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Isolation and Partial Characterization of High-Density Lipoprotein HDL_1 from Rat Plasma by Gradient Centrifugation

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The lipoproteins isolated from rat plasma by flotation in the density range 1.019–1.063 g/ml were further characterized. Using rate zonal ultracentrifugation, we isolated two lipoproteins in almost equal proportions from this density range. Similar isolations may be accomplished with density gradients in a swinging-bucket rotor. On isopycnic-density-gradient ultracentrifugation one component banded at $\rho = 1.031$ g/ml and the other at $\rho = 1.054$ g/ml. More that 98% of the apoprotein of the lighter component was B protein, and hence this particle is LD (low-density) lipoprotein. Of the apoproteins of the $\rho = 1.054$ g/ml particles, designated lipoprotein HDL_1, over 60% was arginine-rich peptide, and the remainder was A-I, A-IV and C peptides. The molecular weight of these lipoproteins determined by agarose column chromatography was 2.36×10^6 for LD lipoprotein and 1.30×10^6 for lipoprotein HDL_1. On electron microscopy the radius of LD lipoprotein was 14.0 nm and that of lipoprotein HDL_1 was 10.0 nm, in contrast with molecular radii of 10.4 nm and 8.4 nm respectively determined from the gel-permeation-chromatography data. The lipid and phospholipid composition of both particles was determined. Lipoprotein HDL_1 was notable for both the concentration of its esterified cholesterol, which was similar to that of LD lipoprotein, and the low triacylglycerol content, resembling that of HD lipoprotein. The possible origin of lipoprotein HDL_1 is discussed.

As part of our studies of the metabolism of rat plasma lipoproteins (Pottenger & Getz, 1971; Hay *et al.*, 1971; Pottenger *et al.*, 1973), we have undertaken an investigation of the lipoproteins found in the density range 1.019–1.063 g/ml. In previous investigations, rat plasma lipoproteins have been isolated by flotation on subphases of defined density in fixed-angle rotors (Dudacek & Narayan, 1966; Camejo, 1967; Windmueller & Levy, 1967; Faloona *et al.*, 1968; Koga *et al.*, 1969; Lasser *et al.*, 1973; Kuel *et al.*, 1974; Calandra *et al.*, 1975*a*,*b*; Pasquali-Ronchetti *et al.*, 1975; Bar-On *et al.*, 1976) or have been isolated by rate zonal centrifugation in density gradients in either zonal or swinging-bucket rotors (Wilcox & Heimberg, 1970; Hinton *et al.*, 1973; Viikari & Pelliniemi, 1974; Danielsson *et al.*, 1978). Although lipoproteins in the density range 1.019–1.063 g/ml have been reported to contain only LD lipoproteins (Pasquali-Ronchetti *et al.*, 1975; Wilcox & Heimberg, 1970), we have found it difficult to isolate pure rat LD lipoprotein at densities greater than 1.040 g/ml. Many investigators have assumed that proteins other than LD lipoprotein found in this fraction are attributable to HD-lipoprotein contamination (Kuel *et al.*, 1974; Calandra *et al.*, 1975*a*,*b*; Bar-On *et al.*, 1976). However, several studies have suggested the presence of a lipoprotein having an electrophoretic mobility (α) characteristic of HD lipoprotein in the density fraction $\rho = 1.019$–1.063 g/ml (Dudacek & Narayan, 1966; Camejo, 1967; Faloona *et al.*, 1968). In more recent investigations this unique component has been isolated and partially characterized (Weisgraber *et al.*, 1977; Danielsson *et al.*, 1978). From the above work it is clear that the buoyant density of a lipoprotein is not a sufficient criterion to identify it as a member of the class of high- or low-density lipoproteins; its electrophoretic mobility must also be considered. In the present report we describe alternative methods for the isolation and further characterization of a lipoprotein occurring in the classical LD-lipoprotein range ($\rho = 1.019$–1.063 g/ml) but having an α electrophoretic mobility. It is termed lipoprotein HDL_1. This work has been presented in preliminary form (Lusk *et al.*, 1977).

