values, i.e., larger HDLs.

Miniature swine fed diets enriched in saturated fat and cholesterol develop hypercholesterolemia and accelerated atherosclerosis. A lipoprotein called HDL_c (cholesterol-induced) appears in the plasma in appreciable concentrations (Mahley *et al.*, 1975). HDL_c is cholesterol ester-rich, has electrophoretic α-2 mobility, a size intermediate between LDL and HDL_2, and an apoprotein content including the arginine-rich (apoE) and A-I apoproteins.

HDL_c is also induced in other species such as dogs (Mahley and Weisgraber, 1974; Mahley *et al.*, 1974), man (Mahley *et al.*, 1978), and rats (Mahley and Holcombe, 1977) by cholesterol feeding. The composition and size of HDL_c from swine are shown in Table 1 (Mahley *et al.*, 1975; Tall *et al.*, 1977a; Atkinson *et al.*, 1978).

B. Apolipoprotein Structure

A major advance in our knowledge of lipoprotein structure has resulted from the purification and amino acid sequencing of the serum apolipoproteins. ApoA-I consists of a single chain of about 245 residues (Baker *et al.*,

Table I

COMPOSITION AND SIZE OF HDL_c FRACTIONS

	1.02–1.04	1.04–1.06	1.06–1.09
Protein	15.8	20.3	25.7
Phospholipid	17.5	29.3	32.5
Cholesteryl ester	56.3	42.5	35.0
Cholesterol	9.9	7.5	6.2
Triglyceride	0.5	0.5	0.6
Major apoprotein	apoE	apoE, apoA-I	apoA-I
Diameter[a] (Å)	175–225[a] 180[b]	150–200[a]	125–185[a]

[a] From negative stain electron microscopy.
[b] From analysis of X-ray scattering.

1974, 1975; Brewer *et al.*, 1978). ApoA-II has two identical chains linked by a disulfide bond at residue 6 (Brewer *et al.*, 1972). The sequences of the smaller C-apolipoproteins are also known. ApoC-I has 57 residues (Jackson *et al.*, 1974a,b), apoC-II, 78 residues (Jackson *et al.*, 1977), and apoC-III, 79 residues (Brewer *et al.*, 1974). Based on CPK space-filling models of the amino acid-sequenced water-soluble apolipoproteins, Segrest and co-workers (Segrest *et al.*, 1974; Segrest, 1977; Segrest and Feldmann, 1977) speculated that the apoproteins probably contain segments of helix in which one face of the helix contains predominantly hydrophilic amino acid residues, while the other face contains a strip of hydrophobic amino acids. They suggested that in lipoproteins the hydrophobic strip might interact with the fatty acyl chains of the phospholipids, and that there might be ionic interactions between the polar helical face and the zwitterionic phosphorylcholine moiety of the lecithin. The term "amphipathic helix" was coined to describe these lipid-binding helical segments. Experimental validation of this hypothesis has resulted from the synthesis of peptide fragments which have amphipathic polar and apolar faces and which also display the capacity to form lipoprotein complexes with lecithins (Sparrow *et al.*, 1977). Investigations of lipoprotein recombinants using nuclear magnetic resonance spectroscopy (Assmann *et al.*, 1974; Assmann and Brewer, 1974; Stoffel *et al.*, 1974; Finer *et al.*, 1975; Stoffel and Darr, 1976) and chemical cross-linking (Stoffel *et al.*, 1977) have shown the importance of hydrophobic interactions in the stabilization of the complexes, while there is little evidence showing ionic interactions. In fact, if the putative ionic interaction were of primary importance then one might expect marked loss of apoproteins from the lipoproteins during routine isolation in high salt concentrations (Havel *et al.*, 1955).

McLachlan (1977) has reported a high frequency of homology of amino acids between segments (11 or 22 amino acids long) within the apoA-I sequence, suggesting that the sequence may have evolved by internal gene duplication. Such homology may also reflect convergent evolution of sequences of similar amphipathic character.

