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ISOLATION AND ANALYSIS OF HUMAN PLASMA LIPOPROTEINS ACCUMULATING POSTPRANDIAL IN AN INTERMEDIATE DENSITY FRACTION (*d* 1.006—1.019 g/ml)

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Summary

Plasma lipoprotein patterns have generally been determined in the fasting state. However, there is evidence from turnover and dietary studies for short-living "remnants" or "intermediate particles" accumulating postprandial.

In this study we have been able to identify, isolate and partially characterize by immunochemical, chemical and ultrastructural means at least two different lipoprotein populations accumulating 6 h postprandial in the intermediate density fraction *d* 1.006—1.019 g/ml of healthy volunteers.

Introduction

There is abundant evidence that the plasma compartment must be regarded as an important site of lipoprotein metabolism. In the past, substantial evidence accumulated indicating that all different plasma lipoprotein fractions are interrelated in both their protein and lipid portions (for review see ref. 1). Many of the processes involved in this mechanism are mediated through the activity of various lipolytic enzymes, some of which are affected by apolipoproteins acting differently as cofactors or inhibitors [2—10]. Therefore, the metabolic relationship of these conjugated macromolecules seems to be determined and to depend not only on their lipid portion but also on their apoprotein composition. Thus, the evaluation of the relation of plasma lipoprotein

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structure and their metabolic pathways may allow for a better understanding of lipid metabolism and clinical disorders where plasma lipoproteins are elevated.

In the past, plasma lipoprotein patterns have generally been determined in the fasting state. However, since there is evidence from turnover and dietary studies [11—16] for short-living "remnants" or "intermediate particles" accumulating postprandial in the plasma pool, it seems relevant to study such compounds more carefully in order to obtain a better insight into the biodynamics of lipid metabolism in health and disease. In the present study, we have attempted to characterize and fractionate the intermediate density fraction *d* 1.006—1.019 g/ml of healthy volunteers 6 h after a meal.

Material and Methods

Voluntary donors

A total of six healthy voluntary donors, aged 22—40 years and of both sexes, were studied. None of these subjects had abnormal liver or kidney function tests and both their lipid levels as well as their lipoprotein patterns were within the normal range (Table I).

After an overnight fast each volunteer received a meal of 1500 calories (percent calory distribution: protein—fat—carbohydrate, 20:40:40) and 6 h later 200 ml of blood were drawn from the cubital vein, allowed to clot and the serum separated by low speed centrifugation (3000 rev./min for 10 min). EDTA in a final concentration of 1.7 mM was added to all serum samples and lipoprotein fractions.

Chemical analyses

Agarose—agar electrophoresis was performed by a modification [17] of Noble's method [18]. The lipoprotein bands were visualized by the use of polyanionic compounds as previously described [19,20]. The immunochemical properties of isolated lipoproteins were studied by double diffusion [21] and immunoelectrophoresis [22] in 1% agar (Difco Bacto Agar, Detroit, Mich.) or 0.8% agarose (Serva, Heidelberg) gels employing a barbital buffer; pH 8.6, ionic strength 0.05. Rabbit anti-sera to apolipoprotein A (apo-A; consisting of the AI and AII peptides), apolipoprotein B (apo-B) and apolipoprotein C (apo-C; con-

TABLE I

SEX, AGE AND PLASMA LIPID VALUES OF THE VOLUNTEERS STUDIED

Initials	Sex	Age (years)	Weight (kg)	Plasma cholesterol (g/l)	Plasma triglycerides (g/l)
S.S.	F	24	60	1.78	0.98
B.E.	F	35	51	2.40	0.55
K.M.	M	22	65	1.38	0.75
H.T.	M	22	71	1.63	1.10
H.F.	M	40	68	2.10	1.73
W.E.	M	23	73	1.80	1.07

sisting of the CI, CII and CIII peptides) were prepared in our laboratory and carefully tested for their specificity as earlier described [23]. Antibodies to human albumin and human gamma-globulins were purchased from Behring—Werke AG Marburg/Lahn. The protein portion of some lipoprotein fractions was also studied by polyacrylamide electrophoresis after treatment with tetramethylurea (TMU) according to a previously described method [24]. Esterified and unesterified cholesterol, phospholipids and protein were determined as described earlier [25]. Triglycerides were measured enzymatically using a standard procedure (Boehringer GmbH, Mannheim).