Abbreviations used: VLD lipoprotein, very-low-density lipoprotein; LD lipoprotein, low-density lipoprotein; HD lipoprotein, high-density lipoprotein.

Materials and Methods

Animals

Two groups of rats (Sprague–Dawley, Madison, WI, U.S.A.) were studied: male rats weighing 250 g,

and female rats weighing 100–125g. Blood was collected from decapitated animals in 1.9mM-EDTA adjusted to pH7 with NaOH. The male rats were fed on Purina rat chow *ad libitum* until the time of decapitation; the female rats, also fed on Purina rat chow, were starved for 16h before decapitation.

Lipoprotein separations

Lipoprotein fractions of $\rho<1.006$g/ml (VLD lipoprotein), $\rho = 1.019$–1.063g/ml (LD lipoprotein) and $\rho = 1.063$–1.21g/ml (HD lipoprotein) were isolated in a Beckman L5-75 ultracentrifuge in a 60Ti rotor, $r_{av.}$ 6.3cm (10°C, 16h, 177700g for $\rho<1.006$g/ml; 10°C, 16h, 215000g for $1.019<\rho<1.063$g/ml; and 10°C, 24h, 255800g for $\rho<1.21$g/ml) according to the basic method of Havel *et al.* (1955). Lipoproteins of $\rho<1.006$g/ml and $\rho = 1.063$–1.21g/ml were re-centrifuged at those respective densities, but lipoproteins of $\rho = 1.019$–1.063g/ml were not refloated.

Rate zonal ultracentrifugation was performed as in the VLD-lipoprotein/LD-lipoprotein isolation method of Patsch *et al.* (1974). The $\rho = 1.019$–1.063g/ml fraction from 300ml of plasma was applied to a linear $\rho = 1.00$–1.30g/ml NaBr gradient in Beckman TiZ14 zonal rotor ($r_{max.} = 6.67$cm). The rotor was centrifuged for 140min at 132000$g_{max.}$, 15°C, after which 10ml fractions were collected and the A_{280} of each fraction was monitored. Each 10ml fraction was concentrated, at the density at which it was isolated from the rate zonal gradient, by flotation in a Beckman 30.2 ($r_{av.}$ 7.9cm) fixed-angle rotor (10°C, 22h, 79500g). Concentrated fractions were dialysed against 0.9% NaCl/0.05% EDTA before chemical analysis was performed.

Similar rate zonal separations were achieved in a swinging-bucket rotor. Rat lipoproteins of $\rho = 1.019$–1.063g/ml from 5–10ml of plasma were concentrated in a Beckman 30.2 rotor to 0.5ml. The lipoprotein solution was adjusted to $\rho = 1.30$g/ml with solid NaBr, placed in the bottom of a Beckman SW 41 rotor ($r_{av.}$ 10.93cm) tube and a $\rho = 1.00$–1.30g/ml linear gradient of NaBr was formed above the sample. The sample was centrifuged for 140min at 118000g at 15°C. Fractions were subsequently collected and the A_{280} was read with an ISCO gradient collector and u.v. monitor (Instrument Specialty Corp., Lincoln, NE, U.S.A.).

Isopycnic banding

The isopycnic-banding procedure used was that of Fless & Scanu (1975). Isolated lipoproteins (1mg/ml) were centrifuged to their equilibrium densities on a linear 0–10% (w/v) NaCl gradient containing 0.05% EDTA and 0.01% NaN_3 in a Beckman SW 41 rotor for 66h, 177000g, at 25°C. Identical gradients with no lipoprotein added were simultaneously centrifuged to determine the density profile. After centrifugation, fractions (0.5ml) were collected, with 20% (w/v) NaCl as the displacement medium, by using an ISCO gradient collector; the A_{280} of each was obtained with the u.v. monitor. The density of the individual fractions from the 'blank' gradients was determined by checking the refractive index at 20°C with a Zeiss refractometer (Zeiss, Oberkochen, Germany).

