OR

Ass. Clin. Sci. Wash. D.C.

11/12-14/1971

see card for details

CONF-71128--1

THE POLYPEPTIDES OF HUMAN SERUM LIPOPROTEIN
AND THEIR CLINICAL SIGNIFICANCE*

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The purpose of this report is to provide a summary on the information currently available on the isolation and properties of the various polypeptides of human circulating lipoproteins. At the outset, it should be recognized that the field is moving at a very rapid pace and that it is not yet possible to arrive at a definitive classification of these various polypeptides in physical, chemical and immunological terms. In this brief survey, I will put primary emphasis on recent development on the subject, referring for more detailed information to pertinent general reviews.⁵

CONSIDERATIONS ON THE METHODS FOR PREPARING POLYPEPTIDES IN LIPID-FREE FORM

In the native molecules, or particles, the polypeptides of serum lipoproteins are combined with lipids, predominantly, if not exclusively, through non-covalent linkages. To permit their characterization, these polypeptides should ideally be obtained free of lipids and in water-soluble form. This goal is not uniformly achieved with all lipoprotein classes and procedures have been devised to overcome difficulties which appear inherent with the intrinsic structural properties of each of these polypeptides. In the case of HDL, the removal of the lipid moiety is more commonly achieved by the use of ethanol-ethyl ether mixtures in the cold.³ Although many variations from the original methods have been used, the protein obtained has been shown to be soluble in aqueous buffers, although still containing a small percentage (less than 1 per cent) of lipid as phospholipids. Other solvent mixtures have been used (ethanol-acetone; methanol-methylal; chloroform methanol) as well as detergents.⁵ Until present, these delipidation products have not been as well characterized as those obtained by ethanol-ether extraction. The solubili-

zation of apo-LDL, regardless of the method for delipidating LDL, has proven a difficult task and may be achieved only by chemical modification of the protein or by the use of anionic or nonionic surfactants or dissociating agents such as urea or guanidine hydrochloride. The reasons for this poor solubility have not been clearly established although it appears to be due, at least in part, to the tendency of apo-HDL to assemble into large molecular aggregates. Delipidation of VLDL yields an essentially lipid-free product which is only partially soluble in aqueous buffer. A large portion of this protein exhibits the solubility characteristics of apo-LDL in terms of requiring detergent, dissociating agents or combinations thereof, to go in solution.

Recent studies from this laboratory have established that some of the polypeptides of VLDL and HDL are partially soluble in the ethanol ether mixtures employed for delipidation,⁴ a fact which accounts for the non-ideal protein recoveries observed with these methods. Appropriate measures have been developed to overcome this difficulty.⁴ In approaching the problem of delipidating any of the serum lipoproteins, one should be aware of the fact that there is no procedure which satisfactorily applies to all serum lipoproteins and that in each individual case the experimental conditions (i.e., protein concentration, buffer pH and ionic strength, ratio of protein to extracting solvent, temperature, etc.) are to be kept rigorously controlled. The same applies for the techniques of solubilization of the delipidated apoproteins. In such a case, consideration ought to be given to the polypeptide heterogeneity now recognized in each major lipoprotein class, a topic which will be dealt with in the following sections.

POLYPEPTIDES OF APO HDL

It is now well established that apo HDL is made of several polypeptide chains which can be separated from each other by chromatographic and/or electrophoretic procedures (Table I). At the present time the information available is still incomplete and the emerging evidence is that the polypeptide composition of apo HDL may be much more complex than previously suspected as supported by the studies, still in progress, carried out in this laboratory. As shown in Table 2, apo HDL, when filtered through Sephadex G-200 columns, equilibrated and eluted with buffers containing 8 M urea,⁷ yields three principal fractions: III, IV and V, each separable into chemically distinct sub-components by ion-exchange chromatography. The general properties of III, IV and V are summarized in Table 3. Work on the characterization of the various polypeptide sub-components is still in progress and the results are proving of great interest both from the structural and functional standpoints. While the reader is referred to a recent review⁵ for the work by others in the field, it must be mentioned that significant advances have been made with studies directed at gradually re-building "in vitro" molecules having characteristics of HDL. Such studies are making use of HDL polypeptides in their lipid-free form and of lipids either isolated from HDL or synthetic ones.⁸ The information thus far obtained, indicates that the two major polypeptide classes of apo HDL, III and IV, retain binding capacity for lipids and, under appropriate experimental conditions, can yield a complex with structural characteristics very similar, at least, to those of native HDL. Such an approach is expected to provide important knowledge on the nature of the protein-lipid interactions in HDL, information which is significantly lacking at the moment.