Electron microscopy

Electron microscopy was performed on various lipoprotein fractions after negative staining at 0° and 25° with a 1% aqueous solution of phosphotungstic acid, adjusted to pH 7.1 with KOH according to standard procedures. Grids were examined in a Siemens Elmiskop 101 electron microscope (Siemens AG Berlin) equipped with a cooling device and double condensor illumination at accelerating voltage of 80 kV. Pictures were taken at a magnification ranging from × 20000 to 50000.

Isolation and fractionation of lipoproteins

The separation scheme is outlined in Fig. 1. The VLDL fraction ($d < 1.006$ g/ml) was separated from the serum by layering the samples under equal volumes of NaCl solutions (d 1.006 g/ml) containing 1.7 mM EDTA and by centrifugation in a type TI 60 rotor of a Spinco model L2 65 B ultracentrifuge for 22 h at 50000 rev./min and 4°. After centrifugation the top fraction was removed by a tube slicing technique and the bottom fraction recentrifuged under identical conditions. The $d > 1.006$ g/ml fraction was then adjusted to a

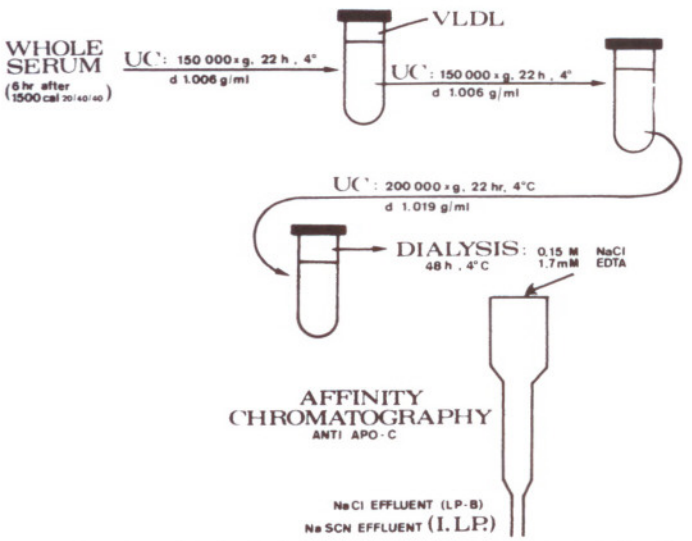

Fig. 1. Procedure for the isolation of two different lipoprotein fractions of the density class d 1.006—1.019 g/ml.

solution density of *d* 1.019 g/ml by adding NaBr in appropriate amounts and then subjected to ultracentrifugation (rotor type TI 65; 60000 rev./min; 22 h at 4°). The obtained *d* 1.006—1.019 g/ml density class was then further fractionated by affinity chromatography [26] on sepharose 4B (Pharmacia Uppsala) containing a covalently linked monospecific rabbit anti-human apo-C globulin fraction. The non-bound beta-lipoproteins (LP-B) were removed from the columns by elution with excess 0.15 M NaCl, pH 7.2, containing 1.7 mM EDTA. The bound and larger intermediate lipoprotein designated (I.L.P.) was then obtained by elution of the columns with 3 M NaSCN. Immediately after recovery this fraction was dialysed exhaustively for 24 h at 4° against 0.01 M Tris buffer, pH 7.2 containing 0.15 M NaCl and 1.7 mM EDTA.

Results

The intermediate density class *d* 1.006—1.019 g/ml isolated 6 h postprandial showes the presence of at least two different lipoprotein families as judged by immunochemical analysis. This holds also for fractions which have been recentrifuged twice at a solution density *d* 1.019 g/ml. The fraction reacts immunochemically with anti-bodies to apo-A, apo-B, and apo-C. However, only partial identify can be obtained between anti-apo-A and apo-C, serum versus anti-apo-B serum (see Fig. 2), suggesting that one compound of the *d* 1.006—1.019 g/ml density fraction consists of apo-A, apo-B, and apo-C, while the second lipoprotein carries only apo-B as protein portion.