Molecular size determination

Gel-permeation chromatography on Sepharose 4B (Pharmacia, Uppsala, Sweden) agarose beads was performed to determine the effective molecular sizes of the isolated lipoproteins. A column (60cm × 1.5cm) was calibrated by using $Na^{125}I$ for determining the total column volume (V_t), bacteriophage T4 for determining the void volume (V_0), and human LD lipoprotein ($r = 10.4$nm), apoferritin ($r = 6.0$nm), human thyroglobulin ($r = 8.1$nm) and aldolase ($r = 4.6$nm) were the standards for the determination of Stokes radii, r (Edelhoch, 1960; Stellwagen & Schachman, 1962; Harrison, 1963; Fisher *et al.*, 1971). Calculation of the Stokes radius was as outlined by Ackers (1967). The formula mol.wt. $= \frac{4}{3}\pi r^3 N/\bar{v}$ (Tanford, 1961) was used for the calculation of the approximate molecular weight of the lipoproteins, N = Avogadro's number, $\bar{v}$ = partial specific volume and r = anhydrous radius. Since the Stokes radius determined from gel-permeation chromatography is that of the hydrated molecule, the conversion factor

$$r_{\text{anhydrous}} = r_{\text{hydrate}}\left[\frac{1}{P_p\,(v-\mu_w)}\right]^{\frac{1}{3}}$$

was used to determine the anhydrous radius, where P_p = isopycnic density and $\mu_w = 0.2$g/g = approximate hydration of lipoproteins. The values for P_p and $\bar{v}$ were determined from the isopycnic-banding experiments.

Electron microscopy

A negative-staining technique, using 1% phosphotungstic acid adjusted to pH7.0 with NaOH, was used for the electron-microscopic examination of the lipoproteins. One drop of lipoprotein solution (1mg of protein/ml) was placed on a Petri dish next to a drop of phosphotungstic acid. A needle was dipped first in the lipoprotein, then in the phosphotungstic acid, and finally to a carbon-coated grid (coated side down); excess liquid was drawn off with filter paper. Grids were photographed by using a Siemens A-1 electron microscope. Microscopic enlargement was $\times 10^4$.

Chemical methods

Protein was measured by the method of Lowry *et al.* (1951), with bovine serum albumin as the standard, and by using three extractions with diethyl ether after colour development to remove

neutral-liquid turbidity. Total cholesterol and triacylglycerol were determined by using methods developed by Rush *et al.* (1970) for the Autoanalyzer II (Technicon Instruments) with modifications specified by the Lipid Research Clinics Program of the Center for Disease Control, Atlanta, GA, U.S.A. (*Manual of Laboratory Operation*, 1974). Cholesterol ester was determined after digitonin precipitation of the free cholesterol as described by Sperry & Webb (1950). Lipid phosphorus was measured essentially by the method of Bartlett (1959); the phospholipid concentration (as phosphatidylcholine) was obtained by multiplying the phosphorus content by a factor of 25.4, which is based on an estimated mol.wt. of 787 for egg phosphatidylcholine. The phospholipid composition was determined by using two-dimensional t.l.c. by the method of Getz *et al.* (1970), followed by elution of the individual phospholipids and measurement of phosphorus concentration (Bartlett, 1959). Agarose-gel electrophoresis was done by the method of Noble (1968), with ACI–Corning (Palo Alto, CA, U.S.A.) supplies. Gels were stained for lipid with Fat Red 7B. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed without prior delipidation by the method of Weber & Osborn (1969) by using both 4% and 7.5% (w/w) polyacrylamide disc gels. Gels were stained for protein with Coomassie Blue by the method of Fairbanks *et al.* (1971); gels were subsequently scanned at 550nm for an estimate of the percentage distribution of protein between apoprotein bands.