Studies of apoprotein conformation in solution have provided insight into apoprotein structure (Scanu, 1969). Jonas (1973) reported that bovine apoHDL has a high degree of exposure of tyrosine and tryptophan residues to the aqueous solvent, suggesting that the apoprotein has an extended conformation. By contrast, in intact HDL tyrosine and tryptophan residues were less exposed to the solvent. Gwynne *et al.* (1974, 1975a,b) showed that apoA-I and apoA-II are denatured in low concentrations of guanidinium hydrochloride. Employing scanning calorimetry and ultraviolet difference spectroscopy, Tall *et al.* (1975, 1976) studied the thermal and urea-induced denaturation of apoA-I and showed that free energy difference (ΔG) between the folded and unfolded states of the apoprotein was small (2.4 kcal/mol at 37°C), compared to other small globular proteins like myoglobin or ribonuclease (~ 10 kcal/mol) (Privalov and Khechinashvili, 1974). Reynolds (1976) confirmed that there was little free energy difference between the native and denatured (completely unfolded) forms of apoA-I or apoA-II in guanidinium HCl, in contrast to intrinsic membrane proteins which are resistant to complete unfolding by the same denaturant. These properties probably account for the cooperative binding of detergents by apoA-I at very low detergent concentrations (Reynolds and Simon, 1974). These findings indicate that the apolipoproteins have a loosely folded conformation in solution with a high degree of exposure of hydrophobic amino acid residues to the solvent. Such conformational properties are important in two respects. First, they probably provide hydrophobic sites for protein–protein self-association known to occur in solutions of isolated apoproteins (v.i.). Second, they probably determine the lipid-binding capacity of the apolipoproteins, by providing ready access of lipid molecules to manifold hydrophobic sites on or within the protein globule.

There is abundant evidence that apoA-I, apoA-II and, apoC-I undergo self-association in solution (e.g., Vitello and Scanu, 1976; Stone and Reynolds, 1975; Osborne *et al.*, 1976, 1977; Gwynne *et al.*, 1975b). Stone and Reynolds (1975) and Jonas and Krajnovich (1977) have presented evidence that self-association does not greatly influence lipid binding. Ritter and Scanu (1977) have shown that monomeric apoA-I forms complexes more readily than multimeric A-I when sonicated with total HDL lipid. Major differences in apoA-I and apoA-II lipid binding have been reported (Assmann and Brewer, 1974; Middelhoff *et al.*, 1976) probably due to differences in their kinetics of interaction with lipid, or to apoprotein self-

association. The earlier reports that apoA-I does not form recombinants with phosphatidylcholines have not been borne out in most subsequent studies (Tall *et al.*, 1975, 1977c; Ritter and Scanu, 1977). No study of the effect of apoprotein self-association on recombination with lipid has adequately differentiated between kinetic effects as opposed to behavior at equilibrium.

Recently Swaney and O'Brien (1978) have studied the self-association of apoA-I and apoA-II, using the cross-linking reagent dimethylsuberimidate. At low concentration apoA-I was monomeric but associated to tetramers and pentamers at concentrations of 0.5 mg/ml or higher. For apoA-II the main oligomeric form was the dimer. These results were in general agreement with the hydrodynamic studies. Interestingly, phospholipid–apoA-II recombinants contained trimers upon cross-linking.