In the electron microscope (Fig. 3a and b) preparations of the *d* 1.006—1.019 g/ml fraction appear to consist of more or less round particles rather heterogeneous in size. The diameter ranges from 20 nm (i.e. that of the normal fasting components of the low density lipoprotein fraction) up to 60 nm which is close to that of fasting very low density lipoprotein particles. No definitive statement on proportions of particles with different diameter can be made, as the diameter of individual particles varies a great deal between the above mentioned limits. On contact with each other the lipoprotein particles of this fraction may undergo some distortion in shape; however, the individual particles are well outlined and no aggregation or fusion can be observed. No distinct surface features or subunit structures can be definitively detected by the applied technique. After elution of the chromatography column by 3 M

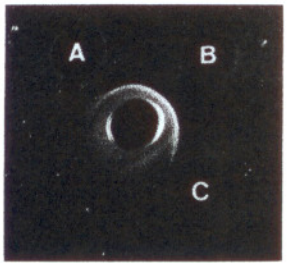

Fig. 2. Immunodiffusion pattern in 1% agar gel of the intermediate density fraction *d* 1.006—1.019 g/ml isolated 6 h postprandial (central well). A, rabbit anti-apo-A serum; B, rabbit anti-apo-B serum; C, rabbit anti-apo-C serum.

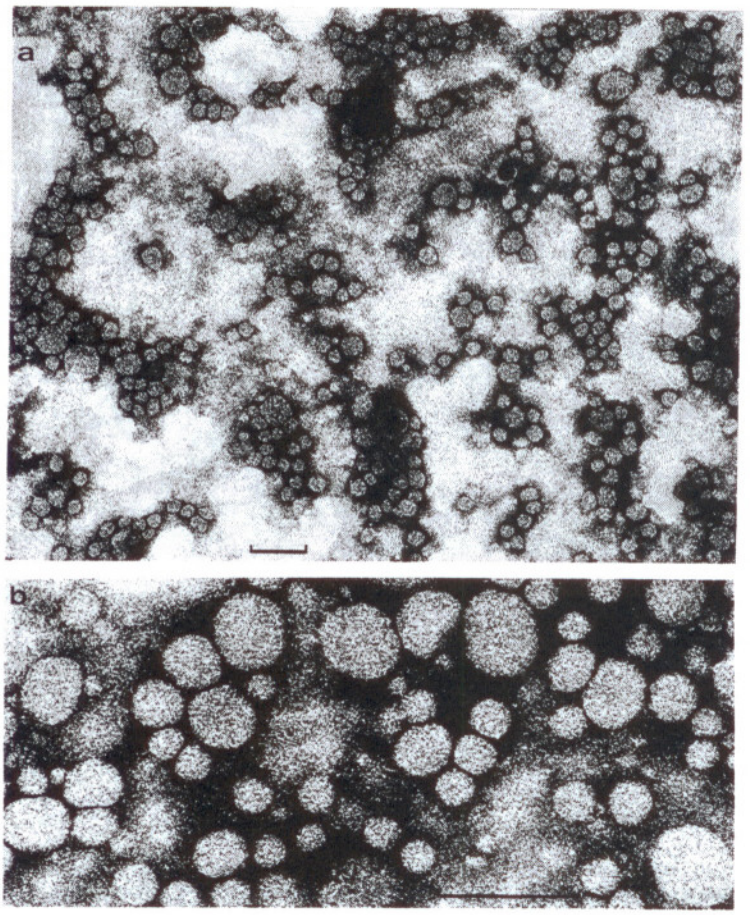

Fig. 3. Electron micrograph of the *d* 1.006—1.019 g/ml density fraction 6 h postprandial from plasma of donor S.S. Negative staining at 25° with potassium phosphotungstate. a, × 72 000; b, × 190 000. Scale bar = 0.1 μm.

NaSCN some structural alterations were observed on the intermediate lipoprotein fraction by electron microscopy.

The total fraction develops beta-mobility on agarose electrophoresis (see Fig. 4) and can be visualized by the use of sodium heparin (0.15%; w/v) and $MgCl_2$ (0.1 M) in a 0.15 M solution of NaCl in contrast to the low density lipoprotein fraction (*d* 1.019—1.063 g/ml) which does not precipitate under these conditions but does when 0.6% (w/v) dextran sulfate 500 (Pharmacia, Uppsala) in a 0.2 M solution of $CaCl_2$ is used (Fig. 4) [20]. Because of differences in their protein portion the two lipoprotein compounds present in the *d* 1.006—1.019 g/ml density class obtained 6 h postprandial can be separated and purified by the use of affinity chromatography with sepharose 4B and specific anti-bodies to apolipoprotein C.