Immunodiffusion

Double immunodiffusion was performed by the method of Ouchterlony (Clausen, 1972). The gels were buffered with 0.05M-Tris adjusted to pH8.6 with HCl. Lipoproteins were checked with antisera against the lipoprotein apoproteins A-I, A-IV, B and arginine-rich peptide. Antisera against apoprotein B and arginine-rich peptide were prepared as described by Pottenger & Getz (1971), and that against apoprotein A-1 was prepared as described by Schonfeld & Pfleger (1974).

Results

Agarose-gel electrophoretograms of rat plasma lipoproteins of $\rho = 1.019$–1.063g/ml revealed two components having the mobility of β- and α-globulin respectively. When this density fraction was applied to a linear rate-zonal gradient, three components were isolated (Fig. 1). When reconcentrated, fractions from the ascending edge of the peak of component I had the mobility of a β-globulin on agarose-gel electrophoresis, and fractions from the descending edge of the peak of component II had an α-globulin mobility. These apparently homogeneous fractions were used for further characterization. Component III did not stain for lipid.

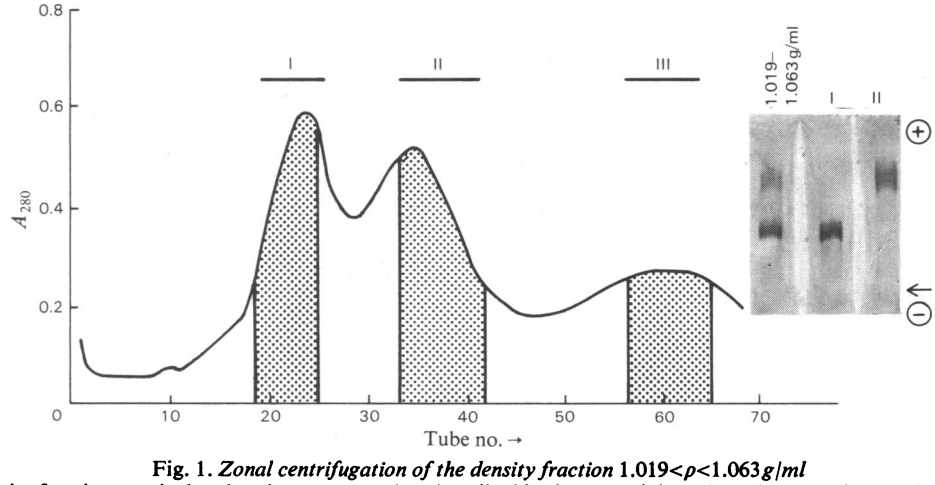

Fig. 1. *Zonal centrifugation of the density fraction* $1.019<\rho<1.063$ *g/ml*

The density fraction was isolated and concentrated as described in the Materials and Methods section, and was loaded on a linear 1.0–1.30g/ml NaBr density gradient in the zonal rotor TiZ14 ($r_{max.} = 6.67$cm) and centrifuged for 140min at 15°C, 132000g_{max}. Fractions (10ml) were collected, monitored at 280nm and concentrated by centrifugation for 24h in a Beckman 30.2 rotor ($r_{av.}$ 7.9cm, 10°C, 22h, 79500g). Each fraction was further examined by agarose-gel electrophoresis (Noble, 1968) and those fractions containing homogeneous components exhibiting the mobility of β- and α-globulins were pooled as indicated on the curve to represent components I and II respectively for further analysis and characterization. The inset illustrates the agarose-gel electrophoretograms of typical fractions.