POLYPEPTIDES OF APO LDL

Contrary to apo HDL, delipidated apo LDL is difficult to dissolve in aqueous buffers. Methods of solubilization rely on the use of anionic or non-ionic surfactants alone or in combination with dissociating agents, such as urea or guanidine hydrochloride, or by appropriate chemical modification of the protein.⁵ Even when in solution, apo LDL forms large molecular aggregates whose individual constituents have been difficult to separate from each other by conventional chromatographic or electrophoretic methods. For the above reasons, the question of the possible chemical heterogeneity of the various polypeptides of apo LDL has not been clearly resolved although some evidence points in this direction. In a discussion of apo LDL heterogeneity, one has to make a clear distinction between impurities which may be present in LDL preparations, from the heterogeneity which may involve the intrinsic polypeptide constituents of apo LDL. The general properties of apo LDL are summarized in Table 4. On the assumption that the minimum molecular weight of apo LDL is 25,000, it has been computed that LDL contains 20 subunits. It has also been suggested that these subunits are arranged in an icosahedral symmetry, based on the interpretation of electron micrographs of negatively-stained preparations of LDL. Corroboration of this concept by other techniques is warranted.

POLYPEPTIDES OF APO VLDL

(Table 5). Particularly through the work of Brown et al.² and later by this laboratory, it has now been established that apo VLDL has a complex polypeptide composition. About 40% of this protein is made of a component with solubility characteristics and immunological properties of apo LDL, whereas the largest percentage represents a class of polypeptides which dissolve readily in Tris buffer

and have a molecular weight less than 10,000 daltons. The presence of small quantities of apo HDL have also been reported. Since such observation was carried out in hyperlipemic subjects, it is not established at present whether such observation has physiological relevance or simply applies to pathological states. Based on their C-terminal amino acids, the Tris-soluble polypeptides of apo VLDL have been named R-Val (or R-Ser), R-Glu and R-Ala, the latter existing in at least three polymorphic forms having no, 1 mole or 2 moles of sialic acid per mole of protein.² It is likely that as studies progress, other polymorphic forms will be detected and the criteria of classification based on C-terminals will prove inadequate to identify these different units. The results thus far obtained are very encouraging and have served to identify a new class of polypeptides which seem specific for VLDL.

INTERRELATIONSHIP AMONG SERUM LIPOPROTEINS

A great limitation in defining the specific structural characteristic of each lipoprotein class is their known tendency to interrelate metabolically with consequent exchange or transfer processes of given components from one lipoprotein class to another. This leads to difficulties in the isolation of "pure" classes by the conventional methods of serum lipoprotein fractionation, and also creates problems in nomenclature and classification. For instance, it has been established that apo HDL and apo VLDL have common polypeptide species and this may also apply to certain LDL classes. For the investigator interested in lipoprotein structure, his task is to distinguish between "constitutive" and "added" components and his goal is to pull them apart by sophisticated isolation procedures generally insuring "pure" products at the expense of heavy lipoprotein losses. For other investigators less

Interested in such problems, they may have to pay for their faith in "established" techniques with the recovery of products containing perplexing polypeptide mixtures which may lead these investigators to either deny the existence of specific lipoprotein classes to believe that his poor laboratory isolates are, in fact, physiological lipoprotein complexes. At this moment, it is not possible to arrive at firm conclusions concerning the nature and classification of circulating lipoproteins. It is anticipated that work in progress in various laboratories on the definition of the nature of the various polypeptides of serum lipoproteins may bring a clarification of the problem and lead to reassessment of conceptual and technological problems.

CLINICAL SIGNIFICANCE OF SERUM LIPOPROTEIN POLYPEPTIDES

The observation that several distinct polypeptides are present in circulating lipoproteins poses the question of their physiological and clinical significance. Although definitive conclusions on the subject are likely to wait for the results of the final characterization of these polypeptides, some information is already emerging, partially through the studies on special enzymatic reactions which appear to involve these polypeptides and partially through the work on the pathogenesis of the various types of dyslipoproteinemias. All of these studies seem to reinforce the concept that lipoprotein proteins play a major role in lipid metabolism as exemplified by the fact that structural derangements involving one or more of these polypeptides are associated with major abnormalities in the distribution of circulating lipoproteins. Tangier's disease has been associated with an abnormal distribution of the major polypeptides of apo HDL, whereas in α - β -lipoproteinemia, the absence of circulating apo LDL has been documented (Table 6). At the present time, little

is known of the polypeptide composition of the various lipoprotein classes in hyperlipoproteinemia and it may not be excluded that they may contribute to the abnormality in lipoprotein distribution present in these disorders.

Some of the lipoprotein polypeptides have been shown to participate as co-factors in the "in vitro" activity of two important enzymes: lipoprotein lipase and lecithin-cholesterol acyl transferase. Should such observations be proven valid "in vivo," the theoretical possibility exists of the occurrence of dyslipoproteinemias secondary to a "co-factor" deficiency. In this respect, particularly relevant would be the study of possible variants of primary or secondary hypercholesterolemia or of disorders characterized by low levels or absence of circulating cholesteryl esters. These types of disorders have been thus far associated with deficiencies in the enzymes lipoprotein lipase or lecithin-cholesterol acyl transferase. Whether this will prove true for all cases, remains to be established.

To sum up, several polypeptides have been found associated with serum lipoproteins. Many of their structural and functional properties are still unknown and much work in this direction is currently under way in various laboratories. In view of this limited knowledge, it is still too early to evaluate the actual role of these polypeptides in the pathogenesis of presently recognized dyslipoproteinemias. It is probable that current concepts and classifications may prove inadequate in the not too distant future.