C. Recombinants of Apolipoproteins and Lipids

Hirz and Scanu (1970) showed that ultrasonically dispersed HDL lipids (phospholipids and cholesteryl esters) could be recombined with apoHDL to produce a particle resembling native HDL. In the absence of phospholipid, ultrasonically treated cholesterol and cholesteryl ester did not recombine with apoHDL. ApoA-I can be recombined with myelin figures of phospholipid (multilamellar liposomes) by simple incubation (Tall *et al.*, 1975), or by consonication, both methods producing an HDL–phospholipid particle of identical composition (Ritter and Scanu, 1977). However, recombination of whole HDL lipids (including phospholipid, cholesterol, cholesteryl ester, and triglyceride) with apoA-I required cosonication, producing an HDL particle of radius 31 Å (two molecules apoA-I per particle) or 39 Å (three molecules of apoA-I per particle). The circular dichroism spectra of HDL apoproteins show an increased helical content of the apoproteins upon recombination with phospholipid (Lux *et al.*, 1972a,b; Jackson *et al.*, 1973), indicating conformational stabilization of the apoprotein in the lipoprotein complex. Studies of HDL recombinants prepared from egg yolk lecithin vesicles and apoC-I show that the increased apoprotein helical content is associated with a movement of tryptophan residues to a more hydrophobic environment, as shown by a 5-nm blue shift in the fluorescence maximum (Jackson *et al.*, 1974b). Differential scanning calorimetry of HDL recombinants prepared from dimyristoyl or dipalmitoyl lecithin and apoA-I showed an increased temperature and enthalpy of denaturation of apoprotein in the lipoprotein complex (Tall *et al.*, 1975, 1977c). The lipoprotein denaturation was a two-state process and gave a calculated free energy of association of DML and apoA-I of 10.5 kcal/mol (37°C).

Under most conditions apoA-I and apoA-II form recombinants with

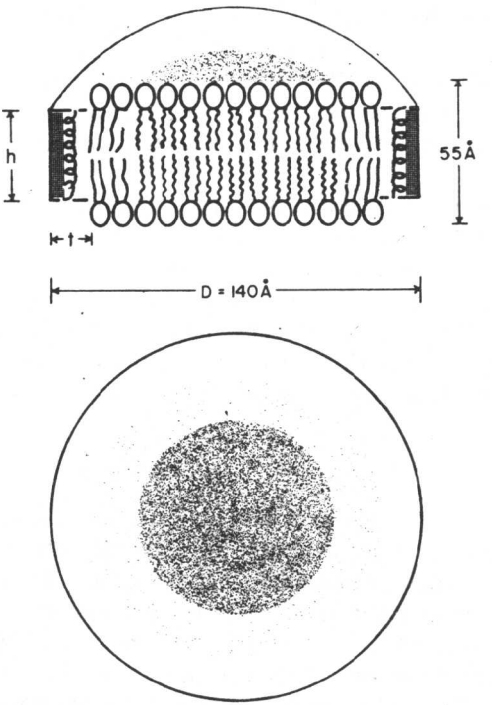

FIG 1. Schematic representation of an apoHDL/DML complex containing about 60% DML; (top) cross-section with three dimensional view; and (bottom) top view. The complex consists of a phospholipid bilayer disc with apoprotein α-helices forming an annulus around the edge of the disc. The hydrophobic surface of the apoprotein amphipathic helices (dotted hatching) contact the phospholipid hydrocarbon chains. D represents the total diameter of discs; h, the length of apoprotein helix in contact with phospholipid; and t, the width of the apoprotein helix. In the top view the outer layer (apoprotein) interacts with a boundary zone of phospholipid one to two molecules thick (light hatching), while the phospholipid molecules in the center (dark hatching) can undergo gel-liquid crystalline transitions. From Tall *et al.* (1977c).

lecithin alone which have the structure of a discoidal phospholipid-bilayer (Fig. 1). Under certain conditions, to be discussed, the product retains the structure of a unilamellar phospholipid vesicle. Forte *et al.* (1971a, b) first demonstrated that the high-density lipoprotein recombinant resulting from the cosonication of egg yolk lecithin and apoA-I had the morphology of a lipid bilayer disc, 150–200 × 45A, when viewed by negative stain electron microscopy. Discoidal or vesicular lipoproteins will stack in rouleaux when dehydrated and examined by electron microscopy. Atkinson *et al.* (1976), studying the complexes of dimyristoyl lecithin with bovine apoHDL