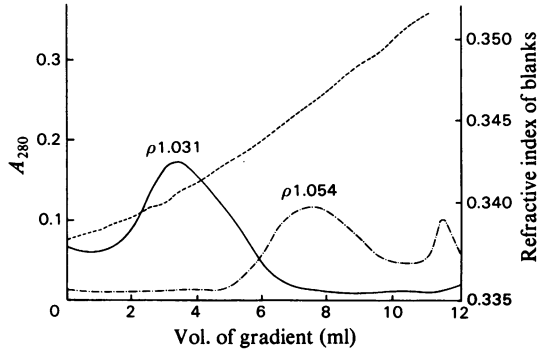

Fig. 2. *Isopycnic banding of components I and II*
Components I and II obtained as described in the legend to Fig. 1 were centrifuged in separate tubes containing 0–10 % (w/v) NaCl gradients in a Beckman SW 41 rotor. Centrifugation was at 25°C, 177000*g* for 66h; 0.5ml fractions were collected and their A_{280} was monitored. ——, Profile of component I; –·–·, profile of component II; ----, density profile through gradient tube.

As determined by triangulation of the zonal-centrifugation profiles, components I and II were present in approximately equal amounts from several independent preparations derived from both fed and starved male and female rats. Thus the ratio of component I to component II in nine preparations from male rats of various ages and nutritional states was 1.22±0.38 (S.D.) and that for three preparations from female rats was 0.88±0.69.

Isopycnic banding of the two components (Fig. 2) showed that component I had a density of 1.031g/ml ($\bar{v}$ = 0.9699) and component II had a density of 1.054g/ml ($\bar{v}$ = 0.9488). We attempted to prepare components I and II from the ρ = 1.019–1.063g/ml rat serum lipoprotein fraction by exploiting this observed difference in their buoyant densities, but met with only limited success. We have isolated the lighter component of ρ = 1.019–1.040g/ml and the heavier component of ρ = 1.050–1.063g/ml, but have always found cross-contamination (Fig. 2). However, some moderate success was achieved by using NaBr gradients in swinging-bucket rotors. The separations of components I and II according to their flotation velocities in an SW 41 rotor were similar to those achieved in the TiZ14 rotor. However, component III, an albumin-containing component, overlapped component II in the swinging-bucket-rotor separations. Attempts to remove the albumin from the ρ = 1.019–1.063g/ml fraction by refloating this fraction at ρ = 1.063g/ml resulted in the loss of much of lipoprotein component II. In spite of the minor albumin contamination, separations in a swinging-bucket rotor seem to be more practical for physiological experiments where small volumes of rat plasma are obtained.

The apparent hydrated molecular diameter as determined from gel-permeation-chromatography data was 20.6nm for component I and 16.8nm for component II. The diameter of HD lipoprotein was 14.8nm in parallel determinations. An Ackers-type plot (Ackers, 1967) of the apparent molecular radius of the standards used (see the Materials and Methods section) against the inverse error-function complement of 1−partition coefficient (determined from their behaviour on gel-permeation chromatography) was linear. The partition coefficients on gel-permeation chromatography of human LD lipoprotein, apoferritin, human thyroglobulin and aldolase were 0.439, 0.653, 0.541 and 0.724 respectively. The calculated molecular weights (Ackers, 1967) from the molecular-diameter values are 2.36×10^6 for component I, 1.30×10^6 for component II and 0.88×10^6 for HD lipoprotein.

The components were also observed by electron microscopy by using the technique of negative staining without fixation. Despite a tendency to aggregate, each fraction appeared to be quite homogeneous. Measurements made from the electron micrographs showed the diameter of component I