While the NaCl effluent develops an immunoprecipitin reaction only with anti-apo B (see Fig. 5) the NaSCN eluted fraction designated in this study as I.L.P. (intermediate lipoprotein) never reacted with anti-bodies to human

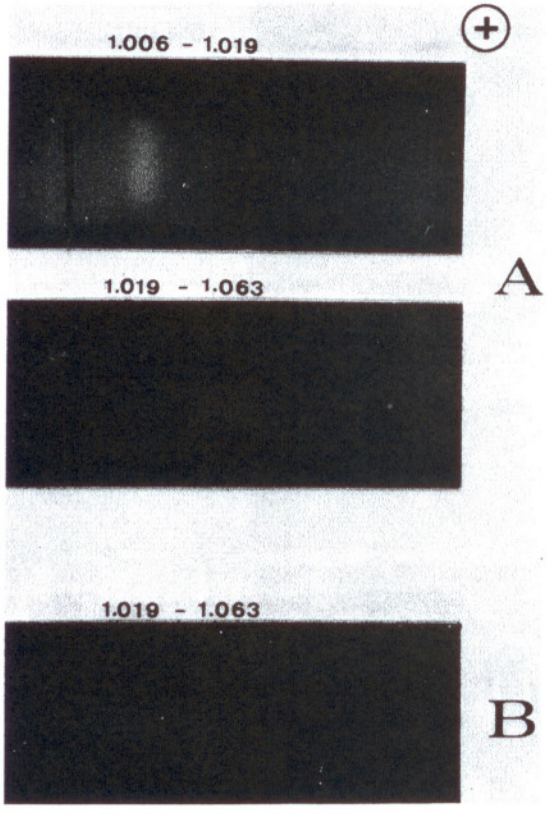

Fig. 4. Lipoprotein electrophoresis of the *d* 1.006—1.019 g/ml and the *d* 1.019—1.063 g/ml density fraction isolated 6 h postprandial. A, polyanion precipitation with a 0.1 M $MgCl_2$ solution containing 0.15% sodium heparin and 0.15 M NaCl; B, the same slide of the *d* 1.019—1.063 g/ml density fraction as in A after precipitation with a 0.2 M $CaCl_2$ solution containing 0.6% sodium dextran sulfate 500.

albumin or human globulins but always showed a precipitin line of complete identity with anti-bodies to apo-A, apo-B, and apo-C in both double immunodiffusion and immunoelectrophoresis (see Fig. 6). This finding clearly indicates that apo-A, apo-B and apo-C form part of the structure of this plasma lipoprotein. The polyacrylamide pattern of tetramethylurea treated I.L.P. revealed all bands typical for the three apolipoprotein C peptides. Studies characterizing

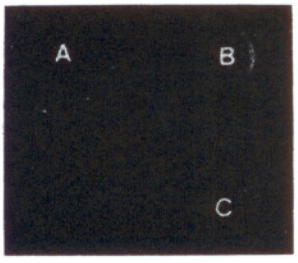

Fig. 5. Immunodiffusion pattern in 1% agar gel of an LP-B fraction, isolated 6 h postprandial from the intermediate density class *d* 1.006—1.019 g/ml (central well). A, rabbit anti-apo-A serum; B, rabbit anti-apo-B serum; C, rabbit anti-apo-C serum.