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FOOTNOTES

*The work reported in this paper was supported in part by grants from the USPHS (HE-08727), the American Heart Association (#70-753) and the Chicago and Illinois Heart Association (A70-17).

Abbreviations: VLDL = very low-density lipoproteins of $d < 1.006$ g/ml; LDL₂ = low-density lipoproteins of $d 1.006-1.019$ g/ml; LDL₁ = low-density lipoproteins of $d 1.019-1.063$ g/ml; HDL₂ = high-density lipoproteins of $d 1.063-1.125$ g/ml; HDL₃ = high-density lipoproteins of $d 1.125-1.21$ g/ml; VHDL₁ = very high-density lipoproteins of $d 1.21-1.25$ g/ml; VHDL₂ = very high-density lipoproteins of $d > 1.25$ g/ml. The prefix "apo" refers to the essentially lipid-free apoprotein obtained by delipidation.

†Recipient of USPHS Research Career Development Award HE-24,867.

‡Operated by the University of Chicago for the United States Atomic Energy Commission.

TABLE 1
CORRELATION BETWEEN DENSITIES AND POLYPEPTIDE
COMPOSITION IN THE HDL CLASS

Lipoproteins		Polypeptides*		
d	Nomenclature	III**	IV**	V**
1.063-1.125	HDL ₂
1.125-1.21	HDL ₃
1.21-1.25	VHDL ₁
> 1.25	VHDL ₂

*Polypeptide nomenclature is operational and derived from their order of elution from Sephadex columns.⁷

**Represents a class made of at least two chemically distinct polypeptides.

The identity of the polypeptides was assessed by chromatographic, chemical and immunological criteria.

TABLE 2
SCHEME FOR THE FRACTIONATION OF THE POLYPEPTIDES
OF HUMAN SERUM APO HDL*

* This scheme is originated from studies on single donors. A greater number of polymorphic forms is expected from the study of pooled sera.

TABLE 3

GENERAL PROPERTIES OF FRACTIONS III, IV AND V SEPARATED
FROM APO HDL BY SEPHADEX CHROMATOGRAPHY IN 8 M UREA

Fraction	Properties
III (60-70% of apo HDL by weight)	MW = ~28,000; apo HDL contains two chains having the same MW, N- and C-terminals and sialic acid content, but differing slightly in amino acid composition. Characteristically, these polypeptides have no or little isoleucine and are immunologically distinct from the other apo HDL fractions.
IV (30-40% of apo HDL by weight)	In its unreduced form, IV has a MW of ~17,000 which, upon disulfide cleavage, yields two polypeptides of MW ~8,500. Apo HDL contains 4 of these low MW polypeptides. Characteristically, they have no or low content in arginine, histidine and tryptophan and are immunologically distinct from III and IV.
V (4-5% of apo HDL by weight)	It represents a class of heterogeneous polypeptides separable from each other by ion exchange chromatography. They are still in the process of being identified; some of them appear identical with the Tris-soluble polypeptides from apo VLDL.

TABLE 4
PROPERTIES OF HUMAN SERUM APO LDL

MW = 25,000 to 80,000 daltons (single chains ?)

N-terminal = Glu; C-terminal = Ser (other minor components detected)

Carbohydrates: 3-4% by weight

Immunological properties dependent on method of delipidation

Optical rotatory dispersion and circular dichroic spectra different from that of the protein in native LDL.

TABLE 5
POLYPEPTIDES OF APO VLDL

Three classes of polypeptides have been identified:

1. A polypeptide class (% by weight = 30-40) soluble in aqueous buffers only in the presence of urea, guanidine hydrochloride or anionic or nonionic surfactants. Immunologically indistinguishable from apo LDL.
 2. A class particularly evident (% by weight = 0-5) in hyperlipidemic patients, having electrophoretic and immunological properties of apo HDL (fractions III and IV).
 3. A class of polypeptides which appears specific for apo VLDL (% by weight = 50-60) with MW of $\sim 10,000$ daltons, readily soluble in aqueous buffers and separable by ion exchange chromatography or isoelectric focusing into at least three different polypeptides differing in amino acid composition and N- and C-terminals. Some of these polypeptides are also found in fraction V of apo HDL.
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TABLE 6

EXAMPLES OF CLINICAL DISORDERS WHICH APPEAR LINKED TO A
STRUCTURAL DERANGEMENT OF A SPECIFIC LIPOPROTEIN PROTEIN

Disease	Postulated Molecular Defect
Tangier's disease (homozygous state)	Abnormal distribution of the two major polypeptides of apo HDL (fractions III and IV according to the nomenclature used in this laboratory).
A- β -lipoproteinemia	Absence in circulation of the major polypeptide of apo LDL. This is probably not the sole defect, since abnormalities in HDL ₂ :HDL ₃ relationship are also present. Apo HDL fraction V appears normal.
?	Other abnormalities are likely to be uncovered as a more thorough knowledge is obtained on the structure and function of the various lipoprotein polypeptides.