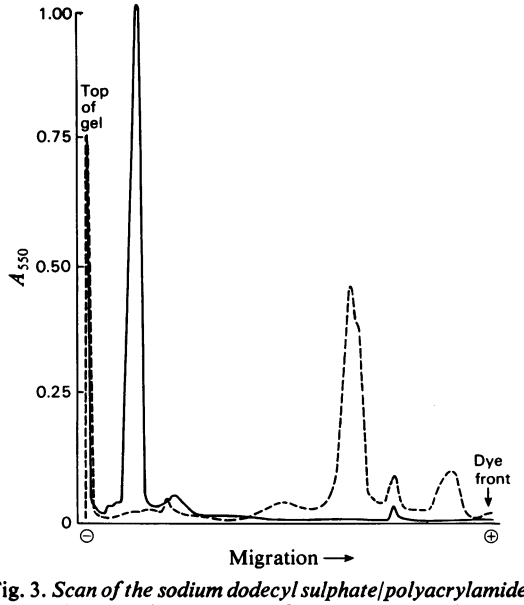

Fig. 3. *Scan of the sodium dodecyl sulphate/polyacrylamide-gel electrophoretograms of components I and II*
To permit B apoprotein to enter the gel, 4% acrylamide gels were used. Scans were performed at 550nm of Coomassie Blue-stained gels loaded in the gel linear-transport attachment of the Gilford 2400 spectrophotometer. Traces: ——, component I (LD lipoprotein); ----, component II (lipoprotein HDL_1 or ρ=1.054g/ml particle).

Table 1. *Composition of isolated rat lipoprotein particles*

LD lipoprotein (component I) and lipoprotein HDL_1 (component II) as well as HD lipoprotein were isolated by gradient centrifugation from the pooled plasmas (derived from 12 or more rats per pool) of old (250g) fed male rats (M) or young adult (100–125g) starved female rats (F). Protein, phospholipids, triacylglycerols, esterified and unesterified cholesterol as well as phospholipid composition were measured as described in the Materials and Methods section. Abbreviation: tr, trace.

	Percentage composition (by wt.)				
Lipoprotein particle	Protein	Phospholipids	Triacylglycerols	Unesterified cholesterol	Cholesterol ester
LD lipoprotein (M)	25.1	28.1	17.0	9.0	20.8
LD lipoprotein (F)	28.8	26.9	16.7	8.5	19.1
Lipoprotein HDL_1 (M)	30.4	37.1	1.3	10.6	20.7
Lipoprotein HDL_1 (F)	34.7	36.8	2.2	7.0	19.3
HD lipoprotein (F)	50.2	26.3	0.7	2.3	20.6
VLD lipoprotein (F)	8.0	15.9	70.3	3.7	2.0

	Phospholipid composition (% of total phospholipid P)					
Lipoprotein particle	Phosphatidyl-ethanolamine	Phosphatidyl-choline	Lysophosphatidyl-choline	Phosphatidyl-inositol	Sphingomyelin	Other
LD lipoprotein (M)	7.0	61.7	10.0	3.6	18.5	—
Lipoprotein HDL_1 (F)	tr	72.8	7.1	5.3	14.6	—
Lipoprotein HDL_1 (M)	3.0	62.2	6.9	5.7	16.4	6.0

to be 28.0±2.6nm and that of component II to be 20.0±1.2nm.

The analysis of the apoprotein composition of components I and II by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis revealed that, although component I contained almost exclusively apoprotein B (over 95%), component II was rich in an apoprotein having a mobility identical with that of isolated arginine-rich peptide (characterized by its amino acid composition). This peptide represented more than 60% of the total peptide loaded on the gel, and lesser amounts of A-I, A-IV and C peptides (less than 15% each) were also present. Except for C peptides the presence of each of the other peptides was established by double immunodiffusion against the appropriate antisera. The apoprotein profiles of components I and II are illustrated in Fig. 3. We used 4% acrylamide to permit apoprotein B to enter the gel. As seen from the scan shown in Fig. 3, there was very little B protein in component II. Thus on immunodiffusion gels only 10μl of undiluted component II (at a concentration of about 1mg of protein/ml) reacted with antiserum to LD lipoprotein, whereas the sample could be diluted more than 100-fold and retain its reactivity with anti-(arginine-rich peptide) serum. Conversely component I reacted with anti-(arginine-rich peptide) serum only in a similar undiluted state, but retained its reactivity with anti-(LD lipoprotein) serum after more than 100-fold dilution.