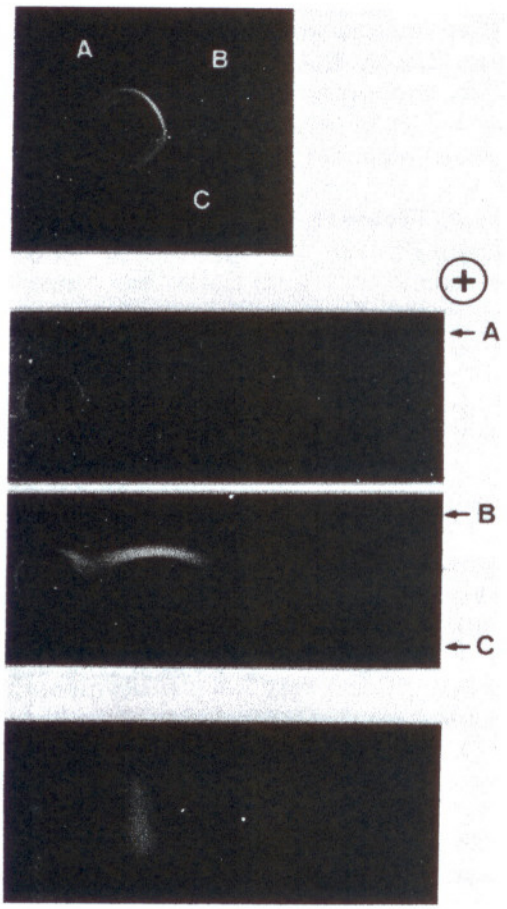

Fig. 6. Immunochemical and electrophoretical behaviour of isolated I.L.P., 6 h postprandial. Immunodiffusion pattern (1% agar gel) and immunoelectrophoresis pattern (1% agarose gel): A, rabbit anti-apo-A serum; B, rabbit anti-apo-B serum; C, rabbit anti-apo-C serum. Lipoprotein electrophoresis pattern in 1% agarose gel after polyanion precipitation with a 0.1 M $MgCl_2$ solution containing 0.15% sodium heparin and 0.15 M NaCl.

TABLE II

PERCENT PROTEIN—LIPID COMPOSITION OF I.L.P. ISOLATED FROM THE DENSITY CLASS *d* 1.006—1.019 g/ml 6 h POSTPRANDIAL

Protein (n = 6)	Cholesterol		Triglyceride (n = 6)	Phospholipid (n = 6)
	total (n = 6)	% ester (n = 2)		
22.1 ± 2.2	26.1 ± 1.4	61;64	33.5 ± 2.3	18.1 ± 1.9

the precise chemical nature of the different polypeptides present in the I.L.P. as well as the determination of their relative amounts are in progress.

Like the entire *d* 1.006— 1.019 g/ml density fraction, the isolated I.L.P. developed beta-mobility on agarose electrophoresis and precipitated with heparin (0.15%; w/v) $MgCl_2$ (0.1 M) in a 0.15 M solution of NaCl. This behaviour was earlier described to be characteristic for VLDL of fasting serum [20].

The percent protein—lipid composition (Table II) of purified I.L.P. differs significantly from fasting VLDL, fasting LDL and fasting HDL. Its unique chemical composition of 34% triglycerides and 27% total cholesterol suggests its intermediate nature between triglyceride-rich lipoproteins of hydrated density $d < 1.006$ g/ml and fasting beta-lipoproteins which are primarily found in the density class *d* 1.019—1.063 g/ml. The limited amount of purified LP-B isolated postprandial from the *d* 1.019—1.063 g/ml density class did not allow us to establish the exact percent protein—lipid composition of this fraction.

Discussion

In the 12 h fasting plasma of normal subjects only small amounts of lipoproteins are found in the intermediate density fraction *d* 1.006—1.019 g/ml [27]. It was earlier shown that postprandial or after heparin administration the concentration of lipoproteins increases in this fraction [13,16,27] and a precursor—product relationship between human plasma very low density lipoproteins and lipoproteins of higher density has been demonstrated in the past for both the protein and lipid portion (for review see ref. 1). However, the fate of the different very low density lipoprotein constituents seems to be different and to follow different mechanisms [13].

The immunochemical and ultrastructural results of our study suggest that 6 h postprandial the intermediate density fraction *d* 1.006—1.019 g/ml of healthy volunteers contains at least two different lipoprotein populations of different size and different protein portion. The smaller particles up to 25 nm contain only apo-B, the larger particles (probably those ranging from 35 up to 60 nm) designated I L.P. carry all major apolipoproteins. The intermediate nature of I.L.P. between VLDL and lipoproteins of higher density is suggested by the unique lipid composition and precipitation behaviour with polyanionic compounds [20] of this fraction.

Since the *d* 1.006—1.019 g/ml density fraction isolated from fasting subjects seem to contain only lipoproteins in form of "association products" [28] consisting of apo-B and apo-C the apparent differences to the postprandial state as demonstrated in this study may reflect different ways of conversion of VLDL to LDL. This possibility is supported by recent turnover studies of VLDL apolipoproteins [13].

Because of the specific and different action of the various apolipoproteins upon the various lipolytic enzymes and because of the role of the apolipoproteins in stability and structure of various lipoprotein families it seems relevant to evaluate the lipoprotein spectrum not only in the fasting but also during the postprandial stage. Such studies may yield structurally different "intermediate particles" in the various forms of hyperlipoproteinemia at different time inter-

vals and under different dietary and drug managements. It will be a task of the future to isolate and characterize such intermediate particles and to determine their interaction with isolated lipolytic enzymes.

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