The chemical composition of components I and II is shown in Table 1. For comparison, the chemical compositions of rat VLD lipoprotein (ρ<1.006g/ml) and HD lipoprotein (ρ = 1.063–1.21g/ml), as determined in our laboratory, are included. Component II had a protein content intermediate between that of LD lipoprotein and HD lipoprotein. The most notable difference in lipid composition of these two particles was in the very low triacylglycerol content of component II, similar to that of HD lipoprotein. Component II had a higher weight proportion of phospholipid than did other lipoprotein, including HD lipoprotein. The proportion of unesterified cholesterol in component II was higher than in HD lipoprotein, but it had a cholesterol ester content similar to those of both LD lipoprotein and HD lipoprotein.

Discussion

In the present study, the alternative procedures, involving rate zonal gradient centrifugation using either the zonal rotor or tubes in swinging-bucket rotors, have been described for the isolation of HD lipoprotein separately from LD lipoprotein. The new findings in this study establish the isopycnic point of lipoprotein HDL_1 as 1.054g/ml and suggest that the cut-off density for the separation of lipoprotein HDL_1 from rat lipoprotein HDL_2 should be at 1.075g/ml rather than 1.063g/ml. Also, the relationship between the size of the lipoprotein HDL_1 particle determined by electron microscopy and gel-permeation chroma-

tography is reported. The phospholipid composition of the HDL_1 particle has been reported for the first time.

Many investigators have reported that rat LD lipoprotein isolated at $\rho<1.063$ g/ml is not homogeneous. Windmueller & Levy (1967) and Koga *et al.* (1969) lowered the 'cut-off' density to $\rho = 1.040$ g/ml, Lasser *et al.* (1973) used $\rho<1.050$ g/ml and Pasquali-Ronchetti *et al.* (1975) chose $\rho<1.045$ g/ml. Lipoproteins isolated between these densities and $\rho = 1.063$ g/ml were considered to be HD-lipoprotein contamination.

Camejo (1967) and Faloona *et al.* (1968) showed that concentrated $\rho = 1.006$–1.063 g/ml fractions contained two major peaks. Indeed, Danielsson *et al.* (1978) reported the isolation of lipoprotein very similar to component II described here, by rate zonal ultracentrifugation of whole rat plasma. However, in contrast with our observation for the isopycnic point for lipoprotein HDL_1 (1.054 g/ml), these last authors reported that the density of this particle was 1.08 g/ml.

Dudacek & Narayan (1966) isolated lipoproteins in the density ranges 1.030–1.040 g/ml and 1.050–1.063 g/ml. The lighter lipoprotein was designated LD lipoprotein and the denser lipoprotein HDL_1 on the basis of their mobilities in 3.75% polyacrylamide gels. We obtained similar fractions containing predominantly LD lipoprotein and lipoprotein HDL_1 which resemble the components I and II separated on gradients according to their different flotation velocities, but have found that the lipoprotein HDL_1 recovered after isopycnic centrifugation in the density range 1.050–1.063 g/ml is invariably contaminated with LD lipoprotein. Gradient separations yield larger recoveries of purer fractions in much shorter times.

Component II isolated from both normal male and female rats, whether starved or fed, has an isopycnic point of 1.054 g/ml, the electrophoretic mobility of an α-globulin on agarose, all of the same apoproteins as are found in HD lipoprotein, and a lipid composition resembling that of HD lipoprotein. All of these properties justify the designation lipoprotein HDL_1. However, it differs substantially from the major HD lipoproteins (HDL_2 and HDL_3) in molecular size, isopycnic point (1.10 g/ml reported by Camejo, 1967) and proportion of various constituent apoproteins. Arginine-rich peptide predominates in lipoprotein HDL_1, whereas A-I is the major apoprotein in lipoproteins HDL_2 and HDL_3.

HDL_1 particles similar in physical and chemical properties to those described here were isolated by zonal ultracentrifugation of whole rat plasma (Danielsson *et al.*, 1978), and by a combination of ultracentrifugation and Geon–Pevikon block electrophoresis from normal dog plasma (Mahley & Weisgraber, 1974) and from normal rat plasma (Weisgraber *et al.*, 1977). Though lipoprotein HDL_1 is present in both normal rat and dog plasma at a concentration similar to that of LD lipoprotein, both are still minor lipoproteins compared with lipoproteins HDL_2 and HDL_3. On the basis of the similar contents of LD lipoprotein and lipoprotein HDL_1 in the plasma of the rat, the latter represents about 5–10% of the total HD-lipoprotein protein (Eder & Roheim, 1976) and only about 2% of total dog HD-lipoprotein protein (Mahley & Weisgraber, 1974).

The relatively low concentration of LD lipoprotein in the rat is probably the result of the efficient removal and degradation of plasma VLD-lipoprotein B protein in the liver (Faergeman *et al.*, 1975). Whether this process relates to the steady-state concentration of lipoprotein HDL_1 in the plasma of normal rats remains undetermined. It is worth noting that the dog and the rat are also two species requiring extraordinary manipulation for the experimental induction of atherosclerosis. In both, thyroid ablation and/or cholate administration are necessary to induce significant atherosclerosis. Lipoproteins containing arginine-rich peptide bind to the high-affinity LD-lipoprotein receptors of fibroblasts (Bersot *et al.*, 1976; Goldstein & Brown, 1977) and indeed may have some structural feature in common with LD lipoprotein (Mahley *et al.*, 1977). The steady-state concentration of lipoprotein HDL_1, with a high content of arginine-rich peptide, accessible to the LD-lipoprotein receptor in the presence of low concentrations of competing LD lipoprotein may exert a direct or indirect effect on the peripheral uptake of cholesterol or on the genesis of hypercholesterolaemia.

The origin of lipoprotein HDL_1 is uncertain. Available experimental evidence does not permit one to distinguish between several mutually exclusive possibilities. Among the latter are a maturation of nascent HD lipoprotein (Marsh, 1976; Hamilton *et al.*, 1976), which lipoprotein HDL_1 resembles, especially in its apoprotein composition. However, the spherical shape and high esterified-cholesterol content of lipoprotein HDL_1 contrast with the disc shape and predominant unesterified cholesterol of nascent HD lipoprotein (Hamilton *et al.*, 1976), which argues against the latter as a source of lipoprotein HDL_1. Alternatively it is possible that lipoprotein HDL_1 originates as a product of the catabolism of chylomicrons, especially those transporting relatively large amounts of cholesterol. Chylomicrons contain all the apoproteins found in lipoprotein HDL_1, though several of these, notably arginine-rich peptide and apoproteins C, are acquired, not through intestinal synthesis (Wu & Windmueller, 1978), but by exchange with HD lipoprotein (Imaizumi *et al.*, 1978).

HDL_c, a lipoprotein similar in both its lipid and apoprotein composition to lipoprotein HDL_1, is

induced by feeding cholesterol to dogs (Mahley *et al.*, 1974), pigs (Mahley *et al.*, 1975), rabbits (Shore *et al.*, 1974) and rats (Mahley & Holcombe, 1977). Formation of lipoprotein HDL_c may require chylomicrons as an intermediary particle. In those species that do not have steady-state concentrations of lipoprotein HDL_1 or whose mature HD lipoprotein contains little or no arginine-rich peptide, nascent HD lipoprotein in normal animals or lipoprotein HDL_c in cholesterol-fed animals may serve as a source of chylomicron arginine-rich peptide. In the normal rat and dog, lipoprotein HDL_1 may represent a readily detectable pool of recycling arginine-rich peptide involved in chylomicron metabolism. In cholesterol-fed animals an expanded recycling pool of this peptide may appear as lipoprotein HDL_c.

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